Decidual sialylation shows species-specific differences in the pregnant mouse and rat

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Biotinylated lectins from *Sambucus nigra* (SNA) and *Maackia amurensis* (MAA), which bind to α 2,6-linked and α 2,3-linked sialyl residues, respectively, were used as probes to study glycan terminal modifications associated with decidualization in the uterine stroma of pregnant rats and mice. Binding of lectins from *Erythrina cristagalli* (ECA), *Phaseolus vulgaris* (leukoagglutinin, L-PHA), *Triticum vulgaris* (WGA) and *Bandeiraea simplicifolia* (BSA-1B₄) was also examined. Tissues from rats between day 5 and day 8 of gestation and mice between day 5 and day 7 of gestation were fixed in Bouin's solution and embedded in wax prior to lectin histochemistry. On day 7 in rats and day 6 in mice, there was a marked reduction in the binding of SNA in the subluminal decidua surrounding the implantation site. In rats, MAA binding to enlarged decidual cells around the implantation chamber was increased markedly, but there was no change in mice. In both species there was *de novo* binding of ECA in the SNA-negative area, suggesting that the loss of α 2,6-linked sialyl residues unmasks terminal *N*-acetyl lactosamine. These findings are consistent with previous evidence of a close structural and functional similarity between the artificially induced deciduoma and true decidua of rats and show identical changes to the glycosylation patterns previously found in differentiating rat deciduoma. In both species, therefore, decidua exhibits regionally specific terminal glycosylation. However, the species-specific expression of α 2,3-linked sialyl residues suggests distinct patterns of steroidally modulated sialyl transferase expression.

Introduction

In rodents, implantation of embryos is accompanied by marked changes in the morphology and metabolism of endometrial stromal cells, which become enlarged and polyploid with a concomitant reduction in extracellular space. This transformation is known as the decidual cell reaction, or decidualization. It can also be induced by artificial stimuli to produce a response called a deciduoma that seems to be both morphologically and biochemically identical to the decidua found in pregnant animals (O'Shea *et al.*, 1983; Welsh and Enders, 1985; Glasser *et al.*, 1987). Lectin histochemistry was used to examine glycosylation changes during decidualization, and striking changes in the distribution of sialyl residues in the differentiating deciduoma of the rat were demonstrated (Jones *et al.*, 1993). Alterations were also seen in non-reducing terminal residues recognized by *Erythrina cristagalli* lectin (ECA) and in *N*-linked glycans bound by *Phaseolus vulgaris* (leukoagglutinin, L-PHA). These findings suggest alterations in glycoprotein biosynthesis and sialyl transferase activity as the stromal cells differentiate. In this study, we investigated whether similar changes occur in pregnancy in rats and mice or if the presence of the embryo modifies this pattern. Illustrative

comparisons are presented of approximately similar stages in the development of the two species: rats at day 6 with mice at day 5, rats at day 7 with mice at day 6, and rats at day 8 with mice at day 7, as development in rats is 12 h or more behind that of mice by the peri-implantation period.

Materials and Methods

Mice and rats

Nine MF1 mice and seven Sprague–Dawley rats were obtained from Harlan Olac (Bicester, UK). For pregnant animals, females were housed individually with males of their own strain and mating was established by checking for a vaginal plug (mice) or spermatozoa in vaginal smears (rats). Pregnancy was confirmed either by flushing embryos from part of one uterine horn on day 5 of pregnancy or by identification of decidual swellings on later days. Mice were killed by cervical dislocation and rats by chloroform anaesthetic overdose, after which the uterine horns were dissected and cut into 4–6 pieces or individual decidual swellings where these were visible. They were fixed in Bouin's fluid overnight at 4°C, dehydrated through a graded series of alcohols and, after clearing in xylene, embedded in paraffin wax. Sections, 5–7 μ m thick, were cut, mounted on slides and dried at 60°C.

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

Acronym	Source	Major specificity	References
SNA	<i>Sambucus nigra</i> Elderberry bark	NeuNAc α 2,6Gal/GalNAc-	Shibuya <i>et al.</i> , 1987; Taates <i>et al.</i> , 1988
MAA	<i>Maackia amurensis</i>	NeuNAc α 2,3Gal β 1-	Wang and Cummings, 1988; Knibbs <i>et al.</i> , 1991
L-PHA	<i>Phaseolus vulgaris</i> (Leukoagglutinin) Kidney bean	Tri/tetra-antennary, non-bisected complex <i>N</i> -linked sequences	Hammarström <i>et al.</i> , 1982; Green and Baenziger, 1987
WGA	<i>Triticum vulgaris</i> Wheatgerm	Di- <i>N</i> -acetyl chitobiosyl, <i>N</i> -acetyl lactosamine (especially if clustered) and some sialyl residues	Allen <i>et al.</i> , 1973; Bhavanandan and Katlic, 1979; Gallagher <i>et al.</i> , 1985
ECA	<i>Erythrina cristagalli</i> Coral tree	Gal β 1,4GlcNAc β 1-	Iglesias <i>et al.</i> , 1982; De Boeck <i>et al.</i> , 1984
BSA-1B ₄	<i>Bandeiraea simplicifolia</i> Griffonia	Gal α 1,3Gal β 1,4GlcNAc β 1-	Wood <i>et al.</i> , 1979

Lectin histochemistry

Staining was carried out using the method of Jones *et al.* (1992) with a carefully selected panel of lectins (Sigma, except for SNA and MAA which were from Boehringer-Mannheim, Lewes, UK; see Table 1 for their major sugar specificities). Sections 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 (BDH, Poole, UK) 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 µg biotinylated lectin ml⁻¹ in TBS plus 1 mmol CaCl₂ l⁻¹, pH 7.6 (TBSC). Washing for 15 min in TBSC (three changes) was followed by incubation in 5 µg 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; then sections were washed in three changes of TBS over 15 min. Sites of lectin binding were visualized using 0.05% (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).

As a negative control, sections were incubated in TBSC in place of the lectin. ECA was incubated in the presence of 0.2 mol galactose l⁻¹ while no simple sugars were available to compete for the binding of L-PHA. As competing sugars would alter the acidity or ionic strength of sialic acid-binding lectins, SNA, MAA and WGA were controlled by incubation of 0.1 iu neuraminidase ml⁻¹ (Type VI from *Clostridium perfringens*, Sigma) for 2 h at 37°C in 0.2 mol acetate buffer l⁻¹ pH 5.5, with 1% (w/v) calcium chloride to cleave off terminal sialic acid residues. Sections of mouse (day 6) and rat (day 7) uterus were also stained with BSA-1B₄ to identify trophoblast giant cells which have been reported to bind this lectin in mice (Azuma *et al.*, 1991). All observations were made using a minimum of two implantation sites from two different mice or rats for each lectin on each day.

Results

To provide a direct comparison with the deciduoma model used by Jones *et al.* (1993), we used a similar panel of lectins and equivalent stages in decidual differentiation. Descriptions of lectin binding are mainly restricted to the resident stromal cells of the endometrium and their associated extracellular matrix to give a general overview of the patterns of lectin staining; binding of glandular and luminal epithelial cells, and capillary endothelium is described only where evident in the figures. Staining of the embryo will form the subject of a future report. The results have been summarized in Table 2, which shows the representative lectin binding patterns of rat and mouse decidual, in regions proximal and distal to the implantation site.

Rats at day 5 of pregnancy

Beneath the highly folded uterine lumen, there was strong membrane staining of anti-mesometrial subluminal stromal cells with SNA, and some cytoplasmic staining (Fig. 1a). Distal to the lumen, strongly staining cells were more scattered and showed up darkly against a pale background of extracellular matrix. Tissue in the subluminal area bound MAA (Fig. 1b) and L-PHA faintly, mainly to the cell membranes. Apart from occasional, isolated cells, there was virtually no binding of MAA to the stroma. With ECA, these isolated cells stained strongly (Fig. 1c) and there was also a diffuse, speckled staining of the stroma, especially in peripheral regions. L-PHA showed some cytoplasmic and extracellular staining, especially in the peripheral stroma adjacent to the myometrium. WGA showed very strong membranous and cytoplasmic staining in the mesometrial subluminal area, and weaker binding to the extracellular matrix and scattered stromal cells more peripherally in the endometrium.

Mice at day 5 of pregnancy

With SNA, intense binding was present throughout the endometrium (Fig. 1d), especially in the cytoplasm of some stromal cells, with a slight diminution of cytoplasmic staining

Table 2. Lectin-binding patterns of rat and mouse decidua, proximal and distal to the implantation site (excluding capillaries, glands and endothelia)

Lectin	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
	Day 5		Day 6		Day 7		Day 8	
Rat								
SNA	2-3	1-2	2-3	2-3	0-1	3	1-2	3-4
MAA	1	1	1	1	0-1	0-1	3	1-2
L-PHA	1	1-2	0-1	0-1	0-1	0-1	3	1
WGA	2-3	2-3	2-3	2-3	3	2-3	3-4	3
ECA	1-2	1-2	0-1	0-1	0-1	1-2	2-3	1
Mouse			Day 5		Day 6		Day 7	
SNA			3-4	4	1-2	4	1-2	3-4
MAA			2	2	2	2	2	2
L-PHA			2-3	2	1-2	1-2	3-4	1
WGA			3-4	3-4	3-4	3-4	3-4	3-4
ECA			2-3	2	3	1-2	3-4	2

Key to staining intensities: 0: negative; 1: weak; 2: moderate; 3: strong; 4: intense. The figures in bold show that in rats both SNA and MAA change on days 7 and 8, whereas in mice only the SNA changes.

around the uterine lumen, where staining was mainly pericellular. MAA staining was faint and uniform throughout the endometrium (Fig. 1e), with a stippled appearance in and around the cells and some membranous staining. Scattered individual cells also bound this lectin strongly. With L-PHA, there was light to moderate staining throughout the endometrium that was mainly granular but with some binding to the cell membranes; the staining reaction was marginally stronger around the antimesometrial uterine lumen. There was also membranous and cytoplasmic staining of the scattered cell population. WGA stained uniformly and strongly throughout the endometrium, with individual scattered cells staining particularly intensely. ECA showed faint to moderate binding (Fig. 1f), increasing slightly near the uterine lumen, especially antimesometrially adjacent to the implanting embryo, with strong staining of the scattered cells.

Rats at day 6 of pregnancy

The uterine lumen exhibited a smooth, oval profile, and surrounding stromal cells were variable in their binding of SNA. Membrane staining was less prominent than before. There was little change in MAA binding, while L-PHA showed diminished staining intensity, especially near the myometrium. WGA bound uniformly throughout the bulk of the stroma, apart from some variable staining of subluminal cells and strong binding to scattered stromal cells. ECA also bound to a distinct population of widely dispersed cells, but showed diminished binding elsewhere in the stroma.

Rats at day 7 of pregnancy

By this stage, the uterine lumen was narrow and slit-like at the implantation site, with the embryo in contact with both

lateral walls. Staining with SNA was altered markedly (Fig. 2a), with a more or less complete loss of binding to approximately six layers of cells adjacent to the implantation chamber (Fig. 2b). Peripherally, SNA staining was moderate to strong. MAA showed little change (Fig. 2c), and there was virtually no staining with L-PHA (Fig. 3a). A tongue of increased binding extending from the antimesometrial tip of the decidual crypt was prominent with WGA (Fig. 3b), and strong staining with this lectin extended back along the sides of the implantation chamber, delineating an area more or less equivalent to that showing diminished SNA binding. The rest of the stroma was stained with moderate intensity, increasing slightly at the border with the myometrium, and individual cells showed a range of binding intensity. The staining with ECA showed no change from day 6 (Fig. 3c), while BSA-1B₄ did not bind to trophoblast giant cells.

Mice at day 6 of pregnancy

There was a marked reduction in binding of SNA in the primary decidual zone except for a narrow band adjacent to the implanting embryo and the epithelial remnants in the decidual crypt (Fig. 2d). More peripherally, adjacent to the myometrium and in the interimplantation sites, strong cytoplasmic and extracellular matrix staining was still present. MAA, however, showed little change in expression over most of the decidua (Fig. 2e). Slightly stronger MAA staining was found in a narrow band, 5-6 cells in depth, around the implantation site including decidual cells and trophoblast giant cells. Beneath this band, MAA staining was prominent in maternal capillaries (Fig. 2f). Trophoblast giant cells also bound BSA-1B₄ (Fig. 2f, inset). With L-PHA, there was again a ring of strong staining immediately around the implantation chamber in the primary trophoblast giant cells (Fig. 3d) and

Fig. 1. Lectin histochemistry of rat (a–c) and mouse (d–f) uterus on day 5 of pregnancy. (a) Lectin from *Sambucus nigra* (SNA) binds mainly to the area under the uterine lumen. The glands (G) and uterine epithelium (Ep) also stain strongly. (b) There is only faint binding of the lectin from *Maackia amurensis* (MAA). (c) The lectin from *Erythrina cristagalli* (ECA) stains in a speckled fashion, with strong staining of occasional scattered cells near the myometrium (arrows). (d) There is strong staining by SNA with a slight subluminal diminution of binding. Part of the embryo (E) in its implantation chamber can be seen. (e) Binding with MAA is faint and uniform throughout the endometrium. (f) There is faint binding of ECA, increasing slightly near the uterine lumen, antimesometrially. (AM: antimesometrial; M: mesometrial pole of uterus.) Scale bars represent 250 μ m.

decidual capillary endothelium was also prominently stained. Beyond this, L-PHA showed decreased binding around the implantation area with little granularity evident, apart from occasional darkly stained cell processes. The binding of WGA (Fig. 3e) continued to be uniform and strong throughout the

endometrium, with intense staining around the implantation chamber, including binding by the embryo and trophoblast giant cells. With ECA, there was a wide area of moderate to strong binding to decidual cells extending from the implantation site to midway across the endometrium. Staining was

Fig. 2. Lectin histochemistry of uterus of rat on day 7 (a–c) and mouse on day 6 (d–f) of pregnancy. (a) There is a marked reduction in the binding of *Sambucus nigra* (SNA) around the uterine lumen (L). Part of the embryo (E) can be seen. Only a narrow tongue of cells extending from the antimesometrial tip of the decidual crypt exhibits faint binding (arrow). (b) Higher power view of part of the uterine lumen (L) and area of decidual crypt showing loss of SNA binding (*). (c) There is little change in the binding of MAA. (d) In this transverse section, the reduction in the binding of SNA can be seen clearly. The embryo (E) and area immediately adjacent to the implantation site are well-stained. (e) There is little change in the binding of *Maackia amurensis* (MAA) over most of the endometrium. (f) In this higher power view, strong staining of the trophoblast giant cells (T) and surrounding capillaries (arrows) with MAA can be seen. Inset: the lectin from *Bandeiraea simplicifolia*-1B₄ (BSA-1B₄) binds strongly to the trophoblast giant cells. Scale bars represent 200 μm (a, c, d and e) and 50 μm (b and f).

strong adjacent to the implantation chamber (Fig. 3f), while adjacent to the myometrium it was faint. Isolated cells stained darkly with L-PHA, WGA and ECA were mainly restricted to an area adjoining the myometrium.

Rats at day 8 of pregnancy

The area of diminished SNA binding was now extended considerably (Fig. 4a), comprising most of the large cells of the

Fig. 3. Lectin histochemistry of uterus of rat on day 7 (a–c) and mouse on day 6 (d–f) of pregnancy. (a) Apart from some binding by the luminal epithelium (Ep), there is virtually no staining with the lectin from *Phaseolus vulgaris* (leukoagglutinin) (L-PHA). (b) The lectin from *Triticum vulgaris* (WGA) stains strongly, especially the luminal epithelium and in the antimesometrial region beneath the lumen (AM), extending back along the sides of the implantation chamber. (c) There is strong staining of scattered cells with *Erythrina cristagalli* (ECA). The rest of the stroma binds only weakly. Part of the lumen (L) is visible. (d) With L-PHA, there is strong staining around the implantation chamber and of the capillary endothelium (C); part of the embryo (E) can be seen. (e) WGA stains the trophoblast giant cells and embryo particularly intensely. (f) ECA binds around the implantation chamber, with moderate staining of the decidual cells. Scale bars represent 100 μ m.

decidual zone adjacent to the implantation chamber and decidual crypt. These showed only very faint binding of SNA, mainly at their surfaces, in contrast to the deeper layers, which bound SNA strongly. In the antimesometrial decidual crypt,

trophoblast showed moderate staining, while maternal capillary endothelium also stained strongly. With MAA, the decidual cells around the implantation chamber showed intense binding (Fig. 4b), with intracellular stippling and strong pericellular

Fig. 4. Lectin histochemistry of uterus of rat on day 8 (a–b) and mouse on day 7 (c–d) of pregnancy. (a) The area of diminished binding with *Sambucus nigra* (SNA) has widened compared with day 7, although vessels and the embryo (E) now stain strongly. (b) There is now intense staining of the decidual cells around the implantation chamber with *Maackia amurensis* (MAA). (c) With SNA, there is now an extensive zone of reduced binding by decidual cells around the implantation site, although capillaries stain strongly. Part of the embryo (E) can be seen. (d) Staining with MAA is pale and uniform apart from the primary trophoblast giant cells (arrows). Scale bars represent 100 μ m.

staining. Towards the mesometrial pole, the column of positively-stained cells fanned out away from the decidual zone. The decidual crypt was also stained, although it was enclosed by a narrow sheath that showed less binding. The endometrium midway between the implantation chamber and myometrium, on the lateral aspects, was only faintly stained, with a deepening in intensity near the myometrium. There was intense membranous and cytoplasmic staining of a population of cells that clustered around the mesometrial remnant of the lumen. L-PHA showed intense pericellular staining and some intracellular stippling of the enlarged decidual cells (Fig. 5a). There was strong binding to cells within the decidual crypt and around the implantation chamber. Mesometrially, there was less fanning out of the column of positively stained cells than with MAA and the cells around the mesometrial remnant generally lacked staining of the surface membrane. More peripheral areas of the endometrium were almost unstained, apart from the mesometrial pole. WGA showed strong binding throughout the endometrium (Fig. 5b), particularly around the primary decidual zone of the implantation chamber and antimesometrial decidual crypt. The staining pattern echoed that of

MAA, although a more extensive area showed intense binding; this was more prominent near the embryo, where it was both pericellular and more faintly cytoplasmic. More peripherally, it was mainly cytoplasmic. The population of cells around the mesometrial remnant was again strongly stained with this lectin. There was a marked increase in the binding of ECA (Fig. 5c), which now showed a light to moderate staining of the primary decidual cells that was more intense towards the antimesometrial pole. The decidual cells had pale, diffuse cytoplasmic staining decreasing towards the myometrium. Only faint binding was present over the rest of the endometrium, and the population of cells that bound MAA and L-PHA intensely were not stained by this lectin.

Mice at day 7 of pregnancy

At day 7 there was an extensive zone of reduced binding of SNA around the implantation site, except for a narrow strip of cells adjacent to the implantation chamber (Fig. 4c). Invading trophoblast cells in this region were stained as well as

Fig. 5. Lectin histochemistry of uterus of rat on day 8 (a–c) and mouse on day 7 (d–f) of pregnancy. (a) There is strong staining with *Phaseolus vulgaris* (leukoagglutinin) (L-PHA) mainly around the implantation chamber and the embryo (E). (b) *Triticum vulgaris* (WGA) binds strongly, especially around the primary decidual zone of the implantation chamber. (c) There is a marked increase in the binding of *Erythrina cristagalli* (ECA) to the primary decidual cells around the implantation chamber. (d) L-PHA shows intense binding to the area adjacent to the implantation chamber and to surrounding capillaries (C). The embryo (E) is unstained. (e) There is uniform, strong binding of WGA throughout the endometrium. (f) There is increased binding of ECA around the implantation chamber apart from unstained trophoblast giant cells (arrows). Scale bars represent 250 μm.

superficial maternal capillary endothelium. More peripherally, a population of decidual cells retained intense SNA binding, producing a patchwork effect. Near the myometrium, where cells were smaller, staining intensity was again high. With MAA, staining was relatively pale and uniform (Fig. 4d) apart

from the cells adjacent to the implantation chamber, where intense binding was seen on primary trophoblast giant cells. Certain scattered cells again bound MAA, but at this stage they were less conspicuous. L-PHA showed intense cytoplasmic and pericellular staining adjacent to the implantation

chamber (Fig. 5d). This binding decreased gradually with increasing distance from the implantation chamber. The area of intense L-PHA staining was narrower than that showing decreased SNA-binding activity (compare with Fig. 4c). L-PHA also stained maternal capillary endothelium. The pattern of uniform WGA binding was similar to that seen on day 6 (Fig. 5e). ECA bound strongly to the cytoplasm and cell surfaces of decidual cells around the decidual crypt and implantation site (Fig. 5f), apart from an area immediately adjacent to the implantation site. In this region, trophoblast giant cells were poorly stained in contrast to their strong staining with SNA and MAA (Fig. 4c and d). More peripherally, the area of intense staining decreased gradually and became mainly cytoplasmic. The overall intensity of staining was, however, slightly greater than seen before.

Discussion

These results demonstrate that stage- and regionally-specific glycosylation is a feature of decidual cells in both rats and mice. This is effected under maternal control, as shown by similar findings in artificially induced deciduoma (Jones *et al.*, 1993). The loss during decidual differentiation of terminal α 2,6-linked sialic acid, recognized by SNA, is consistent in both species. In rats, it is accompanied by upregulation of α 2,3-linked sialyl residues recognized by MAA. The observed increase in binding of WGA is probably also accounted for by α 2,3-linked sialic acid terminals (Kronis and Carver, 1982). Increased binding of L-PHA and ECA are also evident. In mice, ECA and L-PHA binding are increased in decidual cells but MAA binding remains unaltered except in a narrow band of 5–6 cells adjacent to the implantation chamber. Thus, it appears that alterations in chain capping sugars occur in both species. The diminished abundance of α 2,6-linked sialic acid may expose new subterminal residues, such as the *N*-acetyl lactosamines that act as ligands for ECA (and WGA). In rats (but not mice), these in turn may act as substrates for addition of α 2,3-linked sialyl residues. Increased amounts of tri/tetra-antennary, non-bisected complex *N*-linked glycan are synthesized in the subluminal stroma in both pregnant rats and mice, as shown by the increased binding of L-PHA. Terminally fucosylated type I and type II chains do not appear to be expressed in mouse (Kimber *et al.*, 1988; Kimber and Lindenberg, 1990) or rat (Kimber *et al.*, 1995) stroma or decidua.

It has been shown that steroid hormones have a marked stimulatory effect on glycoprotein biosynthesis, particularly with respect to *N*-linked structures and lactosaminoglycans (Dutt *et al.*, 1988; Carson *et al.*, 1990). The present data suggest significant changes in the activity of specific glycosyl-transferases, with both similarities and differences between the two species. However, further studies will be required to investigate the mechanisms involved in effecting the observed changes in lectin binding: altered glycosylation of existing components, the production of new glycoconjugates, or both.

Altered glycosylation in decidua may be related to the remodelling of the extracellular matrix (Kleinfeld *et al.*, 1976; Parr *et al.*, 1986; Mulholland and Jones, 1993; Mulholland *et al.*,

1992) and the appearance of electron-dense flocculent material between the enlarging cells. Laminin, which is *N*-glycosylated, has also been found to increase during days 6 and 7 of pregnancy in rats (Glasser *et al.*, 1987; Clark *et al.*, 1993), and this may be related to the increase in L-PHA staining at this time. In addition, decidualizing stromal cells produce new cell surface structures, including adherens and gap junctions (Kleinfeld *et al.*, 1976; Brokelman and Biggers, 1979; O'Shea *et al.*, 1983; Parr *et al.*, 1986; Mulholland and Jones, 1993; Winterhager *et al.*, 1993). Thus, decreased binding of SNA (with a possible reduction of coulombic repulsion) may be related to the development of intercellular contacts. It will be interesting to examine the localization of the newly expressed glycodeterminants at the ultrastructural level and to characterize the macromolecules with which they are associated.

Mouse primary giant cells, identified by staining with BSA-IB₄ (Azuma *et al.*, 1991), react with lectins that bind both α 2,3 and α 2,6-sialyl residues. Trophoblasts of the pre-implantation blastocyst has been demonstrated to express α 2,3 and poly- α 2,8, but not α 2,6-linked, sialyl residues (Shevinsky *et al.*, 1982; Kimber *et al.*, 1993, 1994; S. J. Kimber, unpublished). In contrast to our results with SNA and MAA, expression of poly- α 2,8 sialic acid is downregulated on mouse trophoblast at the time of implantation (Kimber *et al.*, 1994). However, an antibody that recognizes α 2,3 sialyl residues found in brain gangliosides did not react with trophoblasts of day 5–7 mouse embryos (Pennington *et al.*, 1985).

Sialic acids may be functionally important in specific recognition events (as in selectin action), in increasing the net negative charge of the cell surface, which may in turn increase intercellular spaces, and in masking of subterminal glycan recognition structures. Changes in the amount, type or linkage of sialic acid in tumour cell glycoconjugates can affect tumour growth and metastasis (Takano *et al.*, 1994). Removal of α 2,6-linked sialic acid may be important in the preparation of the endometrium for trophoblast invasion in these species. Cell surface sialic acid residues regulate cell adhesiveness negatively in a number of cell lines (Shimamura *et al.*, 1994). Increased SNA staining has been associated with the loss in cell surface adhesiveness that occurs in malignancy. In a study of colonic tumours, Sata *et al.* (1991) found that an increase in α 2,6-linked sialic acid was associated with the histological features of malignancy, while α 2,3-linked residues progressively decreased with the degree of dysplasia in colonic adenoma, although both types of residue were found in carcinoma. Similar effects have been seen in a study of ulcerative colitis (McMahon *et al.*, 1994), with increased staining of SNA in all grades of the disease. Smaller changes were evident with MAA, although binding was lost in severe forms of the disease. Given possible parallels between trophoblast and tumour cells (Kirby, 1965; Lala and Graham, 1990; Graham and Lala, 1992), it will be interesting to examine the effect of modulating trophoblast cell surface sialic acid on blastocyst attachment and outgrowth *in vitro*. In contrast, an increase in intercellular contact and adhesiveness occurs in the maternal stroma during decidualization. Changes in decidual glycosylation must, therefore, be considered in relation both to the local function and integrity of the maternal tissue and its interaction with the developing placenta.

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