Reviews of Reproduction (1997) **2**, 84–93

Adhesion molecules in implantation

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At implantation, trophectoderm attaches to the apical uterine luminal epithelial cell surface. Molecular anatomy studies in humans and mice, and data from experimental models have identified several adhesion molecules that could take part in this process: integrins of the αv family, trophinin, CD44, cad-11, the H type I and Lewis y oligosaccharides and heparan sulfate. The endometrial cell surface mucin MUC1 may play a role in both steric inhibition of attachment and selective glycan display. After attachment, interstitial trophoblast invasion occurs requiring a new repertoire of adhesive interactions with maternal extracellular matrix as well as stromal and vascular cell populations. Human anchorage sites contain columns of cytotrophoblasts in which self-attachment gives way progressively to adhesion to extracellular matrix and then interstitial migration. The β1 integrins are important during these later stages of implantation and placentation.

The presence in rats and mice of a maternally directed receptive phase or 'window' for embryo implantation has been known for over 30 years (reviewed in Psychoyos, 1986). In these species the receptive phase is less than 24 h. In women, the window appears to be of approximately 5 days' duration, from day 20 to day 24 of the cycle (Bergh and Navot, 1992); timing from the luteinizing hormone (LH) peak, which precedes ovulation by about 36 h, gives a receptive phase lasting from approximately day LH + 7 to day LH + 11. In women the operational definition (in terms of pregnancy success after embryo replacement) of the beginning of the receptive phase is not as precise as that of the end: that is, embryos replaced before day 20 may implant, while those replaced after day 24 will not.

Implantation success rates in mice are high. There is an absolute requirement for nidatory oestrogen, and anti-progestin treatment blocks implantation. Thus, a cascade of steroidally triggered events leads to the receptive state. Experiments in which the uterine epithelium is removed or reduced suggest that it is this cell compartment that regulates receptivity (Denker, 1990). Correspondingly, hatched blastocysts readily attach and outgrow in an integrin-dependent process on a variety of surfaces containing ligands found in the endometrial stroma of pregnancy. These include fibronectin, collagens, laminins, entactin, vitronectin, thrombospondin and Matrigel (basement membrane-like) matrix (Armant *et al.*, 1986; O'Shea *et al.*, 1990; Stephens *et al.*, 1995; Yelian *et al.*, 1995). In mice, local maternal epithelial retraction and apoptosis follow attachment (Denker, 1990), whereas in humans, evidence from attachment experiments *in vitro* suggests protrusive penetration of trophoblast through the epithelium (Lindenberg *et al.*, 1986).

Increased knowledge of cell adhesion mechanisms has led to investigation of the molecular events underlying the phenomena of attachment and subsequent interstitial penetration of the embryo. One simple hypothesis for the control of attachment is the steroidal induction of one or more adhesion molecules at the luminal epithelial cell surface (Fig. 1). These receptors would then interact with cognate ligands on the outer trophectodermal surface of the hatched blastocyst (Figs 1 and 2). Considerable advances have been made in describing the composition of these two surfaces in humans and mice (Fig. 2). As a result, a second hypothesis has emerged: that the loss of anti-adhesion molecules may facilitate attachment (Fig. 3; Hey *et al.*, 1994; Surveyor *et al.*, 1995). In either case, a complex interaction with the underlying stroma follows. In mice, decidualization, which occurs with substantial remodelling of maternal extracellular matrix, is already evident at the first stage of interstitial interaction with trophoblast. In contrast, human decidualization occurs about 3 days after interstitial penetration.

In humans a considerable proportion of replaced embryos fail. Could the uterus be imposing a barrier to implantation (Fig. 3a)? There is a relatively high proportion of abnormal embryos in humans and implantation could impose a selection process favouring the healthy ones. A reduction in abundance of the cell surface mucin MUC1, which inhibits adhesion, is observed in endometrium from recurrent spontaneous abortion (Serle *et al.*, 1994; Hey *et al.*, 1995). Such data imply that the selection process is deficient, allowing implantation of embryos that are not competent to develop to term. In this scenario, an abnormal maternal environment leads to the survival of embryos in which *intrinsic* abnormalities are predicted. Placental dysfunction, including failure of intra-arterial migration by trophoblast, has indeed been observed in spontaneously aborting conceptuses (Hustin *et al.*, 1990).

Implantation and placentation in mice have become accessible to study via experimental manipulation of the uterine luminal environment as well as gene knockouts. They provide a fascinating insight into processes that are fundamental to life. Embryo attachment to the uterine epithelium is common to many species, but this does not necessarily mean that the molecular mechanisms are shared. In any case, subsequent events leading to placentation are different. Comparative studies in reproductive biology raise many fascinating questions and hypotheses that challenge our ingenuity in developing approaches to the investigation of human placentation.

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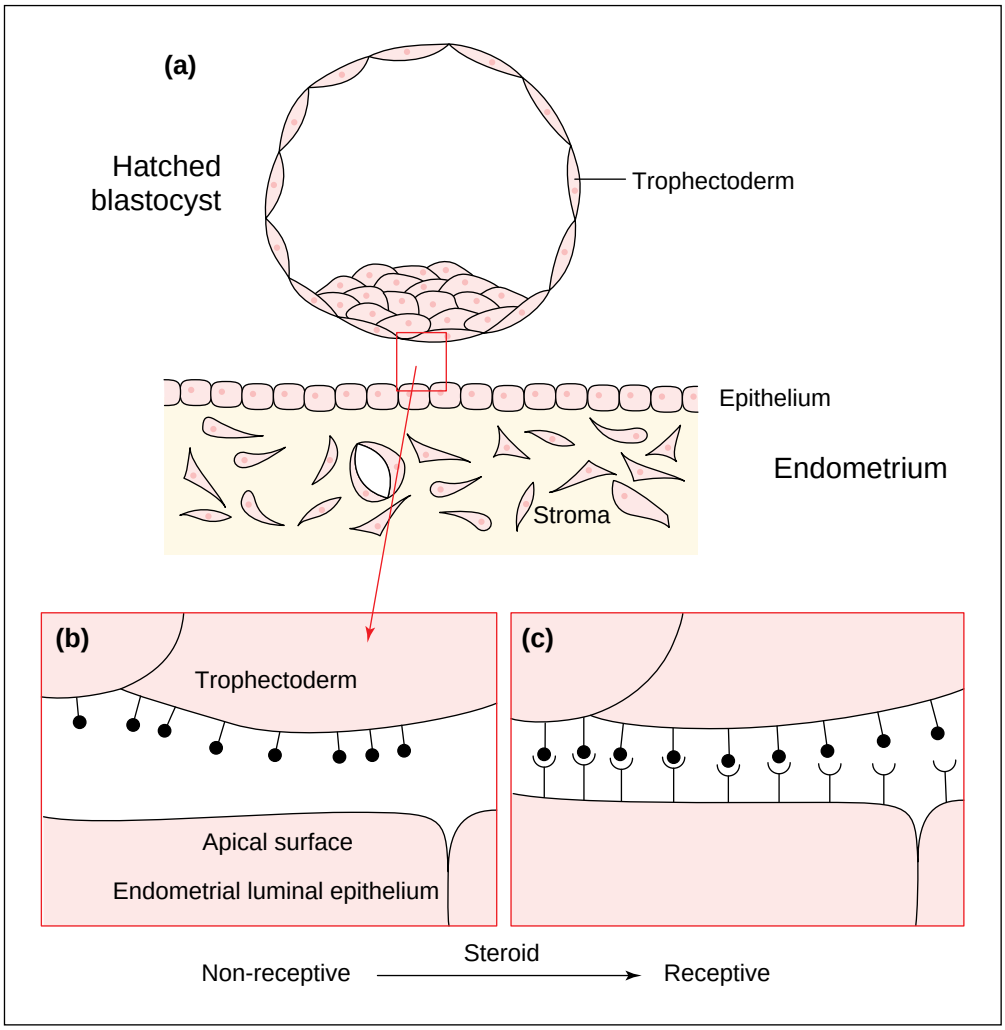

Fig. 1. (a) In humans, the hatched blastocyst becomes apposed to the endometrial luminal epithelium with the inner cell mass oriented proximally. The epithelium is converted by direct or indirect steroidal action from a non-receptive (b) to a receptive state (c). One simple but unproven hypothesis predicts the appearance of an adhesion molecule in the epithelial apical domain to coincide with receptivity. Attachment follows (c).

Embryonic integrins and peri-implantation events in mice

There is continuous expression of integrin subunits α5, α6B, αv, β1 and α3 during development after fertilization in mice (Table 1; Sutherland *et al.*, 1993). Subunit α3 appears from the eight-cell stage, while α2, α6A and α7 all appear at the late blastocyst stage. Localization has been reported of integrins α1, α3, α5, αIIb, βv, β1 and β3 in trophoblast outgrowths *in vitro* (Sutherland *et al.*, 1993; Yelian *et al.*, 1995). Both αv and β1 integrins can be found in focal adhesions at the spreading margins of trophoblast. Thus the mouse blastocyst probably expresses integrins α1β1, α2β1, α5β1, α6β1, α7β1, αIIbβ3 and αvβ3.

Function-blocking experiments have been carried out in which anti-integrin antibodies have been used to inhibit mouse trophoblast outgrowth on substrates of defined composition. Outgrowth on fibronectin appears to utilize integrin α5β1 or αvβ3 or both integrins. Outgrowth on laminin requires an integrin of the β1 family, perhaps α7β1 (Sutherland *et al.*, 1993; Stephens *et al.*, 1995; Yelian *et al.*, 1995; Table 1).

Schultz and Armant (1995) observed that the binding of fibronectin-coated spheres to hatched mouse blastocysts is confined to the abembryonic pole, that is, the site of initial attachment to the uterus. Furthermore, the expression of fibronectin-binding activity depends on prior exposure of the embryo to ligand in solution or on a surface. This finding suggests that blastocysts need to be activated *in situ* to implant (Paria *et al.*, 1993).

Further information on the role of integrins in implantation in mice has come from gene knockout studies. Expression of several integrins has been inactivated by homologous recombination. The most marked is knockout of the β1 subunit where −/− embryos develop normally to the blastocyst stage but fail to implant (Fässler and Meyer, 1995; Stephens *et al.*, 1995). Careful examination of the implantation sites suggests that the block

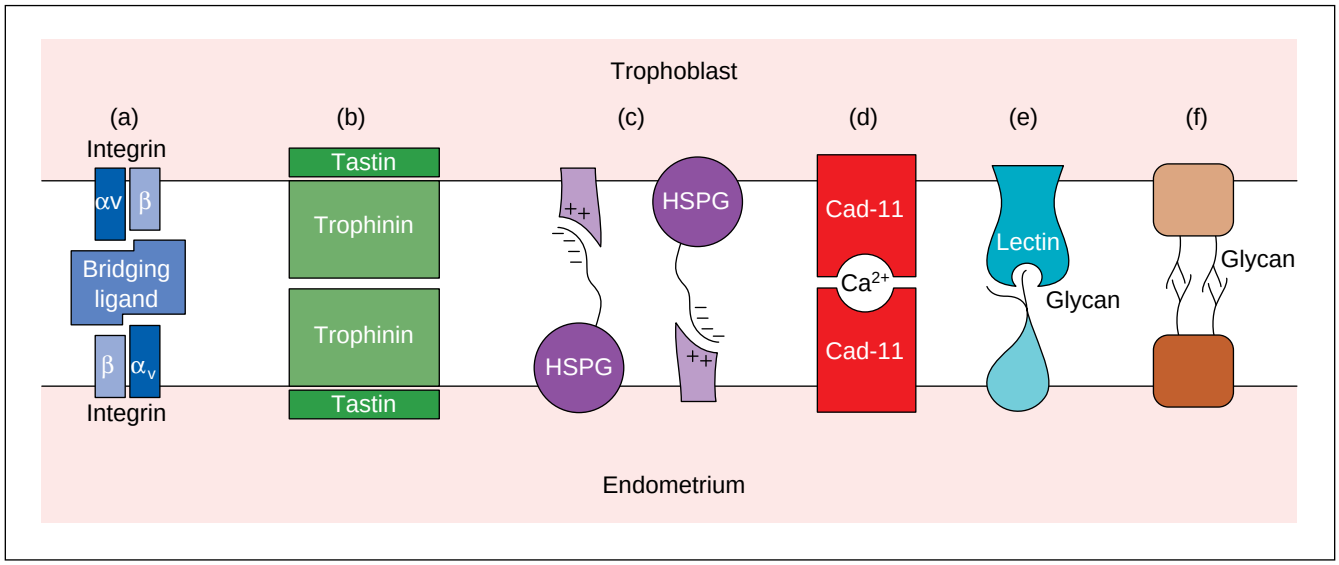

Fig. 2. Possible interactions between trophectoderm and endometrial cells at implantation. Data are from molecular anatomical studies in humans and mice as well as implantation models *in vivo* and *in vitro*. (a) αv Integrin-mediated adhesion via a bifunctional extracellular bridging ligand. (b) Homotypic adhesion mediated by trophinin–trophinin binding with a requirement for cytoplasmic tastin. (c) Heparan sulfate chains of a cell surface proteoglycan (HSPG) interacting with a basic protein at the apposing cell surface. There is evidence that this interaction could occur in either or both directions. A similar type of interaction is possible between CD44 (on either maternal or embryonic surfaces) and chondroitin sulfate-bearing proteoglycans. (d) Homotypic binding mediated by the cadherin, Cad-11. Cad-11 is on the first trimester trophoblast but as yet there are no data on its expression in the blastocyst. (e) Binding of endometrial glycoprotein glycan by an embryonic lectin on the basis of the finding that the mouse endometrial H type I glycan is recognized by a blastocyst surface glycoprotein. (f) Glycan–glycan binding based on data showing interaction between Lewis y and H type glycans.

may occur at the stage at which trophoblast has passed beyond the epithelial barrier and is beginning its interaction with the underlying endometrial stroma. This indicates that interaction between trophoblast β1 integrins and maternal extracellular matrix ligands may be important for placentation. Integrin ligands, including laminin and fibronectin, are abundant in decidua (Aplin, 1989; Church *et al.*, 1996). The –/– embryos outgrow on fibronectin but not on laminin. Thus, β1 integrins are essential in the latter case, but trophectoderm can use integrins of the αv family to spread on fibronectin. However, the αv integrins clearly cannot compensate for the absence of all β1 integrins *in vivo*. Tenascin, which exhibits adhesion inhibiting activities, appears in a restricted region of the subepithelial stroma at the time of implantation in mice, and Julian *et al.* (1994) have suggested that it disrupts interaction of the maternal epithelium with basal lamina.

It should be noted that the β1 null embryos also exhibit significant deficiencies in morphogenesis of the inner cell mass (ICM). Since the presence of normal trophoblast is required for normal ICM development, caution must be exercised in deciding on the factors leading to embryonic failure in β1 null mice.

Further integrin knockout studies have been carried out or are in progress (Hynes, 1996). Several α chains of the β1 family have been inactivated. No implantation-related phenotypes have been observed in embryos lacking α4 (Yang *et al.*, 1995), α5 (Yang *et al.*, 1993), α6 or α9. None of these mice develops to adulthood, so a contribution to implantation on the maternal side cannot be excluded. Implantation deficiencies have not been observed in embryos lacking α1, α3, α7 or αv (Hynes, 1996). Similarly, in the αv family, β5- and β6-null embryos have no implantation-related deficiency; they develop into fertile adults. The α4-null embryo exhibits a failure of placentation owing to the inability of the allantois to fuse with the chorion, a process that appears to require binding of α4β1 to its cognate ligand VCAM-1 (vascular cell adhesion molecule 1; Kwee *et al.*, 1995). This has no direct relevance to the human placenta, in which chorionic mesenchyme has a different origin. The α5-null mouse exhibits generalized mesodermal abnormalities leading to death at about day 10 of gestation (Yang *et al.*, 1993). At present it is not clear whether integrins of the β1 family, some of which can bind to multiple ligands, can compensate or substitute for one another, or whether the β1-null phenotype arises from the loss of a specific integrin the α subunit of which has not yet been identified.

Integrins in human preimplantation embryos

Much less information is available about the human blastocyst. Human oocytes and early postfertilization embryos express integrin subunits α3, αv, β1, β3, β4 and β5 (Campbell *et al.*, 1995a). No evidence has yet been adduced for the appearance of new integrins at the blastocyst stage. Immune co-precipitation experiments are required to establish subunit association patterns. These cannot be performed with adequate sensitivity on the small numbers of available human embryos, so αβ pairings have to be stated speculatively. Available data suggest that the human blastocyst expresses integrins α3β1, α6β4, αvβ3 and αvβ5 (Table 1).

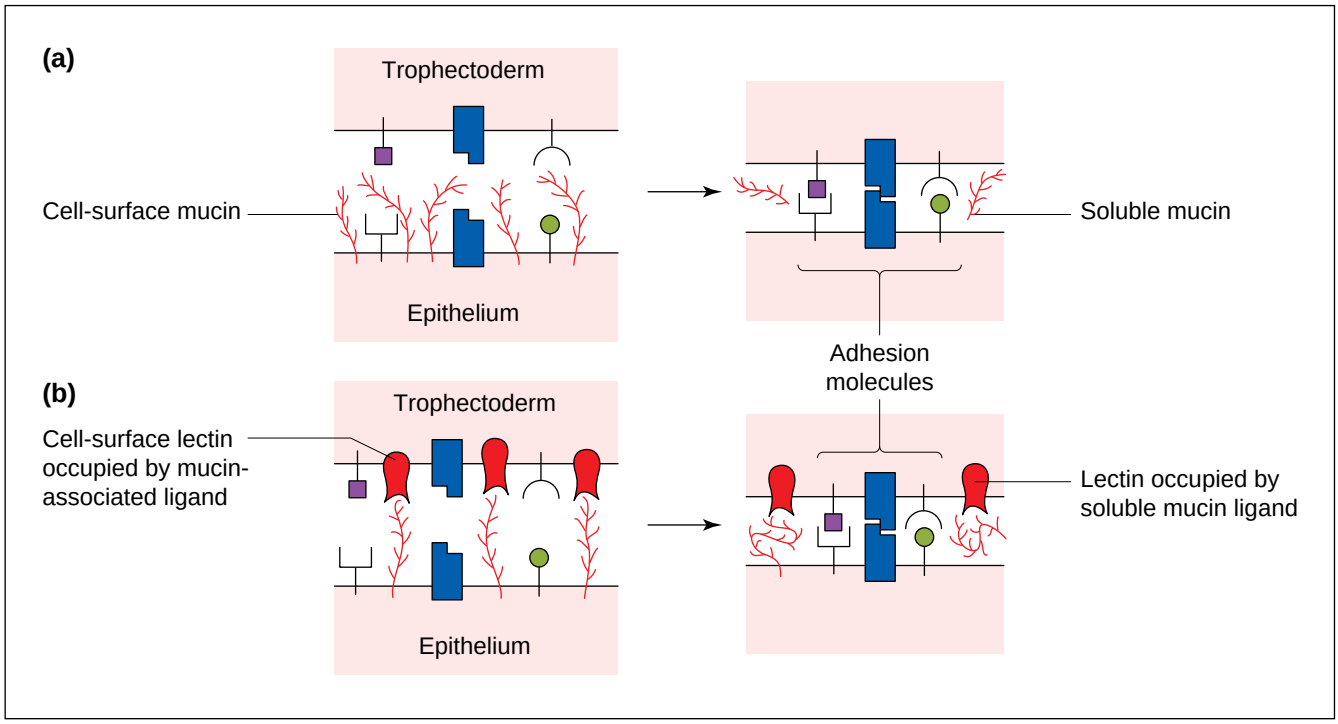

Fig. 3. (a) Possible role of endometrial epithelial surface mucins such as MUC1 in steric inhibition of embryo–endometrial interaction. Attachment occurs as a result of the local reduction in mucin density at the cell surface, which allows access of the trophectoderm to constitutively expressed receptors. Soluble MUC1 may appear in the implantation phase as a result of proteolytic cleavage from the cell surface or secretion of an alternatively spliced form. (b) Initial binding of trophectoderm to glycan structures on endometrial mucin, followed by further interactions mediated by other adhesion systems. Note that (a) and (b) are not necessarily mutually exclusive; a diversity of endometrial mucin glycoforms as well as different mucin densities at the cell surface could allow local variations in receptivity.

Endometrial integrins

Several integrins have been observed in human endometrial glandular epithelium (Table 2; Tabibzadeh, 1992; Albers *et al.*, 1995; Breuss *et al.*, 1995; Aplin *et al.*, 1996; Lessey *et al.*, 1996a). Subunits α2, α3, α6, β1, β4 and β5 are expressed constitutively. Subunits α1, α9, αv, β3 and β6 exhibit regulated epithelial expression in different patterns: αv expression increases after ovulation; α1 increases after ovulation, and then decreases in the late secretory phase; α9 appears after ovulation in the glandular epithelium; β3 appears on day 19 (LH + 6) in the glands; and β6 appears in the secretory phase functionalis, but details of the exact timing are not available.

Subunits α2, α3, α6, α9, β1, β4, β5 and β6 are also present in the luminal epithelium (Tabibzadeh, 1992; Albers *et al.*, 1995; Breuss *et al.*, 1995; Aplin *et al.*, 1996; Lessey *et al.*, 1996a). As in the glands, α1 is upregulated in the secretory phase (Tabibzadeh, 1992). α9 is expressed constitutively in the luminal epithelium in contrast to its behaviour in the glands (Lessey *et al.*, 1996a). β5 has a striking apical distribution (Aplin *et al.*, 1996). There are conflicting reports on the expression of β3 in the luminal epithelium (Albers *et al.*, 1995; Lessey *et al.*, 1996a); however, there is agreement that it is absent in the proliferative and early secretory phase.

These subunit localization data suggest that integrins α2β1, α3β1, α9β1, α6β4, αvβ3, αvβ5 and αvβ6 are expressed by the epithelium. α6β4 is confined to the basal cell surface, while α2β1 and α3β1 appear to be laterally disposed. αvβ5 is expressed in luminal epithelium and has a pronounced apical distribution. However, it is not obviously regulated. αvβ3 and probably αvβ6 are upregulated in the receptive phase. In addition to being expressed in humans, both αvβ3 and αvβ5 are present in mouse endometrial epithelium (Aplin *et al.*, 1996). No information about the role of the β1 integrins in endometrial function can be obtained from the knockout experiment because the mice do not reach adulthood (see above).

Integrins: a role in implantation?

The most likely maternal integrins to be involved in attachment are therefore those of the αv family (Fig. 1). There is evidence that epithelial β3 expression is reduced in infertile women (Lessey *et al.*, 1996b). These integrins share the ability to bind RGD sequences in extracellular ligands including fibronectin, osteopontin, vitronectin and others (Table 2). Injection of RGD peptides into the mouse uterine cavity reduces the rate of implantation (B.A.Lessey, personal communication).

An attachment mechanism involving the αv integrins may require a bifunctional bridging ligand to span between receptors on the embryonic and maternal cell surfaces (Fig. 1). A bridging component may in principle derive from either maternal or embryonic cells. Of the possible ligands, osteopontin is a secretory component of endometrial epithelial cells and localizes to the apical surface region in secretory phase tissue

Table 1. Integrins present in the preimplantation embryo and their ligands

	Mouse embryo	Human embryo	Ligands
α1β1	+		LN, COL, PE
α2β1	+		COL
α3β1		+	FN, COL, LN
α5β1	+		FN
α6β1	+		LN
α6β4		+?	LN
α7β1	+		LN
αvβ3	+	+	FN,VN,OS,vWF, FIB, BSP1, PE, PECAM-1(CD31)
αvβ5		+	FN,VN,OS
αIIbβ3	+		FN, FIB, vWF VN

Ligands: BSP1, bone sialoprotein 1; COL, collagens; FIB, fibrinogen; FN, fibronectin; LN, laminins; OS, osteopontin; PE, perlecan (heparan sulfate proteoglycan); PECAM-1, platelet endothelial cell adhesion molecule; TN, tenascin C; VN, vitronectin; vWF, von Willebrand factor.
See text for discussion of ligands available at the implantation site.

Table 2. Integrins present in human endometrial luminal epithelium (LE), glandular epithelium (GE) or first trimester trophoblast (e, extravillous; v, villous) and their ligands

	LE	GE	Trophoblast	Ligands
α1α1	+r	+r	e	LN, COL, PE
α2β1	+	+		COL
α3β1	+	+	(ev)	FN, COL, LN
α4β1		+r		FN, VCAM
α5β1			e	FN
α6β1	?	?	?	LN
α9β1	+	+r		TN
α6β4	+	+	v	LN
αvβ1	?	?	?	FN,VN
αvβ3	+r	+r	e	FN,VN,OS,vWF, FIB, BSP1, PE, PECAM-1(CD31)
αvβ5	+	+		FN,VN,OS
αvβ6	+r	+r		FN, TN

r, regulated expression during the menstrual cycle. Brackets indicate that α3β1 is expressed in second and third trimester trophoblast only.
Ligands: BSP1, bone sialoprotein 1; COL, collagens; FIB, fibrinogen; FN, fibronectin; LN, laminins; OS, osteopontin; PE, perlecan (heparan sulfate proteoglycan); PECAM-1, platelet endothelial cell adhesion molecule; TN, tenascin C; VCAM, vascular cell adhesion molecule; VN, vitronectin; vWF, von Willebrand factor.
'?' refers to integrins for which the constituent subunits are present but the specific association not demonstrated.

(C. Coutifaris, personal communication). Fibronectin has been described in association with the zona pellucida of human embryos (Turpeenniemi-Hujanen *et al.*, 1995). The heparan sulfate proteoglycan perlecan is present on the outer surface of mouse blastocysts (Carson *et al.*, 1993), and its core protein can act as a ligand for integrin αvβ3. Perlecan heparan sulfate chains may interact with maternal cell surface components (see below). Laminin is found in the same location (Dziadek and Timpl, 1985; Carson *et al.*, 1993). Thrombospondin is a ligand for αvβ3 and is expressed by trophectoderm as well as by glandular epithelium and decidua (O'Shea *et al.*, 1990; Corless *et al.*, 1992). Vitronectin appears not to be essential for implantation, since vitronectin-null adult females display normal fertility (Zheng *et al.*, 1995).

One caveat to these speculative models is that the embryo may create a specialized microenvironment at the implantation site where, for example, maternal epithelial depolarization (with diffusion of laterally displayed surface components into the apical surface domain) or enzymatic modification of apical surface components (blastocyst proteases or glycosidases) may occur (Denker, 1990). There is evidence that cultured epithelial monolayers acquire adhesivity for trophoblast as they become less polarised (Thie *et al.*, 1995). If there are specific and highly localized signalling events or structural modifications at the implantation site, conclusions drawn from studies of endometrium in non-conception cycles or cultured blastocysts may be misleading.

Integrin expression in human placentation

As cells develop from polarized stem cytotrophoblasts attached to the villous basement membrane into columns and then to interstitially migrating cytotrophoblasts, radical alterations occur in cell–cell and cell–matrix interactions (Fig. 4a). Some of the molecules involved are well recognized, while understanding of the underlying regulatory mechanisms is lacking (Aplin, 1991, 1996; Vićovac *et al.*, 1995). The villous cytotrophoblast layer expresses the α6 and β4 integrin subunits (Fig. 4b; Korhonen *et al.*, 1991; Damsky *et al.*, 1992; Aplin, 1993). Cytotrophoblast cells that leave the villous basement membrane to form cell columns also undergo a striking downregulation of α6 and β4 integrins accompanied by an upregulation of α5 and β1 integrin subunits and, in more distal regions of the columns, of α1. This presumably gives rise to the heterodimers, α5β1 and α1β1, which act as receptors for fibronectin and laminins/collagens, respectively (Table 2; Fig. 4b). An alternative laminin receptor, integrin α6β1, may be present and integrins of the αv subfamily are also seen in the extravillous lineage (T.D. Burrows and B.A. Lessey, personal communication). It is important to note that cells can control integrin-mediated adhesion not only by the amount expressed at the cell surface, but also by modulation between high and low affinity states (Mould *et al.*, 1995).

Cultured first trimester cytotrophoblasts can be inhibited from migrating across a Matrigel barrier by the addition of fibronectin, while antibodies to integrin α5 or β1 stimulate migration (Damsky *et al.*, 1994). The data suggest an anchoring role for the fibronectin–α5β1 interaction. Antibodies to laminin or collagen IV or to integrin α1 inhibit cells from crossing the barrier, suggesting a role in migration for the α1β1–laminin or –collagen IV interaction. The insulin-like growth factor binding protein 1 (IGFBP-1), which is a secretory product of decidual cells, contains an RGD sequence motif that binds α5β1 and modulates trophoblast–fibronectin interaction, resulting in increased migratory rates *in vitro* (Irving and Lala, 1995). In the light of the above observations, it is particularly interesting that integrin β1-deficient mouse embryos penetrate the uterine epithelium at implantation but fail to progress further in placental development (Fässler and Meyer, 1995; Stephens *et al.*, 1995).

In pre-eclampsia, intravascular cytotrophoblast invasion is abnormally shallow with increased proliferation and decreased

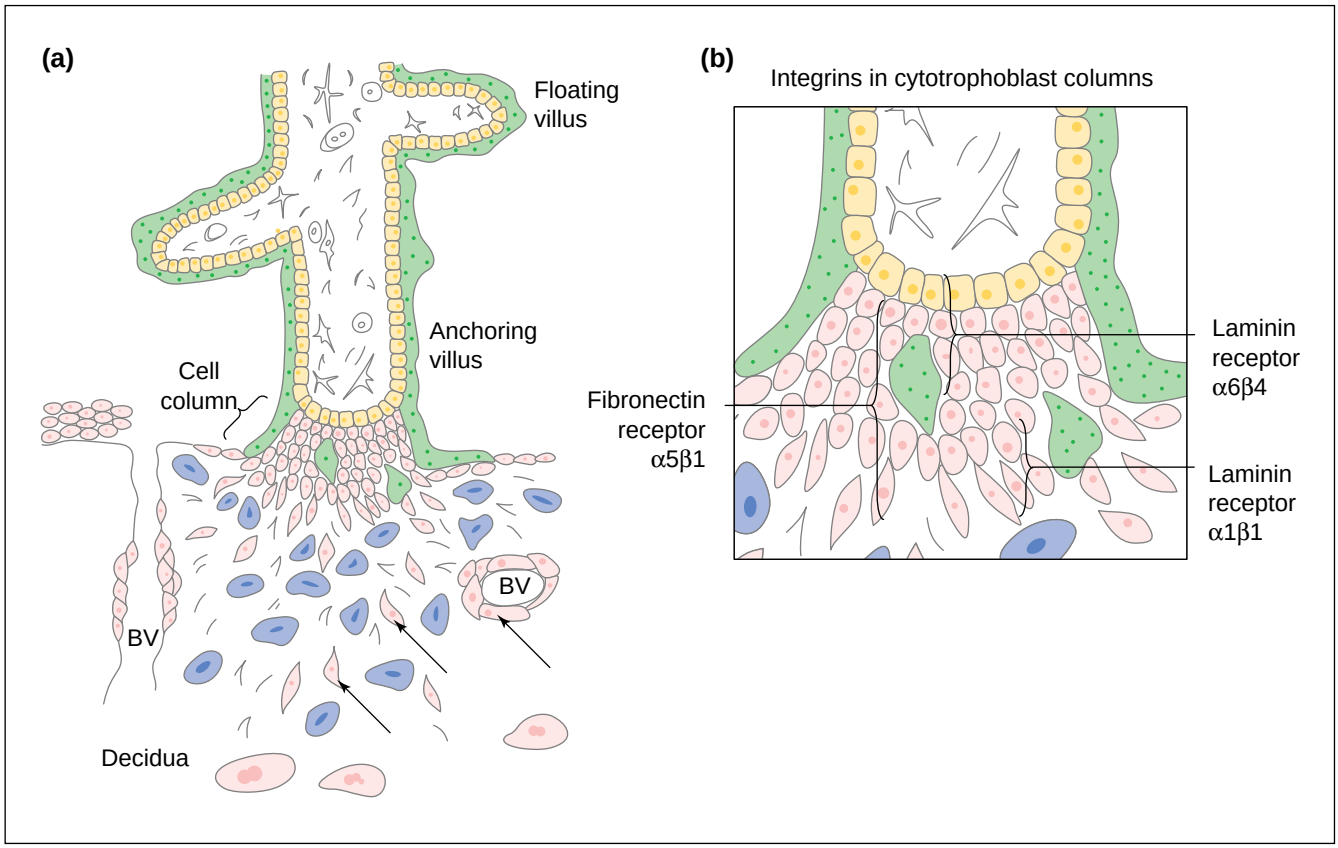

Fig. 4. (a) Anchoring villus at the periphery of a first trimester placenta. Cytotrophoblast stem cells (yellow) give rise either to the syncytiotrophoblast layer (green) that covers floating (transporting) villi or, at sites of anchorage to maternal decidua (stromal cells shown in blue), to columns of extravillous cytotrophoblast (red). From the distal columns, cytotrophoblast infiltrates the decidual interstitium and penetrates maternal arteries. Eventually the migratory cytotrophoblast undergo terminal differentiation into placental bed giant cells. (b) Detail from (a) showing some of the alterations in integrin expression that occur during the development of cytotrophoblast columns. In addition to being a receptor for laminin, integrin α1β1 binds collagen type IV. BV, blood vessel. Modified from Vićovac and Aplin (1996).

differentiation of extravillous cytotrophoblast, and the transformation of maternal arteries is incomplete (Pijnenborg, 1994). The downregulation of integrin α6β4 and upregulation of α1β1 may fail in placental bed cytotrophoblast in this condition (Zhou *et al.*, 1993), but data have not been confirmed independently (Divers *et al.*, 1995) and remain controversial. In efforts to model the pre-eclamptic placenta, first trimester cytotrophoblasts have been cultured in hypoxic conditions in which they show increased proliferation, reduced invasion of Matrigel-coated filters and a less pronounced induction of integrin α1 expression (Genbacev *et al.*, 1996).

Extracellular matrix

Trophoblasts at the villus attach via their basal surface to a basement membrane that contains laminins, collagen IV and heparan sulfate proteoglycan. As the cells proliferate at anchorage sites to form columns, they become depolarized, and an unusual intercellular matrix appears. This contains no fibrillar collagen, as assayed by electron microscopy (Enders, 1968), but is rich in basement membrane components including collagen IV, heparan sulfate proteoglycan and laminin (Damsky *et al.*, 1992; Church *et al.*, in press). There are also rich deposits of fibronectin, which carries a characteristic oncofetal glycopeptide epitope (Feinberg *et al.*, 1991).

Once the cells leave the columns and enter the maternal interstitial environment, less is known about their production of extracellular matrix. At sites where columns disperse to form migratory cells, the trophoblastic extracellular matrix is intimately associated with the extracellular matrix of the maternal decidua, and with another extracellular deposit known as 'fibrinoid'. This is ultrastructurally distinct from fibrin (Frank *et al.*, 1994). It seems possible that, in these specialized locations, fibrinoid acts instead of interstitial-type collagens as a structural matrix upon which components are displayed for the purpose of cellular interactions. The intra-arterially migrating cytotrophoblast effects extensive degradation of the musculoelastic matrix of the vessel walls which is also replaced by fibrinoid. This allows a greatly increased flow of blood to the intervillous space and also renders the blood supply to the placenta independent of vasoconstrictors. Intriguingly, pregnancy in fibrinogen Aα-deficient mice results in fatal uterine bleeding at about day 10 of gestation (Suh *et al.*, 1995). Intravascular trophoblasts express a highly sialylated form of neural cell

adhesion molecule (NCAM) which may act to stabilize their self-association (Burrows *et al.*, 1994). Cytotrophoblastic plugging of superficial maternal arteries in early pregnancy followed by this vascular conversion, effected by a combination of intra-arterially and interstitially migrating cells, is likely to be the major function of the extravillous trophoblast population.

Decidual interstitial matrix contains collagens I, III, V and fibronectin, while individual decidual cells elaborate a pericellular basal lamina of collagen type IV, laminins 2 and 4 and heparan sulfate proteoglycan (Aplin, 1989; Church *et al.*, 1996).

Cadherins, trophinin–tastin, CD44

A novel adhesion complex including a cell surface-associated glycoprotein – trophinin – and a cytoplasmic protein – tastin – has been identified by expression cloning of cDNAs in a model in which trophoblastic teratocarcinoma cells attach to endometrial carcinoma monolayers (Fukuda *et al.*, 1995). This appears to occur via a homotypic interaction (Fig. 1). Trophinin is absent from human proliferative phase tissue but is present in secretory endometrial epithelium and also in macaque implantation sites in both trophoblast and endometrium. It is cell surface-associated, but also appears in endometrial glandular secretions. It contains 69 tandem decapeptide repeats rich in serine and threonine, accounting for more than 90% of the protein, and therefore has mucin-like properties.

CD44 is present up to and including the blastocyst stage of human preimplantation development; in contrast, it is absent from first trimester trophoblast (Campbell *et al.*, 1995b). This suggests an involvement in peri-implantation interactions. CD44 can recognize polyanionic glycans including hyaluronan and chondroitin sulfate (Toyama-Sorimachi *et al.*, 1995); sulfated and sialylated oligosaccharides are abundant on the endometrial apical epithelium (Hoadley *et al.*, 1990; Graham *et al.*, 1994; Hey and Aplin, 1996), and could act as ligands. CD44 is expressed in endometrial epithelium and in stromal cells both during the cycle and in pregnancy. The epithelium expresses larger variant CD44 isoforms that arise through alternative splicing (Behzad *et al.*, 1994). CD44 also binds osteopontin (Weber *et al.*, 1996), which could therefore bridge from integrins of the αv family, which recognize its RGD site.

MacCalman *et al*. (1996) have carried out a PCR homology screen for members of the cadherin (cad) family, which mediate calcium-dependent homotypic intercellular adhesion, in term human placenta. Two major trophoblast-associated products were detected: E-cad and cad-11. E-cad is in villous cytotrophoblast from which it disappears after fusion both *in vivo* and *in vitro* (Coutifaris *et al.*, 1991), while cad-11 exhibits the opposite pattern of behaviour, appearing during differentiation either into syncytial cells or extravillous cytotrophoblast. E-cad seems to play an important role in early (mouse) development since transgenic mice lacking the gene fail to form trophectoderm and do not implant (Larue *et al.*, 1994). E-cad and cad-11 also show opposite patterns of behaviour in the extravillous differentiation pathway: E-cad is present only at the base of first trimester cytotrophoblast columns, while cad-11 appears in the medial and distal columns (MacCalman *et al.*, 1996). Cad-11 may be important in anchorage, especially since it is present in endometrial epithelium (Fig.1) and is upregulated during decidualization in stromal cells (MacCalman *et al.*, 1996). In contrast, P-cadherin is absent from human trophoblast and decidua, but is expressed in these cells in mice (Kadokawa *et al.*, 1989). E-cad and P-cad are found in endometrial surface and glandular epithelium, where expression appears to be independent of the stage of the menstrual cycle (Tabibzadeh *et al.*, 1995). The melanoma cell adhesion molecule, Mel-CAM, is expressed by extravillous cytotrophoblast within the columns as well as by the infiltrating cell populations of the placental bed (Shih and Kurman, 1996).

Other adhesion molecules that have been studied in human embryos include ICAM-1, NCAM and VCAM-1, all of which are present at early preimplantation stages of development but have not yet been identified in blastocysts (Campbell *et al.*, 1995a).

Carbohydrate-mediated interaction

The blood group H type I structure (Fucα1-2Galβ1-3GlcNAcβ1-Gal) is expressed on mouse endometrial epithelium at the time of implantation; there is a receptor for this ligand on polar trophectoderm; and the oligosaccharide partially inhibits embryo attachment to cultured epithelial monolayers (Lindenberg *et al.*, 1988).

Zhu *et al.* (1995) showed that the Lewis y antigen (Fucα1-2Galβ1-4[Fucα1-3]GlcNAc) is present at the surface of mouse blastocysts and endometrial epithelium, and that anti-Lewis y antibodies can inhibit implantation if introduced into the uterine lumen shortly before attachment. Lewis y binds to the H type I and H type II oligosaccharides, suggesting a carbohydrate–carbohydrate interaction at attachment (Fig. 1).

Mammalian lectins are candidates for the mediation of cell–cell interactions involving a carbohydrate ligand. The L14 lectin (also known as galectin 1) binds β-galactosides and is present on trophectoderm of the expanded mouse blastocyst, but L14-null embryos implant normally (Poirier and Robertson, 1993).

Heparan sulfate is present at the surface of attaching mouse blastocysts *in vivo* (Carson *et al.*, 1993), and can mediate embryo attachment to surfaces containing platelet factor 4, which binds to it (Farach *et al.*, 1987). The mouse uterine epithelium also bears apical heparan sulfate (Tang *et al.*, 1987). Human choriocarcinoma (JAr) cells attach to monolayers of the endometrial carcinoma cell line RL95 by means of a heparan sulfate-dependent mechanism, and binding is mediated by a basic protein of 24 kDa present in both cell types (Rohde *et al.*, 1996). Thus, the possibility arises of a two-way heterotypic interaction (Fig. 1). A further interaction involving heparin-like glycans has been demonstrated by Raab *et al*. (1996) who showed that a cell line bearing the membrane-anchored form of heparin-binding epidermal growth factor (HB-EGF), which is a ligand for the EGF receptor (EGFR), adheres to the surface of hatched mouse blastocysts. The interaction is abolished by pretreating the embryos with heparitinase. HB-EGF is expressed at the uterine epithelial surface at the time of implantation.

MUC1 and anti-adhesion effects

As it approaches the epithelial surface, the attaching embryo encounters the glycocalyx (Fig. 2; Hoadley *et al.*, 1990; Aplin and Hey, 1995; Aplin, 1996). One component of this layer is the cell surface-associated mucin, MUC1 (reviewed in Aplin and

Hey, 1995). MUC1 is particularly abundant on the microvilli and cilia that extend from the apical cell surface of endometrial epithelial cells. MUC1 is a type 1 intercalated plasma membrane molecule with a large extracellular domain and a short cytoplasmic sequence. The extracellular domain contains a variable number tandem repeat (VNTR) sequence of 20 amino acids, including three serine and two threonine residues and is highly *O*-glycosylated. The number of repeats varies from about 20 to 80 in the normal population, and individuals carry two codominantly expressed alleles. As a result, the core protein varies in the range 120–220 kDa; with glycosylation this can rise to over 400 kDa. Variant forms of MUC1 arise by alternative splicing of mRNA. These include a secretory variant (MUC1/S) that lacks the cytoplasmic domain (Fig. 2).

MUC1 is expressed in endometrium both in the proliferative and secretory phases of the cycle (Hey *et al.*, 1994, 1995). However, there is increased abundance in the secretory phase in both glandular and luminal epithelium, at the cell surface and in secretions. Uterine flushings from normal fertile women show a striking increase in concentration from day 7 after the LH peak (Hey *et al.*, 1995). High concentrations of MUC1 persist in the flushings until day 13 after the LH peak. MUC1 contains a variety of glycans including sialyl Tn, sialyl Lewis x, sulfated and sialylated lactosaminoglycans (Hoadley *et al.*, 1990; Graham *et al.*, 1994; Hey and Aplin, 1996) and these are regulated with increased expression in secretory phase epithelium and secretions.

The function of the secreted form of MUC1 is unknown. Along with other mucins it may play a role in forming a protective barrier (for instance to prevent infection) in the upper genital tract; it may also have a role in relation to sperm access. It is presumably a component of the fluid environment of the implanting embryo. At the cell surface, MUC1 is predicted to take on an extended conformation resulting from its long hydrophilic VNTR domain rich in proline, threonine and serine residues and extensive glycosylation. Thus, it probably extends outward from the apical cell surface further than receptors (such as integrins or cadherins) that mediate cell–cell adhesion (Fig. 2a). A high density of cell surface MUC1 can inhibit cell–cell interactions by simple steric hindrance of ligand access to the cell. Therefore, it may inhibit the interaction of the embryo with adhesion molecules present at the maternal apical epithelium at implantation (Fig. 2a), raising the possibility of a uterine barrier to implantation (Aplin, 1996).

Consistent with this, in mouse uterine epithelium, the homologue Muc1 is regulated with reduced expression at the time of implantation (Braga and Gendler, 1993; Surveyor *et al.*, 1995). Thus, Muc1 may play a role in defining the onset of a 'receptive window'; it is not responsible for subsequent loss of receptivity, since it does not reappear in the epithelial compartment after day 5. However, in humans, expression is high 1 week after ovulation, the time implantation would be expected to occur in a conception cycle. This presents a contradiction if MUC1 is indeed inhibitory. However, the balance between cell surface-associated and secreted MUC1 is critical in this respect; considerable heterogeneity is observed in cell-associated immunoreactivity, and it may be that a small area of low expression could define a receptive site. It is also possible that MUC1 carries glycans that are recognized by the embryo (Fig. 2b). Such interactions could be followed by integrin- or cadherin-mediated adhesion in a cascade (Fig. 2b). This role is not necessarily inconsistent with an inhibitory function, since there is clearly considerable microheterogeneity of glycosylation; localized areas on the epithelium could display recognition structures, while other regions are inhibitory. Finally, the avidity of the trophoblast–epithelial interaction in primates could be reduced by the presence of MUC1, thus allowing attachment to be followed by efficient migration of the embryo across the epithelial barrier.

Conclusions

Much remains to be learned of the interactions that regulate implantation. Adhesion systems probably only function in the correct spatio-temporal sequence in the context of other processes such as signalling by growth factors, extracellular matrix deposition and proteolysis. The results of adhesion gene knockouts are intriguing but when the result is a normal implantation phenotype, this cannot be taken as conclusive evidence of non-involvement: a multiplicity of receptor–ligand interactions, organized into a cascade, is a likely scenario. There is one example of an integrin knockout – β1 (Stephens *et al.*, 1995) – in which implantation of homozygous null embryos is blocked, but a whole subfamily of the integrin repertoire is lost. There is good evidence that the β1 integrins are important in the anchorage of human extravillous trophoblasts to extracellular matrix. In knockouts of integrin α4, VCAM1 and fibrinogen, placental abnormalities are evident. Results from studies of animals exhibiting haemochorial placentation provide hypotheses for testing in humans. Here, progress will rely on careful detailing of molecular anatomy coupled with disease studies and *in vitro* modelling.

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