Patterns of sialylation in differentiating rat decidual cells as revealed by lectin histochemistry

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Lectin histochemistry was used to demonstrate changes in the surface glycan distribution of uterine stromal cells as they differentiate to form decidual cells. Decidualization was induced in hormone-treated, ovariectomized rat uteri by needle scratch. Uterine tissue from days 2 to 8 of decidual development was examined with a panel of lectins specific for terminal non-reducing structures in *N*- and *O*-linked classes of glycoprotein glycan, including α 2,3- and α 2,6-linked sialic acid residues. Immunostaining for desmin was used to identify decidual cells. An increase in *N*-linked glycans associated with the cell surface and recognized by lectins from *Phaseolus vulgaris* (leukoagglutinin) (I-PHA), *Pisum sativum* (PSA) and *Triticum vulgaris* (WGA) was found during the early growth of decidual cells. As decidualization progressed regionally from the antimesometrial to mesometrial uterus, an increase in α 2,3-linked sialic acid residues was followed by a loss of the α 2,6-linked form. The results suggest that as stromal cells differentiate, glycoprotein biosynthesis and glycosyl transferase activity are altered. These changes in patterns of glycosylation may give rise to altered decidual cell–matrix and cell–cell interactions during differentiation and play a role in the modulation of decidual cell interactions with trophoblast during early placentation.

Introduction

After embryo attachment in the rodent uterus, stromal cells underlying the epithelial attachment site begin a process of differentiation known as decidualization, which was first described morphologically in the rat uterus by Krehbiel (1937). The first cells to differentiate form a cup-shaped area beneath the antimesometrial epithelium known as the primary decidual zone (PDZ). Differentiation then progresses deeper and laterally into the antimesometrial stroma to form the secondary decidual zone, and then subsequently into the mesometrial stroma where it forms the decidual basalis of pregnancy. Cells in the basal stroma, adjacent to the myometrium, do not differentiate.

The proposed functions of decidual cells have been reviewed (De Feo, 1967; Parr and Parr, 1989; Glasser, 1990) and probably include the provision of a physical environment conducive to placentation, allowing access of trophoblast to maternal blood, while regulating trophoblast invasion, modulation of the local immune system, paracrine and endocrine hormone secretion, and serving as a direct source of nutrients for embryos.

In rodents, decidualization can be induced in hormonally prepared animals by artificial stimuli. The uterine response to an artificial stimulus is termed a decidualoma and appears to be morphologically and biochemically identical to the decidual of pregnancy (O'Shea *et al.*, 1983; Welsh and Enders, 1985; Glasser *et al.*, 1987). Decidualoma can be used to study the effects of steroid hormones on stromal cell differentiation and to examine

this process in the absence of embryonic tissue. An optimal decidual response can be obtained by treating ovariectomized animals with a regimen of oestradiol and progesterone that mimics secretion of these hormones during the preimplantation period and then maintaining the induced decidual tissue by continued treatment with these hormones (Psychoyos, 1973). Decidualization in the antimesometrial stroma reaches a maximum 4 days after induction. As the mesometrial stroma begins to differentiate (5–6 days after induction), the antimesometrial tissue regresses (Bell, 1983; Hong *et al.*, 1991). The decidualoma model has been used in this study to examine changes in the expression of lectin-binding molecules in differentiating stromal cells.

Although patterns of glycosylation are often sensitive indices of differentiative change in many tissues and have been extensively studied in epithelial cells of human and rodent endometrium (Glasser *et al.*, 1988; Aplin, 1991), relatively little is known about glycosylation in the uterine stromal compartment. Changes in cell shape, cell–cell and cell–matrix interactions are striking during stromal cell decidualization and may be associated with modifications in cellular and extracellular oligosaccharides. We used lectin histochemistry to investigate changes in glycan distribution associated with the transformation of stroma to decidualoma. Expression of the intermediate filament protein desmin, an established marker for decidual cells (Glasser and Julian, 1986), was used to relate glycosylation patterns to decidual cell development. The main specificities of the lectins chosen are for terminal non-reducing structures in *N*- or *O*-linked classes of glycoprotein glycan which are more likely

to be sensitive to differentiative events than are commonly expressed core structures. The panel includes two sialic acid-binding lectins that detect distinct linkage orientations (Shibuya *et al.*, 1987; Taatjes *et al.*, 1988; Wang and Cummings, 1988; Knibbs *et al.*, 1991).

Materials and Methods

Animals

Virgin female Sprague-Dawley rats, 4 months old, were bilaterally ovariectomized and left untreated for 10 days to clear residual steroids from the circulation before beginning the following treatment schedule: oestradiol ($1 \mu\text{g day}^{-1}$) for 2 days, no treatment for 2 days, progesterone (2 mg day^{-1}) for 4 days, artificial stimulation of decidualization by scratching the length of one uterine horn antimesometrially with a 1.5–2 inch hypodermic needle with a barbed point on day 4 of progesterone treatment, and subsequent injection of progesterone (2 mg) and oestradiol ($0.2 \mu\text{g}$) once a day until the animals were killed. The unstimulated, contralateral uterine horn provided hormonally treated control tissue for each rat. At least three rats were examined at each time point.

Tissue processing

Rats were killed with a lethal i. p. injection of 5 ml Avertin anaesthetic made with 2% tribromoethanol in 1% tertiary amyl alcohol (Sigma) on days 2–8 after decidual stimulation (day 0). Uterine horns were removed, trimmed free of fat and mesentery, and rinsed briefly in phosphate-buffered saline (PBS). Deciduomata and control tissues were fixed in a solution of 85% (v/v) ethanol, 10% (v/v) formalin and 5% (v/v) acetic acid for 1 h at room temperature, rinsed overnight in cold PBS, dehydrated through an ascending alcohol series and embedded in paraffin wax.

Lectin histochemistry

The lectins used were from *Maackia amurensis* (MAA), *Sambucus nigra* (SNA), *Triticum vulgaris* (WGA), *Pisum sativum* (PSA), *Phaseolus vulgaris* leuco-agglutinin (l-PHA) and *Erythrina cristagalli* (ECA) and were obtained from Sigma (Poole) except SNA and MAA which were from Boehringer Mannheim (Lewes). Their major sugar specificities are listed (Table 1). Lectin histochemistry was carried out using the method of Jones *et al.* (1992a). Sections ($4 \mu\text{m}$) were dewaxed in xylene, rinsed in absolute ethanol, and treated for 30 min with absolute methanol containing 0.4% (v/v) HCl and 0.5% (v/v) hydrogen peroxide to inactivate endogenous peroxidase. After rinsing in water and washing in Tris-buffered saline pH 7.6 (50 mmol Tris–HCl l^{-1} , 0.15 mol NaCl l^{-1} , TBS) for 15 min (three changes), sections were incubated for 30 min at room temperature with $10 \mu\text{g}$ biotinylated lectin ml^{-1} ($50 \mu\text{g ml}^{-1}$ for MAA) in TBS plus 1 mmol $\text{CaCl}_2 \text{l}^{-1}$, pH 7.6 (TBSC). Washing for 15 min in TBSC (three changes) was followed by incubation in $5 \mu\text{g}$ avidin–peroxidase ml^{-1} (Sigma) in 0.125 mol Tris–HCl l^{-1} pH 7.6, 0.347 mol NaCl l^{-1} (Jones *et al.*, 1987) at room temperature for 1 h. Sections were then washed in three changes of TBS over 15 min. Sites of lectin binding were visualized using 0.05%

Table 1. Lectins used in this study and their major specificities

Lectin	Source	Major specificity
MAA	<i>Maackia amurensis</i>	NeuNAc α 2,3Gal β 1-
SNA	<i>Sambucus nigra</i> Elderberry bark	NeuNAc α 2,6Gal/GalNAc-
WGA	<i>Triticum vulgaris</i> Wheatgerm	Di-N-Acetylchitobiosyl and some sialyl residues
PSA	<i>Pisum sativum</i> Garden pea	α -D-Mannose in non-bisected bi/tri-antennary, complex N-linked sequences
l-PHA	<i>Phaseolus vulgaris</i> (leucoagglutinin) Kidney bean	Tri/tetra-antennary non-bisected complex N-linked sequences
ECA	<i>Erythrina cristagalli</i> Coral tree	Gal β 1,4GlcNAc β

(w/v) diaminobenzidine tetrahydrochloride dihydrate (Aldrich Chemical Co., Gillingham) in TBS with 0.015% (v/v) hydrogen peroxide for 5 min at room temperature. Sections were then washed, counterstained with methyl green, dehydrated, cleared and mounted in XAM neutral synthetic mounting medium (Gurr, BDH, Poole).

Immunohistochemistry

For the localization of desmin, sections were dewaxed, rinsed in ethanol and endogenous peroxidase activity was blocked as above. After rinsing in TBS, sections were incubated in mouse anti-desmin monoclonal antibody D33 (Dako, High Wycombe; 1:50 in TBS) for 1 h at room temperature, washed in TBS, then incubated in peroxidase-conjugated rabbit anti-mouse IgG (Dako, 1:50 in TBS) for 1 h. After washing, sections were treated with substrate, counterstained, dehydrated, cleared and mounted as described above.

Histochemical controls

As a negative control, sections were incubated in TBSC instead of the lectin or antibody. An internal positive control for desmin was provided by the presence of the myometrium which strongly expresses this protein.

Sections were stained with lectins in the presence of competing sugars; PSA in the presence of 0.1 mol α -methyl mannoside l^{-1} and ECA with added 0.05 mol *N*-acetyl lactosamine l^{-1} .

Sections were treated with 0.1 U sialidase (neuraminidase, Sigma Type VI from *Clostridium perfringens*) in 0.2 mol acetate buffer l^{-1} pH 5.5, 1% CaCl_2 for 1 h at 37°C before incubation in MAA or SNA.

As a further positive control, sections were treated with concanavalin A, which recognizes mannose residues in a variety of common N-linked core structures.

Results

In this study, descriptions of lectin binding are limited to the resident stromal cells and their associated extracellular matrix;

Fig. 1. Control rat uterus, unstimulated horn. (a) Desmin staining is restricted to the longitudinal and circumferential muscle layers of the myometrium (M), whereas the endometrial stroma is negative. MM: mesometrial stroma; AMM: antimesometrial stroma; L: lumen. (b) Lectin from *Maackia amurensis* (MAA) binds weakly to the endometrial stroma, with slightly increased staining in the basal regions. (c) Lectin from *Sambucus nigra* (SNA) shows moderate staining, especially in subluminal and basal regions of the endometrium, with the intermediate area exhibiting weaker binding. (d) Lectin from *Pisum sativum* (PSA) binds uniformly and intensely to the endometrial stroma. Scale bar represents 200 μm .

results of luminal and glandular epithelium, endothelial cells, bone-marrow derived cells and the myometrium are not reported.

Control uterus, unstimulated horn

Desmin staining was restricted to the myometrium (Fig. 1a). Stromal cells and extracellular matrix in all regions exhibited weak binding of MAA (Fig. 1b), whereas SNA bound more strongly to subluminal and basal regions than to the central area

(Fig. 1c). There was strong binding of PSA (Fig. 1d), moderate binding of WGA, and weak binding of I-PHA and ECA, the latter being hardly detectable (see Table 2).

Day 2 deciduoma

Two days after the decidualizing stimulus, there was slight stromal cell enlargement in the primary decidual zone and some cells began to express detectable amounts of desmin. MAA bound more strongly to these cells than to the rest of the

Table 2. Intensity of lectin staining in the rat uterus (day 4)

Lectin	Decidualized uterus		Control uterus	
	Primary decidua (PDZ)	Secondary decidua	Subluminal stroma	Basal stroma
MAA	++	+ (wings ++)	±	+
SNA	—	+++	+	++
WGA	+++	++	++	++
PSA	+++	++	+++	+++
I-PHA	+++	+	±	±
ECA	++	—	—	±

—, negative; ±, very weak; +, weak; ++, moderate; +++, strong.

MAA from *Maackia amurensis*; SNA from *Sambucus nigra*; WGA from *Triticum vulgaris*; PSA from *Pisum sativum*; I-PHA from *Phaseolus vulgaris* (leukoagglutinin); and ECA from *Erythrina cristagalli*.

stroma; this staining was, however, still faint and was almost completely removed by sialidase treatment.

Cells in the primary decidua bound SNA weakly and even lower amounts of binding were evident in the surrounding subjacent stroma. As in the control horn, the basal stroma was more strongly stained than was the subluminal stroma. This staining was also removed by sialidase treatment.

When compared with nondifferentiated stromal cells, decidual cells gave increased reactivity with WGA, PSA and I-PHA. This was particularly evident with I-PHA, as its binding was mainly restricted to the area of decidualization. There was virtually no binding of ECA in the stroma on day 2 (data not shown).

Day 3 decidualoma

Considerable cellular proliferation and enlargement was evident in the primary decidua which exhibited intense desmin immunoreactivity (Fig. 2a). Immediately beneath the lumen, a narrow band of desmin-containing cells two to three cells thick extended from the primary decidua towards the mesometrial pole. Moderate MAA staining was evident throughout the stroma (Fig. 2b) and this was labile to sialidase treatment. Close examination revealed surface staining of the cells which could be resolved into fine processes or fibrils extending across the intercellular spaces (not shown).

SNA reactivity was similar to that seen on day 2, with moderate staining throughout the stroma (Fig. 2c), weaker binding in the subluminal stroma but slightly stronger reactivity in the primary decidua. Sialidase removed all but a low level of residual binding in the primary decidua.

I-PHA staining was concentrated in the primary decidua zone and in a narrow band of stroma immediately subjacent to the uterine lumen (Fig. 2d). Enhanced binding in the latter area was also observed using WGA (Fig. 2e) and PSA (Fig. 2f).

Day 4 decidualoma

The entire suprabasal antimesometrial sector of the stromal compartment was stained by antibody to desmin, with a narrow band of desmin-positive cells beneath the luminal epithelium

again extending further towards the mesometrial pole (Fig. 3a). MAA now bound strongly to two discrete areas, or 'wings', of subluminal stromal cells located laterally and slightly displaced towards the antimesometrial pole (Fig. 3b), which partly coincided with desmin-weak areas. The primary decidua zone also showed moderate staining apart from a small area at the antimesometrial tip of the lumen, which expressed the highest concentration of desmin, and showed only weak MAA binding. Staining with MAA over the rest of the endometrium showed little difference from previous days.

There was a change in the SNA staining pattern of the primary decidua zone on day 4, with a striking loss of binding in the primary decidua zone (Fig. 3c). The large and often binucleate cells exhibited a completely clear cytoplasm and no surface membrane staining (Fig. 3d). The zone of reduced SNA binding was much less extensive than the desmin-positive area. At the margins of the unstained area, intercellular heterogeneity was apparent, with strongly stained and unstained cells in close apposition (Fig. 3d). Decidual or undifferentiated cells in all other areas of the stroma showed moderate to strong staining with SNA, particularly on the cell surfaces and extracellular matrix. After sialidase treatment, most of this staining was lost.

At this stage, I-PHA also strongly stained the two lateral MAA-positive 'wings' on each side of the lumen and the primary decidua zone (Fig. 3e). Staining with WGA and PSA continued to be strongest around the lumen and in the primary decidua zone (data not shown). ECA staining appeared for the first time in decidual tissue and was localized to the SNA-negative area of the primary decidua zone (Fig. 3f). Observations made on day 4 tissue and the time of maximal growth of the decidualoma are summarized (Table 2).

Day 5 decidualoma

At this stage, desmin-reactive cells occupied the entire antimesometrial half of the endometrium as well as a subluminal area of the mesometrial stroma (Fig. 4a). Lateral subluminal MAA binding was now much more extensive, with 'wings' of reactivity extending deeply into the stroma (Fig. 4b). Relative to day 4, these had also migrated towards the mesometrial pole, thus progressing in advance of the onset of desmin expression in the secondary decidua zone. Cytoplasmic binding was slight or moderate but surface staining was extremely strong. In other areas of the endometrium staining was much weaker, although cell surfaces were still clearly delineated. Most endometrial MAA binding was abolished by pretreatment with sialidase, and a low level of residual staining was detectable in the 'wings' (Fig. 4c).

The area of low SNA reactivity now extended mesometrially on each side of the lumen and towards the myometrium (Fig. 4d). The large decidual cells showed some pale surface staining. The SNA-weak area encompassed desmin-positive cells (although by no means the whole desmin-positive population) and, except in a small region in the lateral subluminal stroma, did not overlap greatly with the MAA-strong cell population.

Stronger SNA binding was observed in the deeper antimesometrial stroma, with clearly defined intercellular boundaries. The smaller cells in the mesometrial segment were less distinctly delimited. Sialidase pretreatment greatly reduced the

Fig. 2. Rat deciduoma, day 3. (a) Desmin reactivity is now intense in the primary decidual zone (PDZ). The mesometrial area is unstained. (b) Lectin from *Maaackia amurensis* (MAA) stains moderately throughout the stroma. (c) Lectin from *Sambucus nigra* (SNA) binds moderately to the stroma, except in the area immediately beneath the lumen, where staining is weaker. (d) Lectin from *Phaseolus vulgaris* (I-PHA) binds strongly to the PDZ (*) and to the cells immediately beneath the lateral luminal epithelium (arrows). (e) Lectin from *Triticum vulgare* (WGA) shows a pattern of binding somewhat similar to that seen with I-PHA. (f) Lectin from *Pisum sativum* (PSA) binds most strongly to the area of stroma immediately beneath the lumen, with moderate binding elsewhere in the stroma. Scale bar represents 400 μ m.

Fig. 3. Rat deciduoma, day 4. (a) Desmin reactivity is present in the whole antimesometrial suprabasal segment of the endometrium. (b) Staining by lectin from *Maackia amurensis* (MAA) is concentrated in two lateral 'wings' (*) with moderate binding elsewhere in the stroma apart from a small area at the antimesometrial tip of the lumen (arrow), which shows reduced activity and corresponds to a site of intense desmin expression. (c) Lectin from *Sambucus nigra* (SNA) shows a dramatic loss of binding in the primary decidual zone (*). (d) High power view of the interface between lectin from *Sambucus nigra* (SNA)-stained and unstained areas of tissue. Between the decidual cells, small deeply stained capillaries can be seen (arrows). The decidual cells in this area are clearly heterogeneous with respect to binding affinity for SNA. (e) I-PHA binds to the antimesometrial decidual cells beneath the uterine lumen as well as to lateral 'wings'. The rest of the endometrial stroma stains only lightly. (f) ECA-positive cells correspond to those showing reduced SNA-binding in the primary decidual zone. Scale bar represents 400 μm (a, b, c, e and f) and 40 μm (d).

Fig. 4. Rat deciduoma, day 5. (a) Desmin staining now includes the whole antimesometrial decidua and the mesometrial subepithelial stroma (arrows). (b) Deeply staining 'wings' of reactivity with lectin from *Maackia amurensis* (MAA) can be seen on each side of the uterine lumen, approaching closer to the mesometrial pole than do the cells expressing desmin. (c) After sialidase pretreatment, most of the MAA binding is lost except for a residue visible in the lateral 'wings'. (d) Binding of lectin from *Sambucus nigra* (SNA) is present in deeper areas of the stroma, but a discrete area of weak binding can be seen beneath the uterine lumen, extending in a mesometrial direction; most of the affected (SNA-weak) cells do not greatly overlap with those binding MAA. (e) Binding of lectin from *Phaseolus vulgaris* (PHA) now includes most of the suprabasal endometrium, with a narrow mesometrial subluminal band staining particularly strongly. (f) The binding of SNA is partially labile to sialidase. Scale bar represents 400 μm .

staining (Fig. 4f). I-PHA binding on day 5 extended to nearly all of the endometrial stroma except the basal region (Fig. 4e). PSA and WGA also stained both decidualized and undifferentiated stroma, whereas ECA continued to bind to those regions showing a decrease in SNA affinity (data not shown).

Day 6 deciduoma

By day 6, cells expressing desmin occupied two-thirds of the stromal compartment and only a narrow band of basal tissue remained unstained along with the mesometrial segment. MAA binding, which as before was pericellular, extended to approximately one-third the depth of the endometrial stroma both anti-mesometrially and mesometrially. The deeper population of desmin-positive decidual cells remained weakly stained for MAA. Most of the staining was lost after sialidase treatment.

At the same stage, a shallow zone of cells, which did not bind SNA, extended almost completely around the lumen. Much of this area was also MAA and ECA positive. The deeper stroma was fairly uniformly stained, although the cell outlines were less distinct. There was faint cytoplasmic and moderate surface and matrix staining which in places appeared rather globular. The bulk of this staining was resistant to sialidase, and the loss of SNA binding following enzyme treatment occurred only in the area adjacent to the myometrium. WGA, PSA and I-PHA gave fairly uniform binding of moderate intensity throughout the endometrial stroma.

Days 7 and 8 deciduoma

Decidual cell regression, characterized by enlargement of intercellular spaces and loss of desmin staining, was evident in the primary decidual zone (Fig. 5a). On days 7 and 8 there was diffuse and patchy desmin staining in the mesometrial stroma.

On day 7, MAA bound strongly beneath the lumen (Fig. 5b). Cytoplasmic staining was strong and granular and intercellular boundaries were difficult to resolve. In deeper areas of stroma, cytoplasmic staining was reduced and cell surface staining clearer. By day 8, MAA staining was still mainly subluminal, but more mesometrially distributed. After sialidase treatment, the bulk of staining was removed in tissue from both days.

Intense and uniform binding of SNA appeared on day 7 over most of the tissue (Fig. 5c). Cytoplasmic staining was granular and cell boundaries were difficult to define except in the basal stroma where cell surface and extracellular matrix staining was particularly intense and sialidase resistant. There was little change on day 8, with staining over the entire endometrial stroma most of which was again sialidase-resistant.

WGA, PSA and I-PHA gave fairly uniform and strong staining patterns on both days, although WGA staining was slightly weaker at the mesometrial pole on day 7. I-PHA, like MAA, gave slightly stronger staining mesometrially on day 8. ECA staining again tended to be strongest subjacent to the lumen, with weak staining in other areas on day 7 (Fig. 5d), although by day 8 diffuse mesometrial staining was also evident (Fig. 5e).

Histochemical controls

The effect of sialidase treatment on MAA and SNA binding are described above. Substitution of TBSC or TBS for the lectin

or antibody abolished the staining completely. Incubation of the lectins PSA and ECA in the presence of competing sugars prevented staining (Fig. 5f). All uterine stromal cells bound concanavalin A, which recognizes core mannosyl residues in *N*-linked structures, at all stages of decidualization. This provided a positive control throughout the study.

Discussion

Striking alterations in cell surface glycosylation during decidualization of the rat uterine stroma have been revealed in this study using lectin histochemistry. SNA binding in a subpopulation of desmin-expressing cells of the primary decidual zone is dramatically reduced 4 days after the induction of decidualization. As decidualization progresses, the SNA-negative area became more extensive and exhibited more ECA binding, suggesting that loss of α 2,6-linked sialic acid residues may expose terminal *N*-acetyl lactosamines. During the same period, two laterally orientated subluminal regions, or 'wings', of decidual cells appeared which strongly bind MAA, and the size of these regions gradually increased during days 4–5 to involve tissue in the mesometrial segment of the endometrium. These cells also showed alterations in the *N*-glycan profile, as detected by greater I-PHA binding, perhaps indicating synthesis of new *N*-linked chains containing terminal α 2,3-linked sialic acid residues. Although the loss of SNA binding sites could result from secreted sialidase activity, these results strongly suggest that alterations in glycoprotein biosynthesis and glycosyl transferase activity are occurring in stromal cells as they differentiate.

This is the first time that alterations in sialylation of decidual cells have been described, although a reduction in the number of sialyl residues resulting in a decrease in the net surface charge of both blastocysts and uterine epithelium during implantation has been reported in both rats and mice (Nilsson *et al.*, 1973; Enders and Schlafke, 1974; Jenkinson and Searle, 1977; Hewitt *et al.*, 1979; Nilsson and Hjertén, 1982; Morris and Potter, 1984). In human endometrial epithelium, progesterone stimulates a major increase in vesicles of the Golgi apparatus (Dockery *et al.*, 1988) as well as the appearance of new sialoglycans during the implantation phase (Hoadley *et al.*, 1990; Aplin, 1991). The expression of sialyl transferases can be regulated by glucocorticoids (Wang *et al.*, 1990; Kolinska *et al.*, 1990) and the data presented here suggest that sensitization of uterine cells by ovarian steroids may similarly prepare them for regionally specific alterations in sialyl transferase expression during decidualization.

The functional significance of the alterations in sialylation of the stromal cell surface as it differentiates into a decidual cell is not known. Sialic acid may serve to mask subterminal recognition structures (Lloyd, 1975), to modulate the affinity of interactions at other binding sites in the same molecule (as in neural cell adhesion molecule; Regan, 1991), or serve as an integral part of a glycan ligand for specific cell adhesion molecules (Crocker *et al.*, 1991; Berg *et al.*, 1992). There is considerable evidence to demonstrate that altered levels and types of sialylation occur in normal differentiation (Taates and Roth, 1990; Aplin, 1991; Regan, 1991; Jones *et al.*, 1992b; Yang *et al.*, 1992) and in pathological processes (Morgenthaler *et al.*, 1990; Sata

Fig. 5. Rat deciduoma, days 7–8. (a) On day 7, decidual cell regression is accompanied by loss of desmin staining, especially in the antimesometrial suprabasal area of the uterus (*). (b) Lectin from *Maackia amurensis* (MAA) stains most strongly around the subluminal region of the stroma, decreasing slightly in the basal areas. (c) Staining by lectin from *Sambucus nigra* (SNA) is generally uniform and intense. (d) Binding of lectin from *Erythrina cristagalli* (ECA) is present over most of the endometrium, but an area of strong staining is visible subjacent to the lumen, especially on the antimesometrial aspect. (e) ECA binding without and (f) with the presence of 0.05 mol *N*-acetyl lactosamine l^{-1} on day 8. The competing sugar has eliminated the majority of the stain. Scale bar represents 400 μ m.

et al., 1991; Le Marer *et al.*, 1992; Vandamme *et al.*, 1992). The expression of sialyl $\alpha 2,3$ -linkages has been associated with normal colonic mucosa, but malignant transformation is accompanied by the *de novo* expression of an $\alpha 2,6$ -sialyltransferase, suggesting a relationship between $\alpha 2,6$ residues and cell growth (Sata *et al.*, 1991). Greater numbers of sialyl $\alpha 2,6$ -linked chain termini may also correlate with *in vivo* aggressiveness of malignant cells and cellular binding of collagen IV (Morganthaler *et al.*, 1990). The reciprocal expression of $\alpha 2,3$ - and $\alpha 2,6$ -linked sialic acid residues, which characterize stromal cell differentiation, may accordingly be related to the remodelling of the stromal extracellular matrix, which is a function of the decidual cell (Glasser *et al.*, 1987; Aplin, 1991; Mulholland *et al.*, 1992). The accumulation of various components of the extracellular matrix, such as collagen IV, laminin and heparan sulfate proteoglycan, and the loss of elements of the original matrix, such as collagen VI and fibronectin (Grinnell *et al.*, 1982; Aplin, 1989; Glasser, 1990; Clark *et al.*, 1992; Mulholland *et al.*, 1992), are likely to be important to the interaction of trophoblast with the decidualizing stroma. However, the relationship of the shift from $\alpha 2,6$ - to $\alpha 2,3$ -sialic acid residues on decidual cell surfaces and remodelling of the decidual extracellular matrix with trophoblast migration has not yet been determined.

In addition to changes in the accessibility and distribution of sialyl residues, there is an increase in several classes of N-linked glycan associated with the plasma membrane of decidualizing cells during their early period of growth and differentiation. The binding specificities of these lectins and the interpretations made from their histochemical behaviour have been discussed in detail elsewhere (Ball *et al.*, 1989; Roberts *et al.*, 1990). It is possible that these changes facilitate localized interactions between decidual cells and trophoblast that are required for the establishment of pregnancy (Bell, 1985; Aplin, 1991; Glasser *et al.*, 1991). If the changes described above for deciduoma also occur in the uterus of the pregnant animal, as suggested by pilot studies on mice in our laboratory, the loss of $\alpha 2,6$ -linked sialic acid and the expression of $\alpha 2,3$ -linked residues at the antimesometrial primary decidual zone and underlying stroma should coincide with the decidual cell reaction which follows attachment of the mural trophoblast. The progressive development of the egg cylinder from the point of original attachment at the antimesometrial pole to the mesometrial locus is, therefore, coordinated with the regional evolution of the decidual cell reaction both morphologically and with respect to sialylation.

Variation in the lectin binding properties of differentiating stromal cells suggests a role for sialoglycans in decidual cell-cell interactions (Jollie and Bencosme, 1965; Kleinfeld *et al.*, 1976; O'Shea *et al.*, 1983; Welsh and Enders, 1985; Parr *et al.*, 1986). The increase in intercellular contact that follows the loss (O'Shea *et al.*, 1983) and remodelling of the extracellular matrix (Mulholland *et al.*, 1992) suggests that decidual cell-cell interactions may play a determinative role both at the sites of interaction with developing trophoblast and deeper in the differentiating stromal compartment. Reduction in the intercellular spaces is accompanied by the appearance of electron-dense, flocculent material (Brökelmann and Biggers, 1979; O'Shea *et al.*, 1983; Parr *et al.*, 1986). Changes in sialylation and the extracellular matrix may also effect reduction in intercellular space (Yang *et al.*, 1992), and increase intercellular adhesion and

communication between the maternal cells (Welsh and Enders, 1987).

The observations reported here also indicate that cells within the uterine stroma can exhibit differential responses to a decidualogenic stimulus. Differences among responsive cells are evident in the bilateral pattern of MAA binding seen in antimesometrial deciduoma (days 8–9). Structural and functional heterogeneity of decidual cells in different regions of the uterus has been described in a number of studies and reviewed by Bell (1985). A striking example is the absence of a decidual response in stromal cells of the basal zone (Krehbiel, 1937; Parr and Parr, 1989). Mesometrial decidual cells fail to achieve the same ploidy ($>4N$) observed in the antimesometrial decidual cells and thus appear to comprise a separate population of differentiated cells (Sartor, 1980). Functional differences between these two populations of cell have also been emphasized by Jayatilak *et al.* (1989), who demonstrated that decidual luteotrophin is expressed in the antimesometrial but not in the mesometrial decidua 8–13 days after induction of decidualization.

The role of these alterations in the glycosylation of the decidual cell surface remains to be elucidated. Decidualization is a critical step in the initiation of placentation and the establishment of pregnancy. The observations described above suggest that regulation of sialyltransferase activity accompanies stromal cell differentiation and may play an important role in producing an appropriate cell surface and extracellular environment for placentation.

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