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Glycosylation at the fetomaternal interface: does the glycocode play a critical role in implantation?

Carolyn J. P. Jones · John D. Aplin

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Abstract During pregnancy, the heavily glycosylated surfaces of the implanting blastocyst and maternal uterine epithelium interact in a highly controlled and specific manner. Examination of this interface in species that show interdigitation of embryonic and maternal surfaces (epitheliochorial placentation) shows that each has its own particular pattern of glycosylation or glycotype, and that closely related and/or interbreeding species *e.g.* horse and donkey or llama and guanaco, have very similar glycotypes. Implantation of interspecies hybrids is facilitated, when the blastocyst has an outer cell layer bearing glycans that are compatible with the maternal host. We refer to this mutual compatibility as a glycocode. The probability that hybrid embryo glycotypes differ from those normally associated with the host species may account for the high pregnancy failure rates seen in interspecies breeding. We suggest the maternal host selects between genotypically distinct embryos, and this selection depends partly on cell surface glycosylation. We infer that the glycocode plays a critical role in implantation, for if the survival of modified genotypes results in fitter offspring with altered placental glycosylation, selection pressure downstream may in turn act to drive adaptations in the maternal surface glycotype to produce a complementary glycocode, thus leading eventually to the creation of new species. We speculate that glycan microheterogeneity plays a specific role in this process.

Keywords Placenta · Fetomaternal interface · Evolution · Speciation · Glycotype

C. J. P. Jones · J. D. Aplin (✉)
Maternal and Fetal Health Research Group, School of Clinical and Laboratory Sciences, University of Manchester,
St Mary's Hospital, Hathersage Road,
Manchester M13 0JH, UK
e-mail: john.aplin@manchester.ac.uk

Introduction

It has been recognized for a considerable time that both the apical surface of the uterine epithelium and that of the outer trophoblast cell layer of an implanting embryo are heavily glycosylated [1–4]. Various studies have indicated that, on initial contact, these surfaces interact in a specific and sensitive manner, such that perturbations of the system generally result in failure of implantation or poor placentation leading to compromised outcomes including embryonic or fetal death.

Interspecies hybridisation studies show that embryonic and maternal cell surface phenotypes are crucial to successful pregnancy

Interspecies transfer of embryos can be used to assess the extent to which recognition events are species-specific. A high proportion of embryos generally fail to implant, a phenomenon attributed partly to errors in recognition between foreign trophectoderm and the maternal uterine epithelium [5]. For example, goat x sheep placentae showed abnormal contact at the feto-maternal junction [6]. Later experiments to produce chimaeras between sheep and goat [7–8] or between two mouse species (*Mus caroli* and *Mus musculus)*, demonstrated that if the implanting embryo were manipulated to produce a chimaeric blastocyst in which the outer trophectoderm cells came from the same species as the recipient, the chances of survival were greatly increased [9–11]. This manoeuvre used compatible trophoblast to protect a foreign fetus from an adverse maternal response. The latter might occur as a result of immune recognition: for example, survival rates of goat-sheep hybrids are lower in goats that have carried sheep embryos before [12].

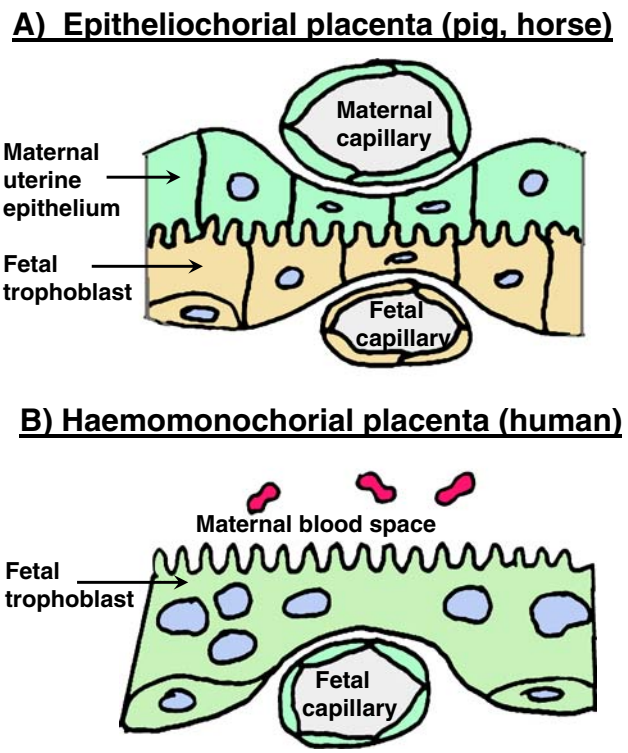

Fig. 1 Diagrammatic representation of **A** epitheliochorial and **B** hemochorial placentation showing the interdigitation of fetal trophoblast with the maternal epithelium in the epitheliochorial placenta. In the hemochorial placenta, the fetal tissues have eroded the maternal epithelium and endothelium to make direct contact with maternal blood

In horses, the type of cross affects the development of the chorionic girdle, the source of specialised binucleate trophoblast "cup" cells which produce equine chorionic gonadotrophin, a glycoprotein hormone that maintains ovarian steroid output. When a horse carries a donkey conceptus, the cells of the girdle fail to invade, and the placental membrane (allantochorion) is not firmly attached [13]; there is no villus formation, and the maternal and fetal surfaces appear to repel one another. The normal microvillous interdigitation between fetal and maternal tissues appears to fail. Paternal imprinting and uterine factors also influence the success or otherwise of hybridisation in horse [14], affecting the normal development and phenotype of the invasive girdle cells that are the source of the endometrial cups.

Different types of placentation

Placental structure is very variable between species, with differences in the shape, morphology of the component parts and degree of invasiveness into uterine tissues [15]. Here, we consider two main types (Fig. 1). In the epitheliochorial placenta, trophoblast microvilli interdigitate closely with those of the endometrial epithelial cells, whereas in the hemochorial placenta, trophoblast erodes the epithelial surface to make direct contact with maternal blood, which circulates around the placental villi, forming a blood/tissue interface. The former type is found in many domestic ungulates such as the pig, horse, camel and alpaca.

In epitheliochorial placentation, intimate interdigitation between the trophoblast and the maternal endometrial epithelium is sustained throughout pregnancy (Fig. 2); in contrast, in hemochorial placentation there is an initial transient contact between the blastocyst and uterine epithelial cell layer, but this is soon lost as trophoblast invades and breaks through vascular endothelium to make direct contact with the maternal blood. The interface is thereafter between the apical surface of the trophoblast and the maternal bloodstream. A second interface is usually maintained between invasive trophoblast and uterine

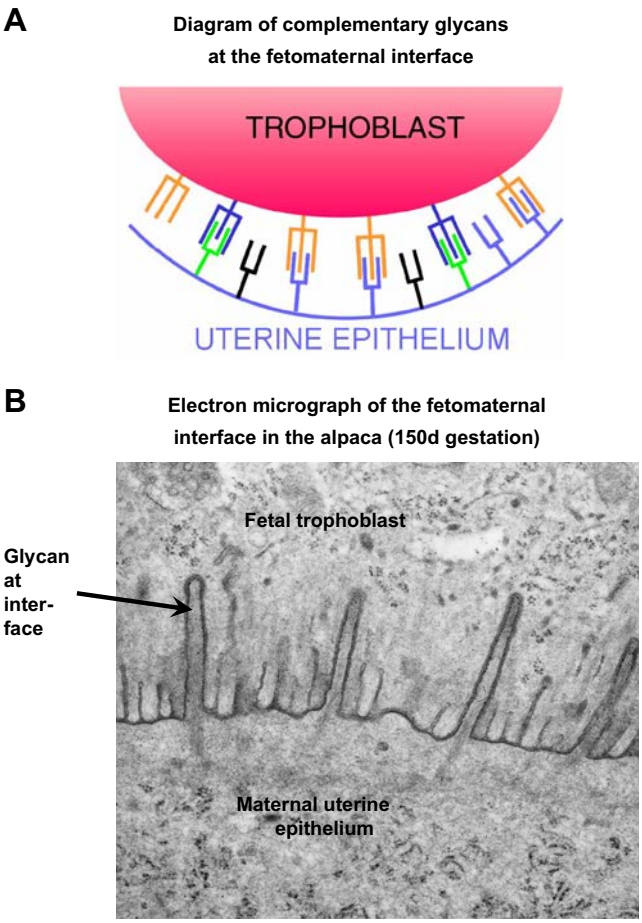

Fig. 2 **A** Diagram showing complementary glycosylated surfaces at the fetomaternal interface. Successful attachment and implantation is dependent on the apposition of appropriate glycotypes as determined by the glycocode. **B** Electron micrograph of the fetomaternal interface in the alpaca showing the close apposition of fetal and maternal microvilli with a dense osmiophilic glycosylated layer between them, suggestive of glycan–glycan interaction

stromal tissue known as decidua. This is a region of active immunological communication.

The maternofetal interface is highly glycosylated in all placental types and this changes with speciation

Studies from our laboratory using lectin histochemistry to probe glycan expression at the fetomaternal interface of diverse species (see Table 1 for lectins used) have shown it to be heavily glycosylated in all placental types [4, 16] (Fig. 2). Examination of closely related species has also shown that speciation is accompanied by subtle changes in glycan expression at this site [17]. Horse and donkey, which can interbreed, show generally similar glycosylation (Fig. 3) except for the presence of non-bisected tri/tetra-antennary complex N-glycan and α2-3-linked sialic acid in the maternal uterine epithelium and a small amount of sialic acid bound by *Limax flavus* agglutinin in the horse trophoblast that is absent in the donkey. This contrasts with the camel, which cannot interbreed with either horse or donkey, and shows more marked differences in fucose, *N*-acetylgalactosamine, and some sialic acids in both maternal and fetal tissues. The pig, which has an epitheliochorial placenta not dissimilar from that of the camel, shows altered glycosylation in trophoblast, while the uterine epithelium is more like that of the horse and donkey. In general, there is more glycan diversity between species in the trophoblast than in maternal uterine epithelium. These data suggest that species-specific glycotypes exist at both the uterine epithelium and trophoblast. [Table 2; 16].

Studies on the fetomaternal interface of camel and alpaca, which diverged 11 million years ago [18] also show more conservation on the maternal side, with variations in the distribution of bi/tri-antennary bisected N-glycan, fucosylated structures such as GalNAcα1-3(Fucα1-2) Galβ1-3/4Glc-NAcβ1, β-galactosyl residues and α2-3-linked sialic acid in trophoblast [19]. Comparison of placental glycosylation of a cama—a camel/llama hybrid produced in Dubai [20]—with that of the parent species as well as the closely-related guanaco and alpaca, showed that the pattern of glycan expression closely followed phylogenetic relationships (unpublished). Analyses of the cytochrome *b* gene sequences and mitochondrial and microsatellite DNA place the llama with the guanaco and the alpaca with the vicuña [18, 21–22]. We found that glycans expressed by llama and guanaco were similar while alpaca showed one major difference from other Lamini—abundant 2,3-linked sialic acid (recognised by binding of MAA, Table 2) that was absent in the others. Fucose in α1–2 linkage was also reduced in abundance in the alpaca. Glycosylation of cama trophoblast was similar to that of llama (the female parent species) and close relative guanaco but showed differences from camel (male parent) and alpaca trophoblast. Hybridisation is difficult to achieve,

Table 1 Lectins used in this study and their major specificities

Acronym	Source	Major specificity
PSA	*Pisum sativum* garden pea	α-D-mannose in non-bisected bi/tri-antennary, complex N-linked sequences
e-PHA	*Phaseolus vulgaris* (erythroagglutinin) kidney bean	Bi/tri-antennary bisected complex N-linked sequences
l-PHA	*Phaseolus vulgaris* (leukoagglutinin) kidney bean	Tri/tetra-antennary, non-bisected complex N-linked sequences
UEA-1	*Ulex europaeus-1* gorse	H type 2 antigen (αL-Fuc(1,2)- Galß1-4GlcNAcß1-) and Le^y
LTA	*Tetragonolobus purpureus* lotus	L-fucosyl terminals (especially where clustered), Fucα1-6GlcNAc >Fucα1-2-Galß1-4 (Fucα1-3)GlcNAcß, $Le^{x,\ y}$
DBA	*Dolichos biflorus* horse gram	GalNAcα1-3(LFucα1-2)Gal- ß1-3/4GlcNAcß1-
MPA	*Maclura pomifera* osage orange	Galß1-3GalNAcα1- >GalNAcα1-
DSA	*Datura stramonium* jimson weed	ß1-4GlcNAc, *N*-acetyl lactosamine >chitotriose
STA	*Solanum tuberosum* potato	ß1-4GlcNAc oligomers
LEA	*Lycopersicon esculentum* tomato	ß1-4GlcNAc oligomers
HPA	*Helix pomatia* Roman snail	Terminal GalNAcα1-
AHA	*Arachis hypogaea* peanut	Galß1-3GalNAcß1- >Galß1-4GlcNAcß1-
ECA	*Erythrina cristagalli* coral tree	Galß1-4GlcNAcß1-
SBA	*Glycine max* soybean	Terminal GalNAcα1- >Galα1
WFA	*Wisteria floribunda* wisteria	GalNAcα1-6Galß1- >GalNAcα1-3Galß1-
SNA	*Sambucus nigra* elderberry bark	NeuNAcα2-6Gal/GalNAc-
MAA	*Maackia amurensis*	NeuNAcα2-3Galß1-
LFA	*Limax flavus* yellow slug	Certain sialyl residues
WGA	*Triticum vulgaris* wheatgerm	Di-*N*-acetyl chitobiose, *N*-acetyl lactosamine (especially if clustered) and some sialyl residues

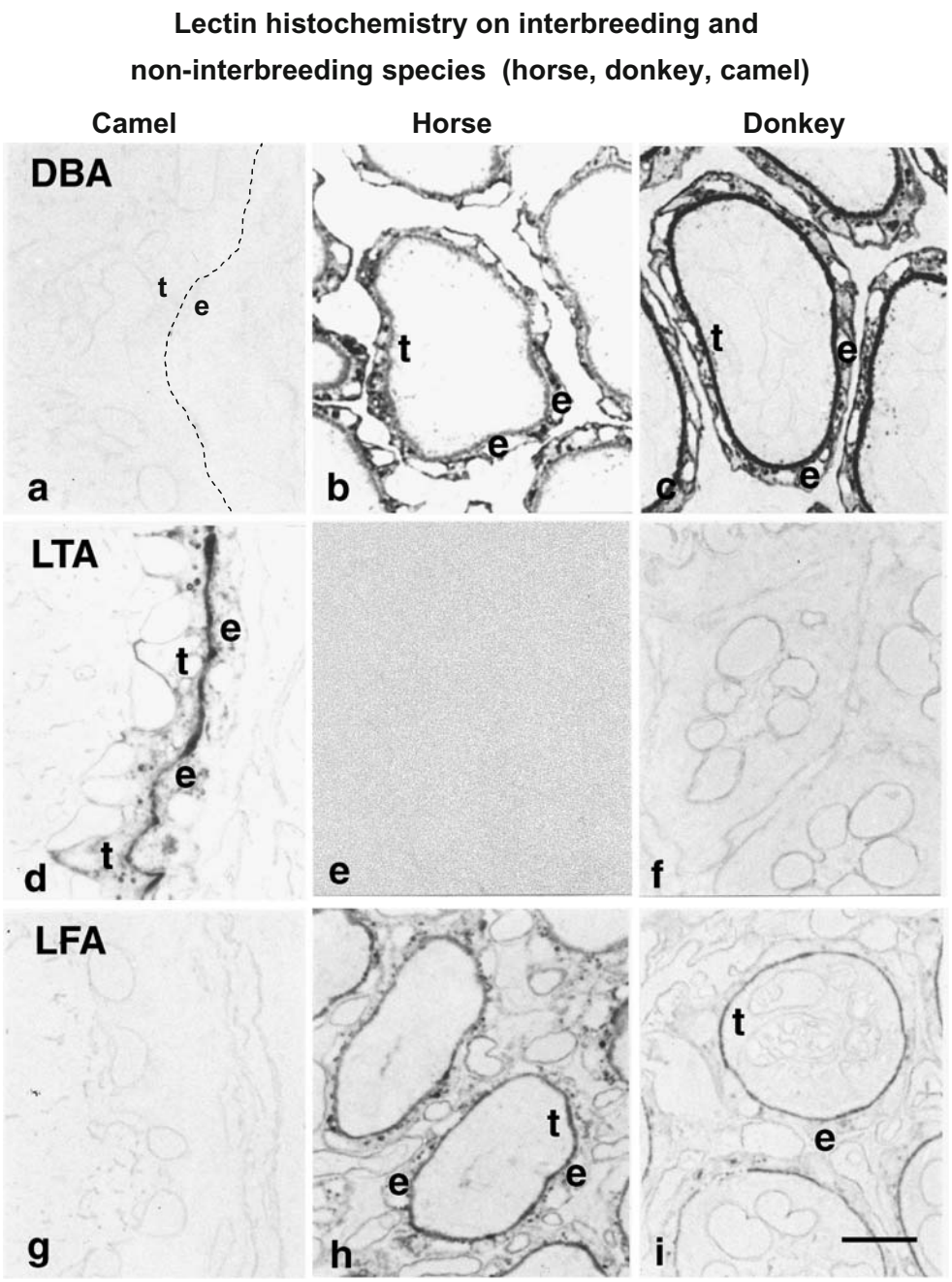

Fig. 3 Comparison of lectin binding at the fetomaternal interface in camel, horse and donkey. **a**–**c** Binding of the lectin from *Dolichos biflorus* to α-linked *N*-acetylgalactosamine residues, often fucosylated, is absent in the camel (**a**) but present in the maternal epithelium of horse (**b**) and donkey (**c**). *Tetragonolobus purpureus* lectin (LTA) binds to fucosyl termini in the trophoblast and epithelium of the camel (**d**), but staining is absent in horse (**e**) and donkey (**f**) tissues. The lectin from the slug *Limax flavus*, which binds to sialyl residues, does not stain the camel (**g**) but binds to maternal epithelium in both horse (**h**) and donkey (**i**). (*t* trophoblast, *e* maternal epithelium). Scale bar: 20 μm. *(Modified from Jones et al., 2000, Journal of Reproduction and Fertility 118: 397,* © Society for Reproduction and Fertility (2000). Reproduced by permission

with a high degree of failure at fertilisation as well as in early and late pregnancy; the fact that the hybrid surviving to term had a trophoblast glycosylation pattern very similar to trophoblast in the maternal species may have contributed to a favourable outcome, allowing stable interaction to occur between the glycans of the trophoblast and the apposing maternal epithelium.

The old world pig and new world peccary diverged about 35 million years ago [23], however, fetomaternal glycosylation is similar [24] apart from slight alterations in fucosyl and sialyl residues on the maternal side and some changes in complex N-glycans. The extent of conservation in glycan expression over a wide evolutionary time span is remarkable, and implies maintenance of glycosyl transferase action. This may be related to a lack of external pressure on the species: pigs and peccaries have similar lifestyles and occupy similar niches in the wild. Conversely, alpaca and camel have come to occupy different ecosystems; here, adaptations necessary to evolve to optimum fitness may have included changes in glycosyl transferases, which in turn may have led to the appearance of different glycotypes. One important factor contributing to glycan alteration during speciation is the binding by pathogens of cell surface glycans, leading to the selection of cells that

Table 2 Lectin staining characteristics of trophoblast from various species with epitheliochorial placentation

Lectin	Horse	Donkey	Pig	Peccary *T. tajacu*	Peccary *T.pecari*	Camel	Llama	Guanaco	Alpaca
PSA	4++	4+++	1++	1-2+	2+++	2+++	3-4++	3+++	3++
ePHA	4+++	4+++	2-3++	1-2+	1-2+	2 diffuse	0	0	0
IPHA	3+++	3++	0	0	0	0	0	0	0
LTA	0	0	0	0	0	2-4++	2-3+++	3+++	2++
UEA-1	0	0	0	0	0	0	2++	4-/+	0
DBA	3+	4-/+	0	0	0	0	3-/+	2+	2-/+
MPA	2+++	3+++	3++	1++	3++	4++++	3+++	3-4+++	3+++
DSA	3+++	3+++	2-3+++	2+++	3+++	2-4++++	2-3++	3++	3+++
STA	4+++	3+++	3+++	2-3+++	3-4++	2++++	3-4+++	3-4++	3+++
LEA	3+++	4+++	2+++	3+++	4++	2++++	4+	4++	4++
HPA	1-2+++	1-2++	3+	3+	4+	3+++/4+	2++	3++	3++
AHA	3+++	2+++	3+++	2++/4+	3+	3-/+	2-3+++	2-3+++	3++
ECA	3+++	2+++	0	3-/+	3+	3-/+	2+	1++	2+
SBA	0	0	2++	1-2++	4++	3+++	3+	2-3++	3+
WFA	4+++	3+++	2++	2+++	2-4++	3+++	2-3++	3+++	3-4++
SNA-1	0	0	0	2++	4+	0	0	0	0
MAA	0	0	0	0	0	0	0	0	2-3+++
LFA	1+	0	0	0	0	0	N/A	N/A	0
WGA	4+++	4+++	2+++	2+++	2+++ /4++	3+++	3++	3-4+++	3-4++

Main differences between pig and peccary

Main differences between camel and llama/guanaco

Main differences between alpaca and llama/guanaco

Related animals are grouped together. Staining intensity from 0 (negative) to 4 (intense) and granule density from −/+ (sparse) to + + + + (abundant). Similarities are evident between closely related species. The most obvious differences are highlighted. Note that since lectins bind families of structurally related oligosaccharides, more subtle species differences may be obscured

N/A Not available

lack receptor and as a result exhibit resistance to infection [25, 26].

Speciation is accompanied by changes in terminal glycosylation

Many of the differences described above involve changes in terminal glycans, particularly fucosyl and sialyl residues, a feature previously noted in systems for which avoidance of pathogen attachment involves alteration in glycan profiles [26]. Fucose plays important roles in several cell recognition processes and is often regulated during ontogeny and cellular differentiation [27–28]. In our histochemical studies, α1-6-fucose is almost ubiquitous in its distribution, whereas fucose in α1-2 linkage is much more selectively expressed. This linkage appears to be of primary importance in separating out different glycotypes. Though not appearing to coincide with an evolutionary change in the repertoire of fucosyl transferase genes encoding enzymes that catalyse the formation of the α1-2 linkage (Fut1, Fut2, Sec1) [29], it may reflect either altered gene expression or alteration in the substrate repertoire with speciation in cells at the fetomaternal interface. Changes in fucosylation are well known in systems that need to produce glycan diversity, for example, dramatic differences have been reported in the fucosylation of glycans on small intestine epithelial cells in mice that lack normal symbionts [30]. The LIF-null mouse fails to down-regulate α1-2-linked fucose in the receptive endometrial epithelium in the pre-implantation phase of pregnancy. In these animals, blastocysts do not attach normally to the maternal epithelium, suggesting that the normal modification of the maternal glycocalyx in preparation for implantation does not occur, resulting in a glycotype mismatch [31].

Sialylation appears to be associated with cell–cell contact or repulsion. Although the mechanisms mediating

close interaction between cells at the fetomaternal interface are unknown, it is interesting to note that, in general, α2,6-linked sialic acid is sparse or absent from trophoblast and uterine epithelial apical surfaces in epitheliochorial placentae, as shown by lack of binding of the lectin from *Sambucus nigra* (SNA-1). In the rat and mouse uterus, loss of this sialic acid moiety has been associated with stromal cell decidualization and the development of close intercellular contacts [32–33], suggesting increased adhesive properties. Conversely, it has been observed that increases in SNA-1 binding are often associated with reduction of cell surface adhesiveness that occurs in malignancy [34–36], while upregulated α2-3-linked sialic acid may lead to increased abundance of selectin ligand and increased adhesion to endothelial cells [37]. In addition to lectin-glycan interactions, cell–cell adhesive interfaces may be stabilised by direct carbohydrate–carbohydrate binding [38–39]. Nuclear magnetic resonance and other studies have provided evidence for carbohydrate-mediated cell–cell interactions between adjacent Lewis^x trisaccharides (Galß1-4[Fucα1-3]GlcNAcß) [40–42]. Similar interactions may occur at the feto-maternal interface to facilitate interdigitation between apposing surfaces.

Hemochorial placentation

In hemochorial placentas, the glycosylated trophoblast surface at the blood–tissue interface must avoid attack by the maternal immune system in the form of circulating lymphocytes. Fas (fatty acid synthase) ligand [43–44] has several N-glycosylation sites [45], is expressed on syncytial apical membranes and is reported to defend the placenta against immune attack. The presence of Siglec-6 at the syncytial surface suggests the possibility that *cis*-interactions with sialylated ligands in the trophoblast may contribute to plasma membrane organisation [46].

There appears to be convergent evolution of glycosylation at this tissue–blood interface [47]. Five widely separated species of mammal with hemochorial placentation (lesser hedgehog tenrec *Echinops telfairi,* spotted hyena *Crocuta crocuta*, nine-banded armadillo *Dasypus novemcinctus,* human *Homo sapiens* and guinea pig *Cavia porcellus*) have marked similarities in glycosylation of the trophoblast apical/microvillous membrane. All species bear abundant bisected bi/tri-antennary complex N-glycan while non-bisected variants are much scarcer. *N*-acetyllactosamine residues are plentiful and all species express NeuAcα2-3 residues. The tenrec, however, which has been geographically isolated from the other species [48], does show some differences, especially in expression of structures recognised by *Dolichos biflorus* agglutinin. This might be related to the presence of cellular trophoblast rather than a syncytium at the fetomaternal interface.

In many hemochorial species there is a second trophoblast population that forms a direct interface with the uterine stroma of pregnancy, known as decidua. Here, major histocompatibility complex (MHC) expression plays a role, in particular human leukocyte antigen G, (HLA-G) which is restricted almost entirely to trophoblast [49]. HLA-G has been shown to be heterogeneous, due to its unusual glycosylation [50] as well as the translation of alternatively spliced mRNAs. These authors postulate that carbohydrate on HLA-G may either interact with maternal immune cells and/or stabilise the molecule. L-selectin on trophoblast may interact with ligand on decidual cells to facilitate trophoblast migration [51].

Decidua has an abundant population of natural killer (NK) cells [52–53] that express several members of the C-type lectin family including CD94/NKG2A, a cell surface component [54]. These receptors have been shown to bind sialyl lewis^x [55]. The interaction between CD94 on maternal cells and HLA-E on trophoblast is thought to contribute to the resistance of invasive trophoblast subsets to lysis and to stimulate the production of angiogenic cytokines by uNK, in turn enhancing the supply of maternal blood to the placenta. N-glycan-dependent binding also occurs between HLA-C, the only polymorphic MHC component found on trophoblast, and Ig family receptors (KIR2DL1) on uNK cells [56]. In the decidua, galectins, glycan-binding proteins that recognize Galβ1-4GlcNAc oligomers on cell surfaces, also play a pivotal role in fetomaternal tolerance, promoting the generation of a tolerogenic dendritic cell phenotype at the fetomaternal interface early in successful pregnancies [57].

Gene-targeted mice may prove informative in experimental tests of questions relating to the glycocode as, for example, an embryo harbouring a null mutation in a glycosyl transferase, and therefore likely to exhibit reduced abundance of the corresponding linkage in trophoblast, may exhibit altered implantation and placentation in a wild-type or heterozygotic mother. Thus it is interesting that mice lacking β1-4-galactosyltransferase (GalT) are smaller at birth than wild-type littermates and exhibit reduced litter sizes [58].

Could a glycocode control the stability of the MF interface by modulating compatibility of interacting surfaces?

Evolutionary forces that promote adaptation to a changing environment appear also to involve adjustments to glycosylation at the fetomaternal interface, in the longer run

leading to speciation. Such alterations must occur in the first place in the zygote. In early pregnancy, the maternal host is, in effect, required to select between genotypically distinct embryos. It is very plausible that selection is, in important respects, dependent on the efficiency of implantation and placental development, which is in turn dependent on cell surface characteristics including glycosylation. If survival of altered genotypes were to give rise to fitter offspring with altered trophectodermal glycosylation, a further selection pressure may in turn act to drive downstream alteration in maternal surface glycotype to produce a complementary glycocode. Complementarity must be maintained throughout gestation, from the earliest attachment phase of the blastocyst [1, 2] through placental development and growth, to term. These speculations are consistent with the observation that glycotypes of related species are more diverse at the fetomaternal interface in trophoblast than in maternal uterine epithelium. In this scenario evolutionary change is dependent on glycan microheterogeneity as well as mutations that produce altered protein sequence.

Concluding remarks

The glycocode shows both evidence of plasticity and a capability of maintaining a stable interface under evolutionary pressures; conversely, the conservation of glycosylation over long evolutionary periods can serve as evidence of a crucial function. Future studies are envisaged in which glycomics is used to investigate evolutionary change in parallel with glycosyltransferase modifications to throw further light on the role of glycosylation at the fetomaternal interface.

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