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Neoglycoprotein-Binding Sites (Endogenous Lectins) in the Fallopian Tube, Uterus and Blastocyst of the Rabbit during the Preimplantation Phase and Implantation

Key Words
Fallopian tube
Uterus
Blastocyst
Implantation
Lectins
Glycoconjugates
Rabbit

Abstract
Regulation of the initial phase of embryo implantation may involve the recognition interplay of glycoconjugates and respective receptors such as endogenous lectins on both cellular surfaces. Whereas changes in glycoconjugate composition have been detected in preparation for embryo implantation and described in detail, knowledge on endogenous lectins has remained scant. Affinity probes (carrier-immobilized carbohydrate structures as ligand part on a histochemically inert backbone) are used in the present investigation in order to gain further insights in this area. Cryostat sections of rabbit Fallopian tubes and uteri in nonpregnant and early pregnant [tubes: 3 days post coitum (d p.c.); uteri: 3, 5, 7 and 9 d p.c.] states were studied for binding patterns of a series of biotinylated (neo)glycoproteins. A high density of binding sites was detected with β-galactosides (with decreasing intensity: β-*D*-galactose-BSA, asialofetuin with its triantennary glycan chains, lactose-BSA). Considerably less binding (but with the same pattern) was obtained with β-N-acetyl-*D*-glucosaminide-BSA and is interpreted to originate from a cross-reactivity of such sites which may bind physiologically to Gal-β1,¾-GlcNAc sequences. In contrast, no evidence for the presence of binding molecules with specificities for a-*D*-mannose-BSA, maltose-BSA, N-acetyl-galactosaminide-BSA and N-acetyl-*D*-neuraminic acid-BSA was obtained in these tissues under the same conditions. The epithelium of the *Fallopian tube* showed a high density of β-galactoside-binding sites at the apical cell poles (including the cytoplasm and membrane region) already in the nonpregnant state. At 3 d p.c., a strong reaction in all epithelial cells of the isthmus and a marked decrease in the ampulla were noted. The putative lectin(s) appear(s) to be synthesized and secreted by the tubal epithelium. A physiological role in forming the mucoprotein layer of the blastocyst coverings by precipitating the appropriate mucin-type molecules can be considered. Within the *endometrium,* the β-galactoside-binding molecules were almost exclusively localized at the apical cell pole of epithelial cells, whereas there was hardly any binding in the epithelial cytoplasm or in the endometrial stroma. The reaction was very weak in the non-pregnant state but increased considerably until 5 d p.c., starting in the luminal-most parts of the epithelium. While the reaction was rather homogeneous at the surface of the luminal epithelium at 5 d p.c., the degree of heterogeneity increased stepwise from 7 to 9 d p.c. In the implantation chamber, the density of these β-galactoside-specific 'receptors' was further enhanced in particular at the epithelial surface of the placental folds. In contrast, the reaction was less intense at the antimesometrial uterine epithelium and in interblastocyst segments of the uterus, and it remained weak in the middle and deep crypts. The *trophoblast* showed a high density of galactoside-binding sites at its surface, and less in the cytoplasm. Neoglycoprotein binding to the *blastocyst coverings* observed at 7 d p.c. was strong in particular at the outer and inner surfaces. Physical factors (e.g. differential texture at surfaces) are discussed to influence the staining patterns of these extracellular coverings. Nevertheless, the observations made on the tubal and the uterine mucosa suggest that the putative lectin(s) detected here is (are) secreted by these epithelia and could be involved in the structural organization of the various layers of the blastocyst coverings with their remarkable content of oligosaccharide chains. This effect on topological aspects of the zona pellucida equivalents may be important for the interplay between trophoblast and uterine epithelium and the cascade leading to implantation initiation.

Abbreviations used in this paper:
ASF = Asialofetuin; BSA = bovine serum albumin; d p.c. = days post coitum; β-*D*-Gal = β-*D*-galactose; β-*D*-GalNAc = β-*D*-N-acetyl-D-galactosamine; β-*D*-GlcNAc = β-*D*-N-acetyl-D-glucosamine; Lac = lactose; LNF-1 = lacto-N-fucopentaose 1; α-*D*-Man = α-*D*-mannose; Mal = maltose; NANA = N-acetyl-*D*-neuraminic acid; PBS = phosphate-buffered saline; TBS = Tris-buffered saline.

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Introduction

For more than two decades, cell surface-bound and secreted glycoconjugates have been assumed to be involved in preimplantation development and in implantation of the mammalian embryo [for reviews, see Denker, 1970a, b; Anderson et al., 1986a; Thie et al., 1986; Morris et al., 1988; Anderson et al., 1990; Bükers et al., 1990; Carson et al., 1990; Chávez, 1990; Foidart et al., 1990; Kimber, 1990; Aplin et al., 1994; Kimber et al., 1995]. Their presence can involve cell-cell recognition phenomena as well as regulation of cell attachment. Since e.g. large-molecular weight glycoconjugates like MUC-1 have recently been found to undergo profound changes in density at the cell surfaces of uterine epithelial cells during the preimplantation phase, regulated by maternal sex steroids, it is an intriguing question whether and how such marked alterations will translate into functional aspects [Aplin et al., 1994; Aplin and Hey, 1995; Carson et al., 1995; Surveyor et al., 1995]. It is conceivable that such 'bulky' molecules could regulate attachment phenomena by sterically hindering access to cell surface-bound adhesion molecules. In contrast, such glycoconjugates can present developmentally regulated determinants to carbohydrate-binding molecules (lectins, glycosyltransferases), thereby mediating cell-to-cell binding. Indeed, attachment and/or outgrowth of mouse blastocysts on endometrial monolayers or various matrices in vitro has been shown to be inhibitable by the oligosaccharide lacto-N-fucopentaose I [Lindenberg et al., 1988] and by heparin [Farach et al., 1987]. Similarly, inhibition experiments performed in a model using human cell lines, JAR (choriocarcinoma) and RL95 (uterine epithelial cells) suggested involvement of heparan sulfate/dermatan sulfate-like molecules in attachment [Rohde and Carson, 1993]. In-vivo experiments along these lines have also been reported, but need to be interpreted very cautiously (see Discussion). In this context, it is noteworthy that such approaches infer the presence of adequate receptor sites whose further characterization obviously warrants attention. To address this problem, carrier-immobilized carbohydrate ligands as histochemical tools can provide valuable insights into the expression of accessible sites on the way to biochemical and immunohistochemical test series [Gabius and Gabius, 1993, 1997].

In the present communication, we report the results of a glycohistochemical study on the changing distribution of carbohydrate-binding molecules (putative lectins) in the Fallopian tube and the uterus of the rabbit during preimplantation and implantation.

Materials and Methods

Animals

Mixed breed rabbits were kept at a light-dark cycle of 12:12 h and fed with standardized pellet chow. Does were mated with two fertile males each. At each of the stages 3, 5, 7 and 9 days post coitum (d p.c.) does were sacrificed by stunning and exsanguination, and the Fallopian tubes and uteri were quickly frozen in liquid nitrogen. Material from nonmated does (0 d p.c.) was obtained similarly. With those neoglycoproteins which bound strongly to these tissues, i.e. β-*D*-galactose-bovine serum albumin (β-*D*-Gal-BSA), asialofetuin (ASF) and lactose-BSA (Lac-BSA), uteri from 2 animals of each stage were studied in detail, while material from only 1 animal of each stage was used for the remaining probes which reacted weakly or not at all, and for the Fallopian tubes.

Neoglycoproteins

Neoglycoproteins used as probes to specifically detect accessible carbohydrate-binding sites were synthesized by coupling *p*-aminophenyl glycosides of 7 mono- and disaccharides (table 1) to BSA and were biotin-labelled as described previously [Gabius et al., 1988; Gabius and Bardosi, 1991]. The oligosaccharide chains of commercial ASF were chemically desialylated by acid hydrolysis at 80 °C and the resulting ASF was also biotinylated.

Histochemistry

Transverse cryostat sections (8 µm thick) of the uteri and tubes were mounted and dried at room temperature for 3 min followed by fixation in acetone at –20 °C for 10 min. Nonspecific binding sites were blocked with 0.2% BSA (Sigma) in phosphate-buffered saline (PBS) or Tris-buffered saline (TBS), pH 7.4 for 5 min at room temperature. The sections were then dried without rinsing for 3 min at room temperature and incubated with the biotinylated neoglycoproteins in 0.2% BSA solution for 30 min at 37 °C [β-*D*-galactose (β-*D*-Gal)-BSA, α-*D*-mannose (α-*D*-Man)-BSA and ASF: 140 µg/ml; lactose (Lac)-BSA, β-N-acetyl-*D*-glucosamine (β-*D*-GlcNAc)-BSA, maltose (Mal)-BSA and β-N-acetyl-*D*-galactosamine (β-*D*-GalNAc)-BSA: 160 µg/ml; N-acetyl-*D*-neuraminic acid (NANA)-BSA: 180 µg/ml]. Subsequently, the sections were thoroughly washed with PBS/TBS (3×) and processed using the avidin-biotin-chromogen method: peroxidase-conjugated streptavidin (Histostain-SP Kit, Zymed Laboratories Inc., San Francisco, Calif., USA, Cat. No. 95-6543, or Dako, Hamburg, Germany, code No. PO397, 1:500 = 0.2 mg/l) for 5–15 min at room temperature, 3× PBS/TBS, 3-amino-9-ethylcarbazole (Histostain Kit, see above) 15 min, or diaminobenzidine (Sigma, Steinheim, Germany), 0.6 mg/ml 0.05 *M* Tris buffer pH 7.6 plus 10 µl/ml 3% H_2O_2, 10 min, incubation at room temperature in the dark, rinsing with distilled water, mounting in glycerol jelly. Blocking of endogenous peroxidases with H_2O_2 appeared unnecessary in the present material and was omitted since controls without streptavidin-peroxidase remained unstained.

Controls

Two types of controls were run in parallel with all assays: (1) replacement of the labelled neoglycoprotein by biotinylated BSA (140 µg/ml) in order to detect any nonspecific binding sites for the protein backbone of the carrier. These controls were always negative. (2) Competitive inhibition: sections were preincubated for 30 min at 37 °C with a mixture of the respective unlabelled neoglycoprotein (approximately 100-fold excess, i.e. 12 µg/ml) plus the appropriate

Table 1. Neoglycoproteins used

Neoglycoprotein	Terminal sugar
β-*D*-Gal-BSA	*D*-galactose
Lac-BSA	lactose (Galβ(1–4)Glc)
β-*D*-GlcNAc-BSA	N-acetyl-*D*-glucosamine
NANA-BSA	N-acetyl-*D*-neuraminic acid
α-*D*-Man-BSA	mannose
Mal-BSA	maltose (Glcα(1–4)Glc)
β-*D*-GalNAc-BSA	N-acetyl-*D*-galactosamine
ASF	galactose; ASF is a desialylated glycoprotein (48 kD) with three triantennary N-linked chains primarily with terminal LacNAc residues and three O-linked disaccharide chains (Gal-β1, 3-GalNAc-α-)

mono- or disaccharide (see table 1; *D*-galactose 105 mg/ml: lactose 200 mg/ml; N-acetyl-*D*-glucosamine 130 mg/ml; NANA 37 mg/ml). After that, sections were treated with a cocktail of the biotinylated neoglycoprotein plus the respective same two types of unlabelled competing molecules, using the complete procedure described above. Suppression of reactivity in these competition experiments was not always complete; only unequivocal suppression was considered as evidence for the existence of neoglycoprotein-binding sites in the sections, whereas it will be mentioned in the Results when no clear suppression was seen.

Since no binding sites were detected for α-*D*-Man-BSA, Mal-BSA and β-*D*-GalNAc-BSA, no inhibition experiments were performed in these cases. In the case of ASF, only unlabelled ASF without any addition of mono- or oligosaccharides was used for the control experiment.

Results

In the studied material, no evidence for the presence of accessible binding sites was detected in cryostat sections with α-*D*-Man-BSA, Mal-BSA (Glc-α1,4-Glc-BSA), β-*D*-GalNAc-BSA and NANA-BSA. In contrast, such sites were detected with β-*D*-Gal-BSA, ASF, Lac-BSA (Gal-β1,4-Glc) and β-*D*-GlcNAc-BSA. Since the spatial reaction patterns were fairly identical with all four reagents, they will be described together (see also table 2).

Tubal Epithelium

In nonpregnant animals, about 50% of the tubal epithelial cells showed strong binding of the four mentioned (neo)glycoproteins, concentrated in the apical part comprising the apical plasma membrane region as well as the apical part of the cytoplasm. This was seen in the ampullary (fig. 1) as well as in the isthmic part (fig. 2) of the tube. The thickness of the used native cryostat sections precluded unequivocal identification of ciliated versus nonciliated cells. At 3 d p.c., the intensity of staining had decreased in the ampullary part (fig. 3), but was enhanced in the isthmus. The staining in this area comprised nearly all epithelial cells and extended into the cytoplasm (fig. 4). The Fallopian tube was not studied at any stage later than 3 d p.c. since thereafter embryos reside in the uterus.

Uterine Epithelium

Binding of the neoglycoproteins was concentrated at the apical plasma membranes of the uterine epithelium, and this is also where impressive stage-dependent changes were seen during early pregnancy. This reaction was clearly diminished in the competitive inhibition experiments and will be described in detail below. Reactivity of the lateral and basal plasma membranes and of the uterine epithelial cytoplasm was low and not as clearly reduced by competitive inhibition.

In the nonpregnant state, the apical plasma membranes of uterine epithelial cells showed a low extent of neoglycoprotein binding which did not markedly differ in the various regions of uterine cross-sections (fig. 5). Until 3 d p.c. binding remained low and uniform in the actively proliferating endometrial crypts but the luminal epithelium showed regionally increased reactivity (fig. 6).

A completely different picture was obtained at 5 d p.c. when nearly all of the epithelial cells (luminal and cryptal) of the proliferated endometrium showed a strong apical reaction (fig. 7, 8). There was no difference in reactivity between the mesometrial and the antimesometrial part of the endometrium.

At 7 d p.c., differences between those parts of the endometrium which were adjacent to a blastocyst (implantation chamber) and those located between blastocysts (interblastocyst segments) became discernible. As compared to the 5 d p.c. stage, reaction intensity had decreased in interblastocyst segments (fig. 10) as well as in certain parts of the endometrium of the implantation chamber (antimesometrial epithelium and middle and deep crypts in the mesometrial parts, fig. 9), whereas it remained strong at the

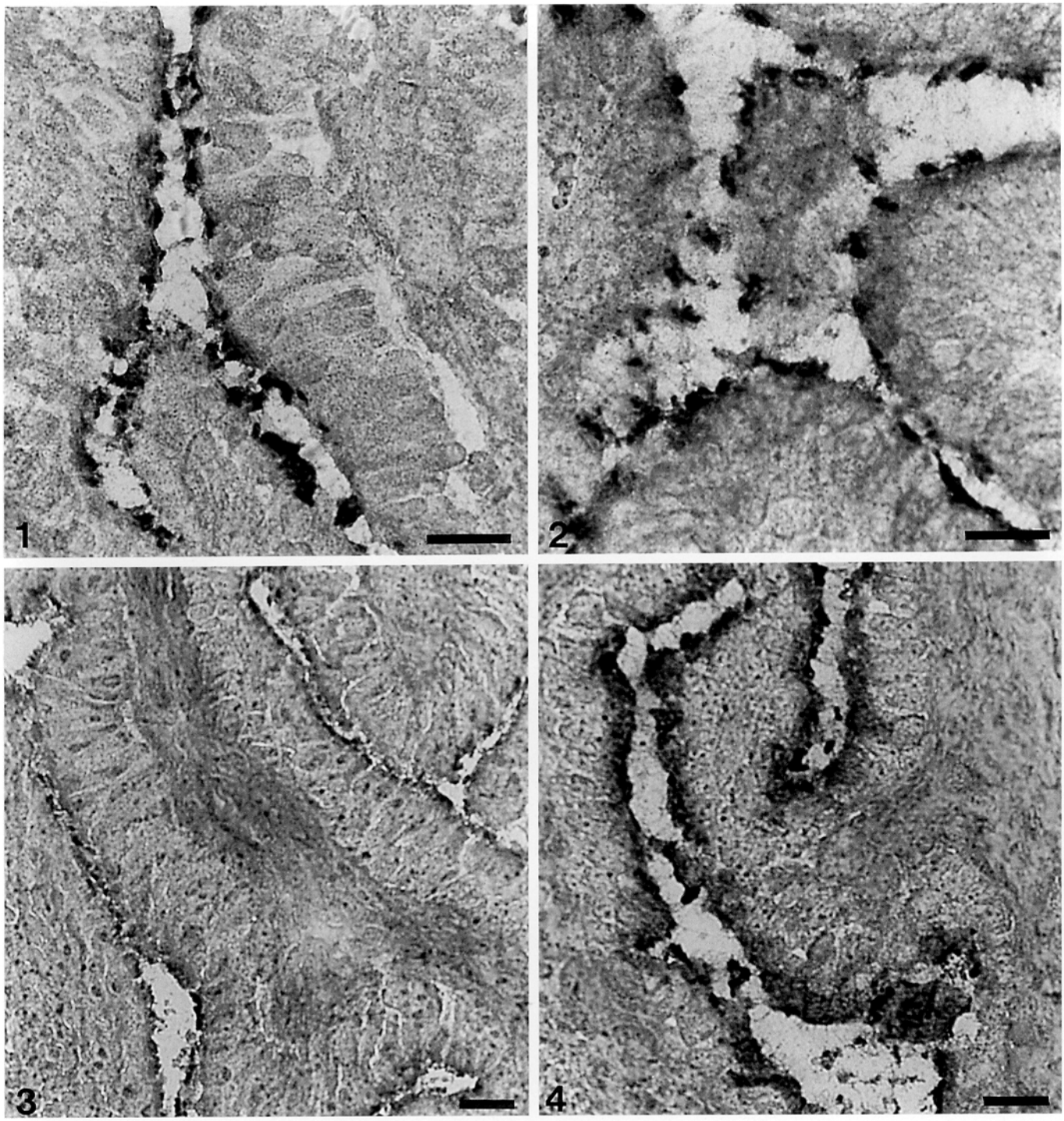


Fig. 1–4. Neoglycoprotein (β-*D*-Gal-BSA) binding in rabbit Fallopian tube. In the nonpregnant state, some of the epithelial cells show strong binding activity at their apical cell pole, while others do not (**1** ampullary region; **2** isthmus). In early pregnancy (3 d p.c.), reactivity is largely lost in the ampullary region (**3**), but increased in the isthmus, where it now comprises almost all cells (**4**). Bar = 20 μm.

Table 2. Major neoglycoprotein-binding sites detected

	Uterus	Tube
β-*D*-Gal-BSA (up to ++++)	epithelium (apical plasma membrane region) – implantation chamber, mesometrial luminal epithelium (0 d<9 d p.c., strongest reaction observed) – implantation chamber, deep crypts, mesometrial and antimesometrial (0 d<5 d>9 d p.c.) – interblastocyst segments (0 d<5 d>9 d p.c.)	epithelial cells of ampulla (0 d>3 d p.c.) epithelial cells of isthmus (0 d<3 d p.c.)
ASF (up to ++++)	blastocyst coverings (7 d p.c.) trophoblast (7 d<9 d p.c.)	
Lac-BSA (up to +++)	musculature (embryo-maternal symplasms, 9 d p.c.: negative) (scattered single cells in the endometrial stroma: only ASF, but inhibition incomplete)	
β-*D*-GlcNAc-BSA	as above, but much weaker reaction	
α-*D*-Man-BSA Mal-BSA β-*D*-GalNAc-BSA NANA-BSA	no binding under the tested conditions	

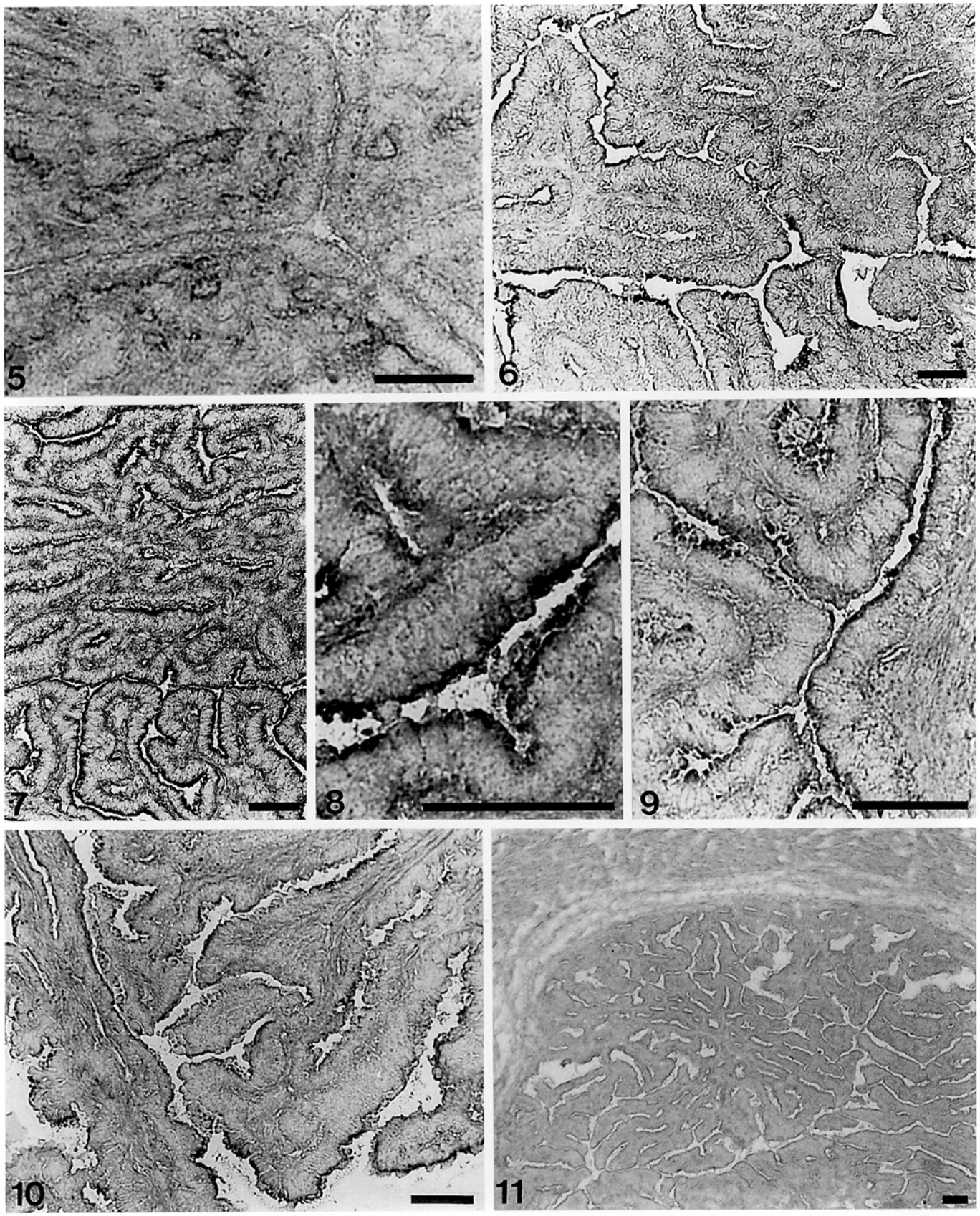
apical plasma membrane of the mesometrial luminal epithelium (fig. 12).

The differences between the various parts of the endometrium were even more pronounced at 9 d p.c. There was little residual reactivity of the uterine epithelium in interblastocyst segments (fig. 11) and in middle and deeper parts of the endometrial crypts at implantation chambers (fig. 13). In contrast, reactivity was strong at the apical plasma membrane of the luminal epithelium, extending somewhat into the uppermost parts of the crypts (fig. 17). However, the extent of the reaction tended to be less in those parts of the epithelium that had fused to form large symplasms, and the reaction disappeared nearly completely where syncytiotrophoblast had fused with the uterine epithelium (fig. 16), i.e. where the apical plasma membranes had disappeared due to the fusion process [Enders and Schlafke, 1971; Denker, 1977]. This is consistent with the interpretation that the reaction is indeed associated with the apical plasma membrane. In the antimesometrial region, where the short-living obplacenta is already degenerating at this stage [Schoenfeld, 1903], unattached parts of trophoblast and uterine epithelium were moderately stained, whereas fused parts reacted poorly.

The stroma of the endometrium as well as of the endosalpinx generally showed only low-degree β-*D*-Gal-BSA, Lac-BSA, β-*D*-GlcNAc-BSA and ASF binding. An exception were single scattered cells that were strongly positive with ASF but not with any other neoglycoprotein. This

Fig. 5–11. Neoglycoprotein binding in rabbit endometrium. In the nonpregnant state (**5**), binding activity of the epithelium is low and homogeneous. In early pregnancy, binding activity increases: spotty staining found predominantly at the surface of the luminal epithelium at 3 d p.c. (**6**), increased and rather homogeneous reactivity of all epithelial cells at 5 d p.c. (**7**). At higher magnification (**8**) staining can be seen in the apical plasma membrane region (possibly including the apical cytoplasm) and also in the luminal contents. In most parts of the uterine epithelium, neoglycoprotein binding decreases again thereafter (**9**: 7 d p.c., middle parts of endometrial crypts at implantation site; **10**: 7 d p.c., interblastocyst segment; **11**: 9 d p.c., interblastocyst segment). This is in contrast to the luminal epithelium of implantation chambers where reactivity continues to increase (see fig. 12–17). **5**, **7–11**: β-*D*-Gal-BSA. **6** ASF. Bars = 50 μm.

Fig. 12–17. Neoglycoprotein binding in rabbit implantation chambers, mesometrial part. Reactivity has further increased in those parts of the uterine luminal epithelium which are close to the blastocyst, at 7 (**12**, **14**) and 9 d p.c. (**13**, **15–17**). Staining has decreased in deeper parts of endometrial crypts (**13**, **15**). Blastocyst coverings (BC) can be seen in **12** and **14**, showing a marked staining at their outer and inner surface while their main parts remain unstained. Coverings have dissolved at 9 d p.c. (**13**, **15–17**). **16** Broad zone of trophoblast (T) attachment to and invasion into the endometrium (IT = invading trophoblast). **17** A narrow point of incipient fusion. While nonattached trophoblast (T) binds neoglycoproteins strongly (**14**, **15**), this reactivity cannot be detected anymore in those parts which have fused with endometrial cells (left hand side of **16**, **17**). **12**, **14**, **17** β-*D*-Gal-BSA. **13**, **15**, **16** ASF. Bars = 50 μm.



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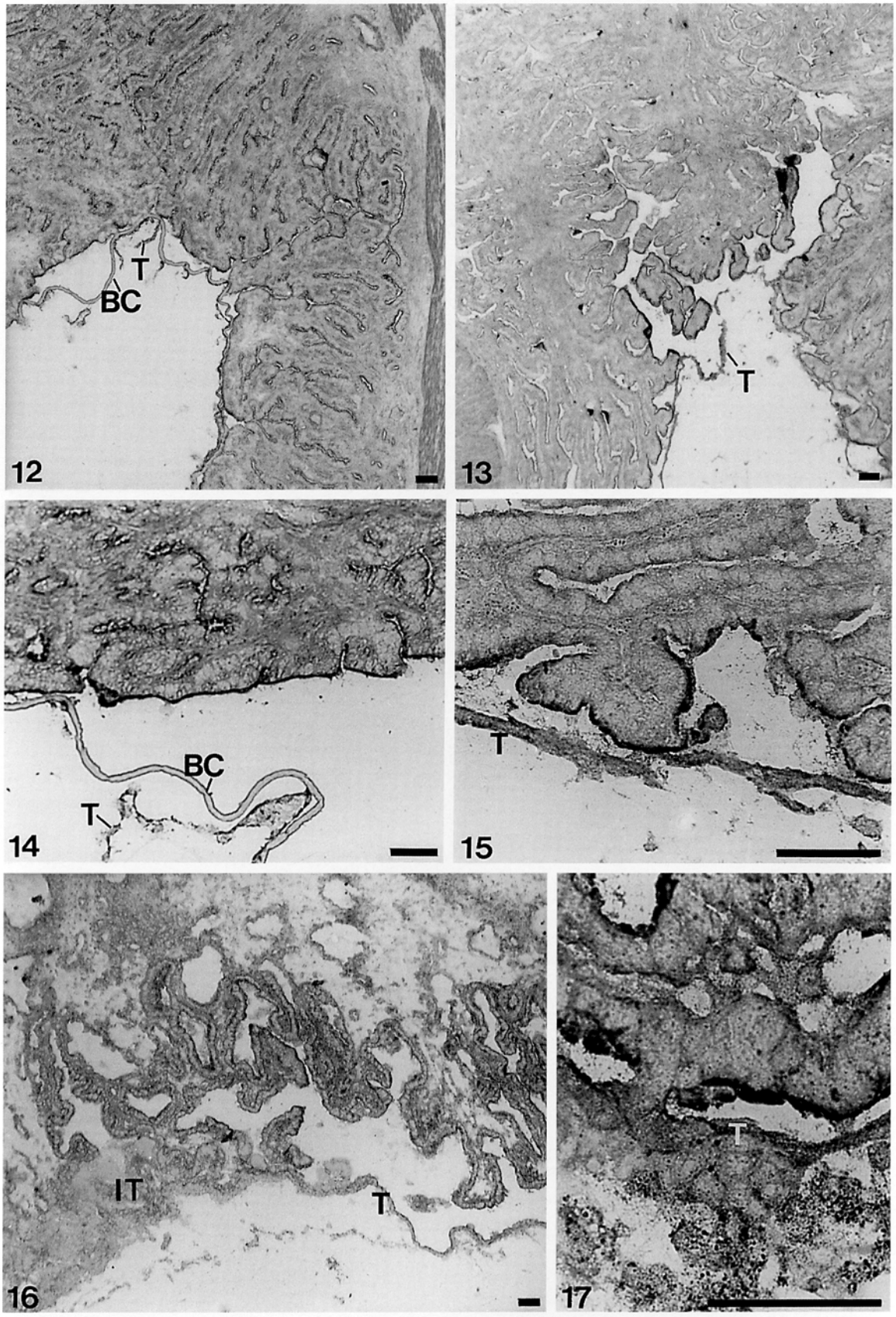

(For legends, see p. 163.)

reaction was only slightly reduced in competition experiments and may be due to a different binding mechanism. According to the distribution pattern these stained cells could be macrophages (see Discussion). Smooth muscle cells did show a positive reaction which, however, was variable and will not be discussed in detail here.

Trophoblast

The trophoblast studied at 7 and 9 d p.c. showed relatively strong neoglycoprotein binding as long as it was not attached to and fused with the uterine epithelium (fig. 14, 15). Since in most parts of the blastocyst the trophoblast is a very flat epithelium it was difficult to judge at the light-microscopic level whether there were any differences in reactivity between the apical, lateral and basal plasma membranes and the cytoplasm. After fusion of the trophoblast with the uterine epithelium (antimesometrially and mesometrially) neoglycoprotein binding could not be detected anymore (fig. 17).

Blastocyst Coverings

The extracellular blastocyst coverings (equivalents of the zona pellucida) could be studied only at 7 d p.c. At this stage, they showed strong staining at their outer and inner surfaces, while the remainder reacted only weakly (fig. 14). The strongly reacting layers were much thinner than the gloiolemma and the neozona previously described on the basis of morphological and histochemical criteria [Denker and Gerdes, 1979]. This reaction may be artifactual since it could not be markedly suppressed in the inhibition experiments (cf. Discussion).

Discussion

Classification of the Sugar-Binding Molecules

With a histochemical approach as used in the present investigation, it is basically possible to describe spatial patterns of accessible sugar-binding molecules. Although a lack of conspicuous Ca^{2+} dependence argues against a participation of C-type lectins, a definitive classification of the molecule(s) certainly requires a biochemical approach [Gabius et al., 1987; Gabius, 1988, 1997]. Neoglycoproteins are a very useful and versatile tool for the detection of sugar-binding molecules within tissues [Danguy et al., 1997; Kannan and Nair, 1997]. From a functional point of view, this type of approach can be considered more attractive than e.g. detection by appropriate antibodies since in the latter case accessibility of binding sites and their functionality are not probed. On the other hand, neoglycoproteins as used in the present studies may not be optimally designed to compete, at the level of the carbohydrate recognition domain of lectins, with high-affinity endogenous lectin ligands. Although the presentation of a number of sugar ligands on the carrier's surface procures a cluster effect [Lee and Lee, 1997], the ensuing avidity may not surpass that generated by in situ oligosaccharides. However, increases of the complexity of the sugar part by chemoenzymatic synthesis has been shown to increase the target selectivity of the probe [Gabius and Gabius, 1992]. In model studies with organs with preferential expression of one lectin from a distinct class, the colocalization of the glycohistochemical probe and the respective antilectin antibody underscores the principal validity of this approach [Bardosi et al., 1989; Gabius et al., 1991].

With respect to the competition experiments performed, the cluster effect, too, should not be overlooked, since the use of free mono- or disaccharides (even in high molar excess) usually did not allow to suppress neoglycoprotein binding completely [Kuchler et al., 1990]. Moreover, it is a common phenomenon from solid-phase assays that competition with the label-free ligand cannot be automatically and predictably computed, e.g. reaching a reduction of 90% at a 9:1 ratio. Therefore, marked decreases of the staining activity in the presence of the inhibitor were judged to indicate a carbohydrate-mediated association. Notably, binding of the conjugate via biotin or the protein carrier part was rigorously excluded by the use of the labelled carrier protein. With respect to other possible artefacts, previous studies [Strunck-Kortenbusch, 1991] had shown that the extracellular coverings of early rabbit embryos show endogenous avidin-binding sites but this was not apparent in the late blastocyst stage (7 d p.c.) studied in the present series.

Binding Specificity

The largely identical binding patterns obtained in our material with β-*D*-Gal-BSA, ASF, Lac-BSA and β-*D*-GlcNAc-BSA suggest that only one type of sugar-binding site (putative lectin) has been visualized with these reagents. A primary specificity for *D*-Gal is suggested by the fact that this sugar is terminal in β-*D*-Gal-BSA, ASF and Lac-BSA. β-*D*-GlcNAc-BSA which showed only very weak reactions could have been bound due to a cross-reactivity with galectins whose prime ligands are Gal-β1,$^{3}/_{4}$-GlcNAc sequences [Gabius, 1997] or by a different colocalized molecule.

Binding of ASF deviated from the otherwise identical pattern only in one respect: the strongly positive labeling of some scattered endometrial stroma cells. According to their distribution pattern, these may be macrophages [cf. Denker, 1971, 1977], although no additional immunohistochemical

tests were carried out to confirm this. Macrophages do react with ASF in the human [Gabius et al., 1987]. The identity of the binding molecule present in these cells in case of the rabbit uterus remains to be defined. Galectins and a C-type lectin with this specificity are known to be present in such immune cells [for a review, see Gabius, 1997].

Since the detected activities did not require the presence of Ca^{2+} ions, it is pertinent to refer to activities known to occur in rabbits. Just as in other species, lectins were found in rabbits some time ago. A lactose-binding lectin from rabbit bone marrow, e.g., is thought to play a role in erythropoiesis [Harrison et al., 1984]. The same group also found a galectin in skeletal muscle, smooth muscle and cardiac muscle, skin, lung, small intestinal mucosa and liver. The distribution patterns change somewhat during fetal development [Catt et al., 1987].

Galectin-1 ('galaptin' = L 14) was detected in the rabbit uterus and located immunohistochemically during preimplantation and implantation in stroma cells, endothelial cells, smooth muscle cells and in the basement membrane region of the epithelium [Hoffman et al., 1993]. The distribution pattern is thus different from the pattern of galactose-binding sites described in this report. Therefore it appears rather improbable that the same molecule was detected in both studies. Hoffman et al. [1993] found stronger reactions in the endometrial stroma, where we have obtained only a very weak staining. In contrast, Hoffman et al. observed no reaction at the location showing strongest neoglycoprotein binding in our study, i.e. at the apical plasma membrane of uterine luminal epithelium during preimplantation and incipient implantation. Therefore, there is no direct evidence for an involvement of galectin-1 studied by Hoffman et al. in the adhesion phase of implantation. It must be kept in mind, however, that the methodology used was different in the two studies (immunohistochemistry vs. glycohistochemistry aiming at the detection of free sugar binding sites).

Functional Aspects

A role of carbohydrate-binding molecules in cell-cell recognition and adhesion phenomena is being discussed for a variety of biological systems like the extravasation of white blood cells [Springer, 1990; Harlan and Liu, 1992; Lasky, 1992; Stoolman, 1992; Springer, 1994; Nelson et al., 1995], hematogenous tumor metastasis formation [Mareel et al., 1993] and sperm penetration into the egg [Saling, 1989; Wassarman, 1992, for reviews]. As mediators of adhesion not only lectin-like molecules (like the selectins in case of white blood cell extravasation) have to be considered, but also e.g. glycosyltransferases, as postulated by Roseman [1970]. A role of β1,4-galactosyltransferase in the penetration of sperm into the egg is discussed [Youakim et al., 1994]. High β1,4-galactosyltransferase activity was found in preimplantation mouse embryos [Sato et al., 1984] and in the mouse uterus [Moran et al., 1986]. Data on a correlation with attachment phenomena at the cellular level are yet missing. Experimental evidence suggesting a role of galactosyltransferase in mouse implantation was presented by Chávez [1990]. A possible involvement of galectin-1 present in mouse trophoblast cells in implantation is being discussed on the basis of a spatio-temporal correlation only [Poirier et al., 1992]. The same lectin is also detectable in the mouse uterus. But the observed spatio-temporal distribution pattern does not provide any evidence for a correlation between the presence of this lectin and the initial phases of implantation [the lectin is present in all tissue compartments except the luminal and glandular epithelium; Phillips et al., 1996]. In contrast, another galectin (Mac-2 = galectin-3) was observed in decidual cells of the mouse immediately after implantation and at later stages of pregnancy, and in placental trophoblast. In nonpregnant animals, there was little evidence for expression of this lectin, and it was not found in the uterine epithelium at any stage [Phillips et al., 1996]. A heparin/heparan sulfate-binding 'receptor' located at the apical plasma membrane of the uterine epithelium is proposed to mediate trophoblast attachment at implantation initiation in the mouse [Farach et al., 1987; Carson et al., 1990; Wilson et al., 1990]. Data from human cell lines (RL 95 and JAR) used as model systems for early phases of implantation [John et al., 1992] also suggest that heparan sulfate proteoglycans may be involved in embryo implantation [Raboudi et al., 1992; Rohde and Carson, 1993].

Trophoblast Attachment to the Uterine Epithelium

The impressive increase in neoglycoprotein binding to the apical plasma membrane of uterine epithelial cells that was observed in the present studies to occur during the preimplantation phase could suggest a possible role in implantation initiation. Expression increases at the luminal epithelium of the placental folds until 9 d p.c. so that there is a correlation with trophoblast attachment [starting at this location 8–8.5 d p.c.; Denker, 1977]. At the antimesometrial side of the uterus, however, where the trophoblast establishes its first contact with the endometrium 1 day earlier (to form the ephemeral yolk sac placenta), this correlation is not that clear: At this location, staining intensity at the surface of the uterine epithelium already starts to decrease at 7 d p.c. The trophoblast attaches to the uterine epithelium in the rabbit through a process of cell fusion. As soon as

this fusion has occurred, neoglycoprotein binding disappears suggesting that this molecule is indeed bound to the apical plasma membranes. The present light-microscopical investigations do not provide any information as to the fate of these molecules (degradation) nor to the route of synthesis and insertion into the membrane.

Not only the uterine epithelium but also the trophoblast of rabbit blastocysts showed accessible neoglycoprotein-binding sites in our study. This should be seen in the context of other 'receptor' molecules identified previously at the trophoblast of periimplantation blastocysts in various species, including binding sites for various extracellular matrix molecules, fibronectin, collagen type IV, hyaluronic acid and in particular heparin/heparan sulfate proteoglycan as discussed above [for a survey of the literature, see Carson et al., 1990; for data in particular concerning the trophoblast of early placentation stages in the human see Aplin, 1991; Korhonen et al., 1991; Damsky et al., 1992; Swann et al., 1993]. Of interest in the context of the present paper is that galectin-1 was found in trophoblast cells of mouse blastocysts [Poirier et al., 1992]. It should be interesting to see, in the course of further investigations, whether galactose-dependent neoglycoprotein binding by rabbit trophoblast as described in the present communication may be due to a related molecule.

Any of these 'receptor' molecules could, alone or in combination with others, be involved in attachment of the trophoblast to the uterine epithelium in the implantation initiation phase, provided that a corresponding ligand molecule is presented by the uterine epithelial surface [cf. Carson et al., 1990]. Terminal Gal and GlcNAc residues are indeed exposed at the uterine epithelial cell surface in the monkey [Anderson et al., 1986b] and in the rabbit [Anderson et al., 1986a; Nalbach and Denker, 1983; Bükers et al., 1990]. However, a positive correlation of the expression of these glycoconjugates at the uterine epithelial surface with receptivity for trophoblast attachment is by no means as clear as originally proposed for the mouse: in the rabbit, where implantation chambers and more remote parts of the endometrium (interblastocyst segments) can be compared very easily and where two phases of attachment (antimesometrial and mesometrial) can be studied separately and subsequently, lectin-binding studies rather suggest that reduced expression of glycoconjugates (including Gal residues) correlates with receptivity, at least at the (mesometrial) placental folds and at least after local signals from the blastocyst have completed the phenotypic changes in the uterine epithelium that are necessary for allowing the trophoblast to attach. This is more consistent with the view already mentioned in the Introduction that bulky molecules containing carbohydrate antennae exposed at the surface of the uterine epithelium and/or the trophoblast during the preimplantation phase may sterically hinder the accessibility of receptor and ligand molecules, and that the reduction in thickness of such a glycocalyx may be an important step towards attaining receptivity or invasiveness, respectively. It is also consistent with the view that a reduction of the polar organization of uterine epithelial cells along the apico-basal axis is a central element to receptivity [Denker, 1990, 1993].

Functional studies performed either in vivo or in vitro partly support, but in other cases do not support the view that carbohydrate-binding molecules may be essential for implantation initiation. Experiments involving intrauterine administration of either sugar derivatives or exogenous lectins have been performed in rodents and were interpreted as evidence for a role of carbohydrate recognition phenomena [Hicks and Guzman-Gonzales, 1979; Wu and Gu, 1981; Sretarugsa et al., 1987; Chávez, 1990]. For technical reasons it is not sure whether the conclusions derived from these experiments are valid as intrauterine administration of any substance can very easily produce side effects disturbing implantation in the rodent model: the uterine epithelium is very easily irritated during receptivity in these species in contrast to the rabbit. Unfortunately, no detailed morphological study of the critical phases of trophoblast attachment to the uterine epithelium has been performed in the above-mentioned investigations [discussed in detail by Friedrich, 1991, and Grütter, 1992]. Experiments in the rabbit involving intrauterine administration of the lectins wheat germ agglutinin and succinylated concanavalin A, including the morphology of implantation stages and a study of the fate of the administered lectins, showed that implantation was indeed not disturbed in this species by those two lectins [Friedrich, 1991; Grütter, 1992].

In vitro experiments, on the other hand, gave clear evidence for a highly specific role of certain carbohydrate chains (lacto-N-fucopentaose 1, Fuc-α(1–2)-Gal-β(1–3)-GlcNAc-β (1–3)-Gal-β(1–4)-Glc, LNF-1) in attachment of mouse blastocysts to endometrial cell monolayers [Lindenberg et al., 1988]. Binding sites for LNF-1 were found at the abembryonic trophoblast [Lindenberg et al., 1990]. The pentasaccharide itself is expressed at the surface of the uterine epithelium around implantation [Kimber et al., 1988]. An enzyme involved in the synthesis of LNF-1, α(1–2)-fucosyltransferase, is highly active in nonpregnant endometrium but its activity decreases during the preimplantation phase to become undetectable at day 6 [White and Kimber, 1994].

Tubal Epithelium and Formation of Extracellular Embryo Coverings

In contrast to the uterine epithelium, the tubal epithelium showed strong neoglycoprotein binding already in the nonpregnant state. Until 3 d p.c. when embryos migrate through the isthmic part of the tube accompanied with deposition of a thick mucoprotein layer around their outside, the reaction decreased in the ampulla but increased considerably in the isthmus. The putative lectin(s) was (were) localized not only at the apical plasma membrane region but also in the apical part of the epithelial cell cytoplasm. These findings suggest that this (these) molecule(s) appear(s) to be increasingly synthesized and possibly extruded into the tubal lumen around 3 d p.c.

The early rabbit embryo is surrounded by conspicuous extracellular coverings, equivalents of the zona pellucida but much more complex morphologically and histochemically that are thoroughly transformed during preimplantation development [Denker and Gerdes, 1979; Leiser and Denker, 1988; Fischer et al., 1991]. The mechanisms of deposition of this glycoprotein material forming the mucoprotein layer in the tube and the neozona and the gloiolemma in the uterus are unknown. As discussed previously [Denker, 1983], one possibility is that lectin-like molecules, secreted by either the tubal and/or uterine epithelium or the trophoblast, could be involved in precipitating glycoproteins that are likewise secreted. The putative lectin(s) detected in the tubal epithelium as well as that expressed by the trophoblast and the uterine epithelium could be involved in deposition of the mucoprotein layer, neozona and gloiolemma, respectively. This assumption will have to remain speculative, as long as appropriate in vitro experiments with isolated secretion glycoproteins and the putative lectin(s) have not been performed.

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