Compartmental distinctions in uterine Muc-1 expression during early pregnancy in cynomolgous macaque (Macaca fascicularis) and baboon (Papio anubis)

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BACKGROUND: Loss of the transmembrane mucin, Muc-1, is a molecular correlate of the acquisition of uterine receptivity to embryo adhesion in most species examined. In macaques, two distinct adhesion events occur at opposite sides of the uterus. Attachment to the secondary site is delayed relative to the primary site. The aim was to determine if Muc-1 is removed at secondary sites prior to trophoblast attachment. METHODS: We examined Muc-1 expression in the uteri of cynomolgus macaque and baboon during early implantation by immunocytochemistry. RESULTS: Luminal epithelia were devoid of Muc-1 at all stages examined at both primary and secondary adhesion sites. Loss of Muc-1 in luminal epithelia was found to be maternally determined, accompanied membrane transformation in both macaque and baboon, and at secondary implantation sites, preceded trophoblast attachment. In contrast, glandular epithelia in pregnant macaques exhibited a temporal and compartmentalized gradient of Muc-1 loss confined to the implantation sites. Glandular epithelia in the pregnant baboon uterus were uniformly negative for Muc-1. CONCLUSIONS: Restriction of the Muc-1 loss in glandular epithelia to conceptual cycles may reflect the fundamental distinctions among epithelia of the various uterine compartments and the differential modulation of Muc-1 that occurs within these compartments in conceptual and non-conceptual cycles.

Key words: endometrium/implantation/Muc-1/primate/receptivity

Introduction
Adhesion of trophectodermal cells of an attachment competent blastocyst to the apical surface of luminal epithelia constitutes the necessary initial event for successful mammalian implantation. Under the control of ovarian steroids, the uterus prepares itself for this event by transitioning from a non-receptive to a temporally restricted receptive state. Receptivity, as originally conceived, was functionally defined: that state of uterine differentiation that is permissive for embryo attachment (Psychoyos, 1986), a definition which has frustrated efforts to establish morphological and molecular correlates of the receptive state, particularly in the human, where ethical and moral constraints prohibit in vivo functional testing for receptivity. In studies on non-human primates, the definition of receptivity has been expanded to include the ability of uterine cells to respond to embryonic signals (Fazleabas and Strakova, 2002). Since the receptive state is followed in many species by a refractory stage, temporal and regional criteria for identification of ‘markers of receptivity’ have been applied, i.e. markers of the receptive state should be confined to the receptive state and must be displayed by the luminal epithelium, the active maternal participant in embryo adhesion. Nonetheless, application of a temporal criterion requires precise definition of the temporal limits of the functionally receptive period in each species examined.

Few morphological and molecular correlates of the receptive state are shared across species (reviewed in Carson et al., 2000; Sharkey and Smith, 2003). Reduction or loss of transmembrane mucin, the major component of the glycocalyx, is a temporal molecular correlate of the receptive state in most species (reviewed in Carson et al., 1998; Lagow et al., 1999; Thathiah and Carson, 2002). By virtue of their highly extended and heavily glycosylated ectodomains, transmembrane mucins form a protective barrier in the non-receptive uterus, limiting access of degradative enzymes and microbes and producing a non-adhesive surface (reviewed in Brayman et al., 2004). In the context of implantation, it has been proposed that removal or down-regulation of transmembrane mucins in luminal epithelia is necessary to produce a surface receptive to embryo adhesion (Braga and Gendler, 1993; Surveyor et al., 1995). Researchers have turned to non-human primate models in hopes of explaining the perplexing observation that humans appear to be a rare species in which
MUC1, the major transmembrane mucin in uteri of most species, does not seem to be reduced in luminal epithelia of the receptive uterus (Aplin et al., 1998; DeLoia et al., 1998). When the temporal and spacial expression of cell-associated Muc-1 core protein was examined in baboon (Papio anubis) uteri during non-conceptual cycles (Hild-Petito et al., 1996), Muc-1 was upregulated in luminal epithelium of baboon uterus during the early luteal phase in response to progesterone as has been observed in the human uterus (Hey et al., 1994); however, unlike the human, continued exposure to progesterone resulted in loss of both Muc-1 and progesterone receptor from luminal epithelia as the transition to receptivity occurred. In the new world monkey, Cebus apella, Muc-1 was detected on luminal epithelium at day 7 post ovulation (PO) of a non-conceptual cycle (Jones et al., 2001). Other than the observation that expression was generally lower in luminal epithelium compared to glandular epithelium, insufficient information on the pattern of Muc-1 core protein expression through the cycle in luminal epithelium of C. apella was provided to allow comparison to the pattern in baboon or human uteri. Comparison is further confounded by differences in day of embryo adhesion (day 5 of luteal phase), length of cycle and uterine physiology. While both of these studies provide information on Muc-1 expression in the cycling uteri of non-human primates during the receptive period, no studies have examined Muc-1 expression during conceptual cycles.

Early implantation in non-human primates differs from that of humans in several respects, two of which suggest that the receptive state may be of longer duration and involve a greater proportion of the luminal epithelial population than in the human uterus. Although initial implantation in both humans and non-human primates is intrusive, implantation in non-human primates is superficial rather than fully interstitial as it is in humans (Enders, 1993). In some non-human primates, exposed trophoblast undergoes a secondary adhesion ~2 days after the initial adhesion takes place (Enders, 1991). At both primary and secondary implantation sites, luminal and glandular neck epithelia hypertrophy to form epithelial plaques. Since the ability to support embryo adhesion and epithelial plaque formation are considered to be functional responses of the receptive state in non-human primates (Fazleabas et al., 1999), the luminal epithilia must remain receptive beyond the initial adhesion event. Attachment to the secondary site is significantly delayed relative to the primary attachment site, providing the opportunity to examine Muc-1 expression at definitive implantation sites. Muc-1 expression was examined in the cynomolagus macaque (Macaca fascicularis) uterus during the early implantation period spanning secondary plaque formation and trophoblast adhesion. Baboon (Papio anubis), which displays a more modest epithelial plaque response and undergoes a single adhesion event, was included as a contrast to the macaque.

Materials and methods

Animals and tissue preparation

Implantation stages were collected from cynomolgus macaques (Macaca fascicularis) and processed as previously described (Enders and King, 1991; Enders, 1995). Table I describes the stages examined in the present study. All procedures were approved by the University of California at Davis Animal Care and Use Committee. Uterine tissues from adult female baboons (Papio anubis) were obtained at hysterectomy or endometriectomy as previously described (Jones and Fazleabas, 2001). Table II lists the source and reproductive status of baboon samples examined in the present study. All procedures were approved by the Animal Care and Use Committee of the University of Illinois, Chicago.

Immunohistochemistry

Formalin-fixed, paraffin-embedded 8-µm sections were deparaffinized by three 3 min rinses in Clearing solvent, citrus based (Cornwell Corp, Riverdale, NJ) and rehydrated in a graded ethanol series, followed by a 5 min rinse in flowing ultrapure water. Rehydrated sections were subjected to the following antigen retrieval protocol: immersion in 250 ml 0.01 M sodium citrate, pH 6.0 in a plastic container and brought to boiling in a microwave oven after 3 min at 100% power, then maintained at boiling with a 10 s pulse at 50% power every 30 s for 6 min. Samples were left for 15 min in the retrieval buffer followed by three 5 min rinses in phosphate-buffered saline (PBS). Samples were blocked for 1 h at room temperature in 2% (v/v) normal donkey serum diluted in PBS. Samples were exposed to primary antibody for 1 h at 37 °C, followed by three 5 min rinses in PBS, and secondary antibody for 40 min at 37 °C, followed by three 5 min rinses in PBS. Samples were mounted in 1 mg/ml paraphenylene diamine in 10% (v/v) PBS/ glycerol at pH 8 (Johnson and Araujo, 1981) and imaged on a Zeiss Axioskop 2 equipped with a Spot cooled color digital camera

Table I. Macaque samples used in this study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Post ovulatory day</th>
<th>Site</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>KE50</td>
<td>d 12</td>
<td>1°</td>
<td>Very early lacunar</td>
</tr>
<tr>
<td>KE55</td>
<td>d 12</td>
<td>1°</td>
<td>Early lacunar</td>
</tr>
<tr>
<td>KE56</td>
<td>d 14</td>
<td>1°</td>
<td>Late lacunar/early villar; includes abembryonic troph prior to 2° adhesion</td>
</tr>
<tr>
<td>KE50</td>
<td>d 12</td>
<td>2°</td>
<td>Plaque present, no troph*</td>
</tr>
<tr>
<td>KE55</td>
<td>d 12</td>
<td>2°</td>
<td>Plaque present, no troph</td>
</tr>
<tr>
<td>KE56</td>
<td>d 14</td>
<td>2°</td>
<td>Plaque present, no troph</td>
</tr>
<tr>
<td>KE57</td>
<td>d 14</td>
<td>2°</td>
<td>Plaque present, troph adherent</td>
</tr>
<tr>
<td>KE59</td>
<td>d 15/16</td>
<td>2°</td>
<td>Plaque present, no troph*</td>
</tr>
<tr>
<td>KE63</td>
<td>d 12</td>
<td>1°</td>
<td>Non-conceptual cycle</td>
</tr>
<tr>
<td>KE63</td>
<td>d 12</td>
<td>2°</td>
<td>Non-conceptual cycle</td>
</tr>
</tbody>
</table>

*The term “no troph” refers to the absence of adhering trophoblast in the sections examined.

Table II. Baboon samples used in this study

<table>
<thead>
<tr>
<th>Animal</th>
<th>Post ovulatory day</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCY 158</td>
<td>d 5</td>
<td>Intact, natural cycle</td>
</tr>
<tr>
<td>PA 6629</td>
<td>d 8</td>
<td>Intact, natural cycle</td>
</tr>
<tr>
<td>PAN 2774</td>
<td>d 9</td>
<td>Intact, natural cycle</td>
</tr>
<tr>
<td>PA 5910</td>
<td>d 10</td>
<td>Intact, natural cycle</td>
</tr>
<tr>
<td>PA 6808</td>
<td>d 12</td>
<td>Intact, natural cycle</td>
</tr>
<tr>
<td>PA 6144</td>
<td>d 14</td>
<td>Intact, cycling, HCG infusion</td>
</tr>
<tr>
<td>PA 6524</td>
<td>d 18</td>
<td>Intact, cycling, HCG infusion</td>
</tr>
<tr>
<td>PA 6526</td>
<td>d 18</td>
<td>Intact, cycling, HCG infusion</td>
</tr>
<tr>
<td>PAN 2019</td>
<td>d 14</td>
<td>Pregnant</td>
</tr>
<tr>
<td>PA6146</td>
<td>d 18</td>
<td>Pregnant</td>
</tr>
<tr>
<td>PA6101</td>
<td>d 22</td>
<td>Pregnant</td>
</tr>
</tbody>
</table>
were examined and displayed similar staining patterns. At least two sections of each site detected by the FITC-conjugated second antibody, remained green-yellow. Examples of this type of fluorescence were seen in the background due to autofluorescence which could not be quenched in the cytoplasmic tail (Pemberton et al., 1992). Attempts to detect macaque Muc-1 with antibodies recognizing epitopes in the tandem repeat region of human MUC1 core protein were unsuccessful. Sections probed with and without antigen retrieval indicated that tissue processed by formalin fixation and paraffin embedding required a retrieval step in order to optimize detection of Muc-1 with the CT-1 antibody. Application of this staining protocol to formalin-fixed, paraffin-embedded sections from cycling baboon uteri produced the same expression pattern for Muc-1 obtained previously when frozen sections were probed with CT-1 antibody and immunoperoxidase detection (Hild-Petito et al., 1996). Controls, which included non-immune rabbit IgG or buffer in place of primary antibody, displayed a background due to autofluorescence which could not be quenched or blocked. The autofluorescence was found to fluoresce across a broad spectrum and could be distinguished from specific Muc-1 staining by visualization through a dual pass filter. Under these conditions, the autofluorescence was orange to brown while Muc-1, recognized by the FITC-conjugated second antibody, remained green-yellow. Examples of this type of fluorescence were seen in the erythrocytes present in the trophoblast lacunae and the vascular elements of the endometrium. At least two sections of each site were examined and displayed similar staining patterns.

**Antibodies**

CT-1, a peptide affinity purified rabbit polyclonal antibody (DeLoia et al., 1998), was used at a concentration of 35 μg/ml in PBS to detect Muc-1. Fluorescein isothiocyanate (FITC) conjugated donkey anti-rabbit IgG, highly cross-adsorbed by the manufacturer against serum proteins of several species to enhance species specificity (Amersham Corp., Arlington Heights, IL), was used at a dilution of 1:10 in PBS and included DAPI at a concentration of 0.1 μg/ml to counterstain nuclei. CT-1 was the antibody of choice since it is not species specific nor affected by the glycosylation state of Muc-1 and would recognize all Muc-1 isoforms except the secreted form lacking the cytoplasmic tail (Pemberton et al., 1992). Attempts to detect macaque Muc-1 with antibodies recognizing epitopes in the tandem repeat region of human MUC1 core protein were unsuccessful. Sections probed with and without antigen retrieval indicated that tissue processed by formalin fixation and paraffin embedding required a retrieval step in order to optimize detection of Muc-1 with the CT-1 antibody. Application of this staining protocol to formalin-fixed, paraffin-embedded sections from cycling baboon uteri produced the same expression pattern for Muc-1 obtained previously when frozen sections were probed with CT-1 antibody and immunoperoxidase detection (Hild-Petito et al., 1996). Controls, which included non-immune rabbit IgG or buffer in place of primary antibody, displayed a background due to autofluorescence which could not be quenched or blocked. The autofluorescence was found to fluoresce across a broad spectrum and could be distinguished from specific Muc-1 staining by visualization through a dual pass filter. Under these conditions, the autofluorescence was orange to brown while Muc-1, recognized by the FITC-conjugated second antibody, remained green-yellow. Examples of this type of fluorescence were seen in the erythrocytes present in the trophoblast lacunae and the vascular elements of the endometrium. At least two sections of each site were examined and displayed similar staining patterns.

**Results**

**Histoarchitecture and compartmental designations of implantation sites**

The morphology and developmental chronology of implantation stages in macaque and baboon uteri preceding those examined in the present study are described in Enders (1993). By day 12 of pregnancy in the macaque, expansion of the trophoblastic plate has raised the embryo and trophoblastic lacunae above the superficial zone (zonal designations are described in Figure 1). The epithelial plaque spans both superficial and principal zone epithelia. At primary sites in the present study, the central portion of the epithelial plaque has been penetrated by trophoblast and usually the lateral portions of the plaques were not contiguous with the luminal surface. At secondary sites, the central portion of the plaque was contiguous with the luminal surface. Luminal epithelia outside the plaque region overlay areas of stromal edema in primary sites. Stromal edema was not present at secondary sites. Generally, the lumena of glands were devoid of secretory material. Glands extended to, but did not penetrate, the myometrial compartment. The endometrial histoarchitecture of baboon uteri was similar to macaque except that the basal portion of some glands penetrated the inner margin of myometrium. Glands of the principal and transitional zones occasionally contained erythrocytes in the lumen.

**Muc-1 expression in the superficial zone**

No studies on early implantation in any primate species, including human, have captured the initial adhesion event in situ. The site of primary adhesion cannot be predicted and there are no reports that a particular area of
the uterus is especially ‘prepared’ to receive the blastocyst. On the contrary, in baboon and macaque the majority of luminal epithelia attain ‘receptivity’. Although the site is usually centrally located in the wall of the macaque uterus, neither wall is favored over the other as the site of primary adhesion (Heuser and Streeter, 1941). Thus, the luminal epithelial cell encountered by the blastocyst and to which it initially adheres is that which develops as the epithelia transitions to the receptive state.

The primary adhesion event in both baboon and macaque has been estimated to occur on days 8–9 PO and functionally defines the initiation of the receptive period (Enders, 1993). For primary adhesion, the approximation is based on time frames between which non-adherent blastocysts may still be flushed from the uterus and the earliest time at which firm adhesion to the uterine surface can be demonstrated. The luminal epithelia encountered by the baboon blastocyst during this period present a domed surface devoid of Muc-1. In baboon the luminal epithelia lose Muc-1 as they transition to a receptive state. Pre-receptive day 5 PO luminal epithelia uniformly expressed Muc-1 at their apical surface, and there appeared to be some intracellular localization (Figure 2A). By day 8 PO, Muc-1 was reduced on some cells and restricted to the apical surface which was protruding or domed (Figure 2B). The apical surface of day 9 PO luminal epithelia expressed no Muc-1 (Figure 2C), a condition which was maintained in luminal epithelia of baboon uteri through day 12 PO (Figure 2D and E) while Muc-1 continued to be expressed in gland neck epithelia on day 12 PO. Similarly, the apical surfaces of luminal epithelia lining both uterine walls of macaque uterus on day 12 of a non-conceptual cycle expressed no Muc-1 and displayed prominent doming (Figure 3A and B). Superficial zone uterine epithelia of day 12 pregnant macaque also lacked Muc-1 (Figure 3C and D). The luminal epithelia proximal to both primary and secondary epithelial plaques resembled the luminal epithelia of day 12 PO cycling uterus, as did those proximal to primary and secondary sites on day 14 (Figure 3E and F) and day 15 (not shown) of pregnancy. Thus, the luminal epithelia outside the implantation site resembled those in stage-matched cycling uteri, and did not appear to have altered their morphology or Muc-1 expression in response to the presence of the implanting embryo.

In one of the gland necks of day 12 PO cycling macaque, progressive stages in loss of Muc-1 were suggested (Figure 4). Initial doming appeared to take place in the central portion of the apical plasma membrane, surrounded by a Muc-1 positive ‘collar’. When the domed area involved the entire apical portion of the cell, Muc-1 was no longer detected, so that cells emerging from the gland opening resembled those lining the uterine lumen (Figure 3A). The cartoon below Figure 4(A) depicts the proposed progression of Muc-1 loss correlated with an apical membrane transformation as just described. A similar progression may occur in the human uterus as suggested by the work of Horne et al. (2002).

**Muc-1 expression in the epithelial plaque**

Formation of the epithelial plaque distinguishes the pregnant from the non-pregnant uterus in both baboon and macaque and is considered a functional response of receptive epithelia (Fazleabas et al., 1999). In response to signals from
the implanting embryo, luminal epithelia and epithelia of the gland necks undergo epithelial plaque formation (Enders et al., 1985; Jones and Fazleabas, 2001). Hypertrophy and endoreduplication produce nests of enlarged epithelia enclosed in a basement membrane and surrounding a tiny lumen. Only sparse microvilli have been described on the apical surface of these cells.

Muc-1 expression in the epithelial plaque was considered separately since it is recruited from and spans two compartments. Although morphologically distinct from the epithelia from which they are derived, the pattern of Muc-1 expression in plaque epithelia reflected their compartmental derivation and subsequent compartmental location. In the primary sites of pregnant macaque uterus, the central portion of the plaque was no longer present and only lateral remnants remained. The luminal epithelia overlying the remaining nests of plaque acini present in the principal zone were devoid of Muc-1 (Figure 5A). Where plaque was contiguous with the luminal epithelium (Figure 5B), plaque cells formed an irregular surface epithelia lacking Muc-1. Epithelial plaques had formed at all secondary sites in the pregnant macaque uteri. Early secondary sites (day 12 PO) displayed a modest plaque formation in response to the proximal presence of trophoblast prior to the secondary adhesion event. In one of the two day 12 sites, the presence of intact abembryonic trophoblast associated with the primary site and absence of adherent trophoblast at the secondary site confirmed that secondary adhesion had not occurred. Although secondary adhesion has been reported to occur on day 11 PO in the macaque uterus (Enders, 1993), adhesion could be confirmed in only one sample from day 14, which contained adherent trophoblast (Figure 5C). The luminal epithelium in this secondary site was negative for Muc-1 as was the luminal epithelia overlying all secondary epithelial plaques included in this study. Muc-1 was not detected in trophoblast at any stage of pregnancy examined (Figure 5A and C). In contrast to the luminal epithelia overlying the plaque, the plaque acini in the principal zone variably expressed Muc-1. Muc-1 was detected in the center of the plaque acini, presumably at the apical surface of plaque cells facing the tiny lumen (Figure 5A and C) and appeared to be intracellular in some plaque cells (Figure 5D). Not all plaque acini contained Muc-1; those with the most intense expression were located at the plaque periphery.

The pattern of Muc-1 expression in epithelial plaque cells of day 18 pregnant baboon was similar to that in macaque (Figure 6). Luminal epithelia overlying plaque (and trophoblast) were negative for Muc-1. The most intense Muc-1 expression was detected in the peripheral plaque acini (compare Figure 6A with B). By day 22 of pregnancy, Muc-1 expression in plaque acini was barely detectable (Figure 6C).

**Muc-1 expression in the principal, transitional and basal zones**

In pregnant macaque uteri, a progressive loss of Muc-1 was observed in the glandular epithelia of the principal and transitional zones underlying the epithelial plaque at both primary and secondary sites, although the progression was delayed at the secondary sites relative to the primary site. In the region of primary plaque on day 12 of pregnancy Muc-1 expression...
was lacking in glandular epithelia of the principal zone underlying the plaque (Figure 7A). A graded expression of Muc-1 was observed in glandular epithelium extending from the base of the plaque toward the myometrium, with the most intense Muc-1 expression evident in the glands of the basal zone (Figure 7C), which displayed an intensity similar to that in basal glands of day 12 PO cycling macaque uterus (Figure 7E). On day 14, the region of Muc-1 reduction in glands underlying the plaque was extended, with only the most basal portion of the glands retaining expression. Glandular epithelia of the principal zone outside the epithelial plaque continued to express moderate levels of Muc-1 (Figure 7B), equivalent to that observed in day 12 PO cycling macaque uterus (Figure 7D). At secondary sites on day 12 PO of pregnancy, principal zone glands, both those underlying and outside the plaque at secondary sites, still retained low levels of Muc-1 expression. Principal zone glands underlying the plaque in secondary sites from later stages (day 14 and day 15/16 of implantation) had lost Muc-1 expression. As at primary sites, Muc-1 was expressed in basal glands of secondary sites with an intensity resembling that in basal glands of cycling uteri, and glandular epithelia of principal and transitional zones outside the plaque continued to express Muc-1. A compartmentalized summary of the staining patterns for Muc-1 in the primary and secondary adhesion sites compared to stage matched non-conceptual endometrium is presented in Table III.

In pregnant baboon uteri, Muc-1 could not be detected in any of the glandular zones. With the exception of the epithelial plaque cells, glandular compartments of pregnant uteri were devoid of Muc-1, including basal glands. In this regard pregnant baboon differed from pregnant macaque. Although cycling baboon uteri expressed Muc-1 in glandular compartments through day 12 PO, expression was graded, with...
the most intense expression evident in basal glands. This pattern was maintained into the late luteal phase (Figure 8).

Discussion

In both baboon and macaque uteri, loss of Muc-1 appeared to precede embryo attachment which is consistent with the proposed role of Muc-1 as an anti-adhesive molecule (DeSouza et al., 1999). The striking differences in Muc-1 expression within the various epithelial compartments observed in the present study emphasize the necessity of adopting a compartmental approach when evaluating expression patterns of the molecular correlates of the receptive state. Functional receptivity is restricted to the superficial zone (luminal epithelium and gland neck epithelium) and, in the cycling primate uterus, reduction or loss of Muc-1 was similarly restricted to the superficial zone as the uterus transitioned to the receptive state. In the cycling baboon uterus, Muc-1 loss in the superficial zone correlates with disappearance of progesterone receptor (Hild-Petito et al., 1996). Receptive stage (day 21 of an artificial cycle) endometrium of rhesus macaque also lacked Muc-1 in the superficial zone (J.Julian, W.C.Okulicz and D.D.Carson, unpublished results). Muc-1 absence persisted in the superficial zone of macaque uterus through day 12 PO in non-conceptual cycles. Thus, loss of Muc-1 in the superficial zone of baboon and macaque uteri is a maternally regulated event that does not require the presence or participation of an embryo.

While restricted to the superficial zone, functional receptivity is not limited within the uterine lumen. The embryo encounters and attaches on day 9 to superficial epithelia uniformly presenting a domed surface devoid of Muc-1 throughout the uterine lumen. Either wall of the uterus may serve as the site of primary adhesion (Heuser and Streeter, 1941). Pregnancy did not alter this state of Muc-1 expression in the superficial zone. Although morphologically distinct, epithelial plaque cells recruited from luminal epithelia devoid of Muc-1 also lacked Muc-1. Muc-1 was uniformly absent in the superficial zones of pregnant macaque and baboon uteri, within and outside both primary and secondary sites of implantation. Adhesion at the secondary site involves cells morphologically and developmentally distinct from those involved in the primary adhesion event. Muc-1 was removed during the initial transition to receptivity and was not re-expressed in the superficial zone before secondary adhesion. Extended maintenance of Muc-1 removal throughout luminal epithelia may be necessary in species producing a bidiscoid placenta resulting from two adhesion events. However, it is not clear why this occurs in species such as baboon where a singular adhesion event occurs.

The membrane transformation that accompanied loss of Muc-1 in the superficial zone is an acknowledged morphological correlate of the receptive state (reviewed in Lopata et al., 2002) and has been observed previously in receptive phase cycling baboon uterus (Fazleabas et al., 1999; Jones and Fazleabas, 2001), day 8 PO macaque uterus (Sengupta and Ghosh, 2002) and day 11 PO pregnant marmoset uterus (Niklaus et al., 2001). The complete membrane transformation is confined to conceptual cycles in most species with a partial transformation occurring during non-conceptual cycles (Murphy, 2000). The primate uterus is unique in that complete membrane transformation takes place in every cycle, regardless of conception. Membrane transformation in the cycling human uterus is both regionally and temporally restricted (Nikas, 1999; Usadi et al., 2003). The same regional and temporal restrictions do not exist in Old World monkeys. Both baboon and macaque exhibited uniform membrane transformation throughout the uterus that was...
Figure 7. Compartmentalized loss of Muc-1 expression in glandular epithelia underlying the epithelial plaque in pregnant macaque uterus. At the primary site of day 12 pregnant macaque, glandular epithelia of the principal zone underlying the plaque (A) had lost Muc-1 expression. Principal zone epithelia outside the plaque (B) continued to express Muc-1 comparable to levels expressed in principal zone epithelia of day 12 non-conceptual cycle (D). Expression of Muc-1 in basal glands was equivalent in sections from conceptual (C, under plaque) and non-conceptual cycles (E). Autofluorescence: orange; Muc-1 staining: green. Magnification (D, scale bar 25 μm) and imaging parameters were equivalent for all panels.

Table III. Summary of epithelial Muc-1 immunostaining in macaque uterus

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Primary site</th>
<th>Secondary site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d12(NC)</td>
<td>d12</td>
</tr>
<tr>
<td>Superficial:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over plaque</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Outside plaque</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Plaque LE*</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Principal:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque acini</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Gland—under plaque</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Gland—outside plaque</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Transitional:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gland—under</td>
<td>NA</td>
<td>+/–</td>
</tr>
<tr>
<td>Gland—outside</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Basal:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gland—under</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Gland—outside</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

NC: non-conceptual
NA: not applicable
– Undetectable
+/– Low intensity and/or variable, cell to cell
+ Moderate intensity
++ Most intense
*plaque continuous with luminal epithelium

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maintained into the late luteal phase of cycling animals. It is unclear whether this is the case in New World monkeys. In marmosets, which produce variations of bidiscoid placentae (Wislocki, 1939), a uniform membrane transformation occurring throughout the uterine lumen has been described in luminal epithelia during the preimplantation period of conceptual cycles, but phase-matched late luteal nonconceptual cycles were not included in the study (Niklaus et al., 2001). The only New World monkey (Cebus apella) in which uterine Muc-1 expression has been examined produces a bidiscoid placenta. Presumably, as in the macaque, adhesion would be delayed at the secondary site. However, only cycling animals were examined during secretory phase and the surface morphology was not described (Jones et al., 2001).

Plaque formation, which has not been reported to occur in humans, is a response of the functionally receptive superficial zone to chorionic gonadotrophin released by trophoblast and, thus, response is confined to conceptual cycles. In the pregnant baboon uterus, plaque formation is less robust than in the macaque and, usually, only a single plaque is formed (Enders et al., 1997). Secondary plaques have been reported in some species of baboon but they do not result in placental formation (Gilbert and Heuser, 1954). Although a receptive state is required for plaque formation, it can be initiated by infusion of CG (Fazleabas et al., 1999) or trauma (Hisaw et al., 1937) and thus does not require direct contact with trophoblast. This observation raises the question whether, in the pregnant primate, plaque formation follows or precedes the adhesion event, and if it precedes adhesion, are plaque cells capable of supporting adhesion? Heuser and Streeter’s original observation that plaque formation follows adhesion at the primary site and precedes attachment at the secondary site (Heuser and Streeter, 1941) has been substantiated by subsequent studies (Enders et al., 1983). In the current study, early secondary plaque formation was evident in both day 12 PO samples that lacked adhering trophoblast. In one case adhesion had not yet occurred since intact abembryonic trophoblast was associated with the corresponding primary site. Thus, it seems that plaque formation precedes attachment at the secondary site. Whether plaque epithelia are able to support adhesion is a moot point since the plaque is the site of secondary adhesion.

In contrast to the superficial zone, it is in the principal and transitional zones of macaque endometrium that distinctions in Muc-1 expression were observed when comparing conceptual to non-conceptual cycles. Since glandular epithelium of the functionalis from non-conceptual cycles uniformly expressed Muc-1 at their apical surface and equivalent expression of Muc-1 was detected in glands outside the implantation site of conceptual cycles, the loss of Muc-1 expression in glands of the functionalis underlying the implantation site appeared to be a response to the implanting embryo. The similar pattern of progesterone receptor expression reported at primary implantation sites in macaque uteri between day 13 and day 17 PO suggests that the loss of Muc-1 is related to the loss of progesterone receptor. A temporally and regionally graded reduction in progesterone receptor was observed, with receptor absent in the superficial and principal zones, significantly reduced in the transitional zone and still present in the basal zone through day 22 (Ghosh et al., 1999). In the earliest stage of pregnancy observed in baboon, day 18 PO, glandular epithelium, including basal glands, were devoid of progesterone receptor while basal glands of a late luteal phase non-conceptual cycle were progesterone receptor positive (Hild-Petito et al., 1992). In this regard, baboons exhibit a subtle distinction from macaques, whose basal gland epithelia continued to express progesterone receptor (and Muc-1) through early pregnancy. However, the apparent correlation between the loss of progesterone receptor and loss of Muc-1 does not provide
an explanation for the temporal distinction in these events displayed by the superficial zone and glandular epithelia in the functionalis. All compartments are presumably exposed to similar levels of steroid hormones. The simple explanation that progesterone downregulates its own receptor is not sufficient to account for the loss of progesterone receptor in luminal epithelia during the transition to receptivity in cycling uteri while glandular loss is restricted to the implantation site of conceptual cycles. The implication is that differences in the respective microenvironments result in compartmental and subcompartmental distinctions in transcriptional responses.

Initiation of the functionally receptive period can be inferred from the timing of embryo adhesion during normal conceptual cycles, but it would not be sufficient to determine when functional receptivity ends in these species. A prolonged period of functional receptivity that is not regionally restricted would offer an explanation for the more efficient rates of implantation observed for natural matings in baboon (Stevens, 1997) and macaques (Ghosh et al., 1997), exceeding 70%, when compared to an estimated rate of 30% per cycle for humans (Wilcox et al., 1988). Rather than reflecting a ‘failure’ to develop a receptive endometrium (Sharkey and Smith, 2003), the low rate of implantation in humans instead may reflect inherent temporal and spacial constraints on the receptive state that have developed evolutionarily as correlates of fully interstitial implantation.

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MUC-1 expression in primate implantation sites


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