The Role of Integrins in Reproduction (44499)

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Abstract. **Fertilization, implantation, and placentation are dynamic cellular events that require not only synchrony between the maternal environment and the embryo, but also complex cell-to-cell communication. This communication involves integrins, a large family of proteins involved in the attachment, migration, invasion, and control of cellular function. Over the past decade, investigators have learned that integrins participate in multiple reproductive events including fertilization, implantation, and placentation in many species. This review will describe: (i) the expression of integrins on gametes and during the establishment and development of the placenta; (ii) regulatory pathways for controlling expression of integrins in the uterus and developing placenta; (iii) the function of integrins as determined by null-mutations; and (iv) reproductive dysfunction in women related to inappropriate integrin expression in the uterus and/or placenta.** [P.S.E.B.M. 2000, Vol 223:331–343]

Over the past decade, insights into mechanisms underlying cell migration, adherence to extracellular matrix, and cell-to-cell attachment have been greatly expanded with the discovery of the cell surface molecules known as integrins (1). Their role(s) in adhesion, migration, invasion, and a multitude of intracellular effects on organization of the cytoskeleton as well as their ability to respond to intracellular and extracellular signals make integrins attractive potential participants in the complex events of fertilization, implantation, and placentation. Pregnancy results from a series of highly coordinated events that begin with oocyte and spermatozoa interaction (fertilization); continues with apposition, adhesion, and invasion of the embryo into the uterine wall (implantation); and includes the development of the placenta.

In all mammals, fertilization occurs when spermatozoa fuse with the oocyte following penetration of the zona pellucida, resulting in formation of the zygote. Implantation and placentation vary greatly among mammals. Specific types of placentation include epitheliochorial (e.g., pigs and horses), where the trophectoderm of the conceptus attaches to the uterine epithelium, and hemochorial (e.g., rodents and primates), where the trophectoderm invades the maternal tissue and is in direct contact with maternal blood (Fig. 1; Table I) (2). This review will focus on current information regarding integrins and their function during fertilization, implantation, and placental development. Similarities and differences among species will be highlighted.

Structure and Function of Integrins

Integrins comprise a large family of cation-dependent heterodimeric transmembrane receptors composed of noncovalently linked α and β subunits (3). Each subunit has a large N-terminal extracellular domain, a transmembrane domain, and a short C-terminal cytoplasmic domain. The single exception is the β4 subunit, which has a cytoplasmic

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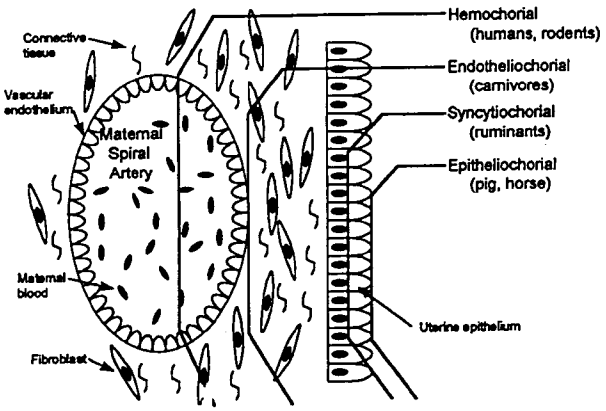

Figure 1. Illustration showing the various modes of implantation and the depth of invasion into maternal tissue. Vertical lines depict the depth of invasion by the trophoblast into the maternal tissue. (Adapted from (2).)

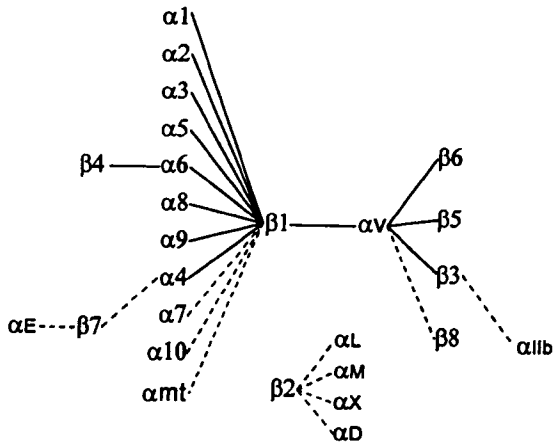

Figure 2. Organization of the integrin family and its potential relevance in reproductive biology. The figure depicts the known integrin subunits and αβ pairings. Connecting lines identify all currently known integrin heterodimers. Solid connecting lines are integrin heterodimers that have been identified in reproductive and early embryonic tissues. Dashed connecting lines indicate integrin heterodimers that have not been identified in reproductive or early embryonic tissues.

domain that is ≈ 45 amino acids longer than the other β subunits. Eighteen α and eight β subunits have been identified, and these subunits form 23 known heterodimers (Fig. 2). The ligand specificity for each heterodimer is determined by the specific combination of the α and β subunits. Most β subunits can associate with more than one α subunit (e.g., the β1 subunit may combine with at least 11 different α subunits). Integrins recognize components of extracellular matrix and cell adhesion coreceptors of the immunoglobulin and cadherin families (3).

The major function of integrins is to mediate cell-to-cell and cell-to-substratum attachment although they may also modulate a number of other cellular functions (3–6). Integrins are intimately associated with the cytoskeleton through the cytoplasmic domain of the β subunit. The complex can bind several cytoskeletal proteins, including α-actinin, paxillin, talin, tensin, and vinculin. These proteins aggregate in discrete assemblies as focal adhesion sites. The focal adhesion site is composed of numerous integrin heterodimers accumulating on the cell surface in response to ligand binding. This accumulation of integrins will recruit the cytoskeletal proteins such as α-actinin, talin, and vinclulin that may act as an anchor for F-actin. The stabilization of the microfilaments will recruit other cytoskeletal proteins such as paxillin and talin (7). In addition to these cytoskeletal proteins, the focal adhesion sites contain signaling complexes involving kinases, such as focal adhesion kinase (FAK), and the integrin-linked kinase (ILK), a serine/threonine protein kinase. Focal adhesion sites can interact with a number of intracellular proteins, including molecules of the MAP kinase pathway, small GTPases (*ras* and *rho*), lipid kinases (PIP 5-kinase and PI3 kinase), and phospholipids (phospholipase C and phospholipase A2), and activation of focal adhesion sites can lead to changes in intracellular pH and Ca^{++} (8).

This network of cytoskeletal and signaling complexes within the focal adhesion site allows for dual control of integrin activity, commonly referred to as inside-out and

Table I. Classification of Implantation and Placental Types in Various Species

Species	Mode of implantation	Depth of invasion[a]	Attachment begins days[b]	Mode of placentation	Type of placenta	Pregnancy duration (days)
Porcine	adhesive, no penetration	central	12–13	epitheliochorial	diffuse	114
Equine	adhesive, no penetration	central	35–40	epitheliochorial	diffuse	340
Ovine	adhesive, no penetration	central	15–18	syndesmochorial	cotyledonary	149
Bovine	adhesive, no penetration	central	28–32	syndesmochorial	cotyledonary	280
Mouse	displacement penetration	eccentric	4	hemochorial	discoid	20
Rat	displacement penetration	eccentric	6	hemochorial	discoid	23
Rabbit	fusion penetration	eccentric	7–8	hemochorial	discoid	32
Cat	intrusion penetration	eccentric	11–12	endotheliochorial	zonary	62
Dog	intrusion penetration	eccentric	14–17	endotheliochorial	zonary	63
Human	??	interstitial	7–9	hemochorial	discoid	266

[a] The extent the conceptus invades into the maternal tissues during implantation: Central–no penetration or erosion of the uterine epithelium; Eccentric–stroma only partially invaded, conceptus is projected into the uterine lumen; Interstitial–conceptus completely invades stroma and surface epithelium becomes restored after implantation.
[b] Day of fertilization is considered gestation Day 0.

outside-in signaling (9). An integrin on the cell surface can respond to changes within the cell by increasing (or decreasing) its affinity for its ligand (inside-out signaling). Alternatively, an integrin binding to its ligand can result in a conformational shift that leads to the activation of signal transduction pathways (outside-in signaling). Both outside-in and inside-out signaling are involved in cell migration, growth, differentiation, inflammation, and cell targeting (5, 9, 10).

Integrins and Reproduction

The term integrin was first coined in the mid-1980s in recognition of the role of these "integral" membrane proteins in connecting the extracellular matrix with the cytoskeleton of the cell (1). Integrins have since been linked to numerous physiological processes including those of interest to reproductive biologists. Integrins are now considered essential for fertilization, embryo implantation, and placental development (11–15).

Integrins and Fertilization. Spermatozoa and oocytes must travel through the female reproductive tract to make contact and ultimately fuse in a process known as fertilization. Both spermatozoa and oocytes express a number of integrins and molecules that contain integrin recognition sites (including extracellular matrix components and fertilin) that may be involved in the binding and fusion of the plasma membranes of the two cell types leading to fertilization. In the following section, the expression of integrins by the spermatozoa and embryo and the potential involvement of integrins during fertilization are discussed.

Spermatozoa. After undergoing the acrosome reaction, spermatozoa release proteases that dissolve the zona pellucida of the oocyte and expose binding sites (or ligands) for the spermatozoa on the zona pellucida (16). Upon penetrating the zona pellucida, the equatorial segment of the spermatozoa is able to bind to the ooplasmic membrane. There is strong evidence that integrins and integrin-like proteins and their ligands are involved in this binding. A prime candidate for gamete binding and fusion is the protein fertilin (PH 30), a member of a family of proteins called ADAMs (characterized by their expression of *a d*isintegrin *a*nd *m*etalloprotease domain) (17), which has been identified in rodents, primates, and humans. Fertilin is a heterodimeric protein composed of α and β subunits. The extracellular domain of the β subunit is characterized by the presence of a "disintegrin" domain that contains the ARG-GLY-ASP (RGD) peptide, a known ligand for many integrin heterodimers (18). Fertilin recognizes an integrin on the surface of the oocyte. Spermatozoa from mice lacking the β subunit of fertilin are deficient in sperm-egg adhesion and sperm-egg fusion (19). Following disintegrin binding and a conformational change in the fertilin heterodimer, the α subunit is exposed, and this mediates fusion of the two membranes (17).

A number of integrins have been found on spermatozoa that may facilitate attachment of spermatozoa to the zona pellucida, migration through the zona, and finally the attachment of the two separate plasma membranes prior to activation of the fertilin protein (18). Integrins have been detected on spermatozoa from all animals studied to date, but human spermatozoa are the best characterized. During capacitation of the spermatozoa, the α5β1 integrin (the classic fibronectin receptor) is upregulated, and following the acrosome reaction, αvβ3 (the vitronectin receptor) is unregulated (20). Coincidental with capacitation, spermatozoa express fibronectin. The importance of this expression is illustrated in the finding that following incubation with antifibronectin antibodies, sperm-egg adhesion and penetration of hamster oocytes by human spermatozoa are significantly reduced (21). Similarly, vitronectin is released from spermatozoa following the acrosome reaction, and blocking peptides (poly RGD peptides that mimic the integrin binding site on vitronectin) impede attachment of human spermatozoa to zona-free hamster oocytes. Furthermore, exogenous vitronectin promotes sperm-egg attachment and sperm aggregation (22). The fibronectin and vitronectin released from spermatozoa appear to facilitate the adhesion of other spermatozoa to the zona pellucida and/or ooplasm by integrins. Several other integrin subunits (α3, α4, and α6) have been identified in human spermatozoa and, like α5, are localized to the equatorial segment (23).

Oocytes. Oocytes express a unique repertoire of integrins. The mouse oocyte contains integrin subunits α2, α3, α5, α6, α6B, αv, β1, β3, and β5 as detected by mRNA and/or protein analysis (24–26) although it is unclear as to which integrins are expressed on the surface of the oocyte. Similarly, human oocytes express integrin subunits α3, α6, αv, β1, β3, β4, and β5 (27, 28). Sperm-egg binding is completely blocked in mice by a function-blocking antibody to the α6 integrin subunit and by a peptide analogous to the integrin ligand domain. However, using an RGD-containing peptide, a nonfunction-blocking antibody to α6, or a function-blocking antibody to αvβ3 has no effect on sperm-egg binding (28). Additionally, Almeida *et al.* (28) have found that spermatozoa bind to somatic cells only if the somatic cells express the α6β1 integrin, and this is blocked by the fertilin analog. Similar to mice, human spermatozoa/oocyte interactions are not blocked by an RGD-containing peptide. However, the use of a function-blocking antibody to β1 partially inhibited sperm-egg fusion in humans (29).

Although the oocyte expresses many different integrin subunits, the data collected thus far highlight the importance of α6β1 integrins on the egg surface where the complex can facilitate fertilization by interacting with fertilin. The activation of α6β1 may lead to intracellular signals that could aid in the development of the embryo. More experiments are needed to study the direct interaction of fertilin and α6β1 in sperm-egg fusion, signal transduction, and expression in other animals.

Integrins and Implantation. Implantation is a highly coordinated sequence of events involving the adherence, apposition, and, in some cases, invasion of the embryo into the uterus. However, the embryo will only implant into the uterus at a precisely regulated time, known as the window of implantation (30). The interaction between the embryo and the uterine epithelium is very similar to leukocyte-endothelial interactions and metastatic processes where integrins are the dominant and final adhesion molecule in the attachment process (12, 31, 32). Once the embryo has attached to the uterine epithelium, it may either bond closely to the uterine epithelium (noninvasive implantation) or bypass the uterine epithelium and invade the uterine stroma (invasive implantation). Recent reviews on this topic include articles from Burghardt *et al.* (33) and Carson *et al.* (34).

Integrins are regulated spatially and temporally within the uterus throughout the reproductive cycle and early pregnancy (12, 35). Most of the data on uterine integrin receptors have come from clinical studies and experiments on rodents, although recent experiments have been reported using porcine (36) and baboon (37) model systems. The developmental regulation of integrin expression in the blastocyst and in the uterus is consistent with integrin involvement in implantation.

The endometrium is composed of the uterine epithelium and stroma. The stroma contains many cellular elements such as fibroblasts, vascular components, and a dynamic array of immune cells. At least 14 integrin subunits are found in the human endometrium (38), 10 integrin subunits in the baboon (37), and 7 subunits in the pig (36). Integrin expression in the mouse uterus as a function of the stage of the reproductive cycle has not been studied. Some integrins expressed by human and porcine uterine epithelial cells display spatial and temporal regulation throughout the reproductive cycle (i.e., menstrual or estrous cycles), whereas others are constitutively expressed (Table II).

Invasive implantation. In humans, integrin subunits α2, α3, α6, α9, αv, β1, β3, β4, β5, and β6 have been identified on the luminal epithelium of the uterus. With the exception of β5 and β6, all of the integrins listed are also expressed in the glandular epithelium, which displays integrin subunits α1 and α4 as well (12, 35, 38, 39). Integrin subunits α5 and α8 have not been found in the human endometrium (12, 38). Thus the possible known heterodimers available at the uterine luminal surface include α2β1, α3β1, α6β1, α6β4, α9β1, αvβ1, αvβ3, αvβ5, and αvβ6.

A remarkable similarity exists between the integrin expression patterns of human and baboon uterine epithelium, both of which employ an invasive implantation strategy (37, 40). Based on their spatial expression and ability to bind to a number of available ligands, three receptors appear to be particularly well suited for the initial process of implantation: α9β1, αvβ1, and αvβ3. These integrins are all members of either the fibronectin or vitronectin family of receptors.

Integrin α9β1 can bind to the fibronectin type III repeat, which is also found in tenascin, and is strongly expressed on the uterine luminal epithelium throughout the menstrual cycle. This integrin is tightly regulated in the glandular epithelium in humans and only expressed during the mid- to late-secretory phase (38). The presence of α9β1 on the luminal surface of the uterine epithelium makes it a potential participant in the initial stages of adhesion. The possibility that the heterodimer αvβ1 exists on the uterine

Table II. Integrins and Their Ligands Expressed in Embryos, Trophoblast, and Uterine Epithelium of Species that Use Different Strategies for Implantation and Placentation

Integrin	Ligands	Mouse embryo	Human embryo	Murine trophoblast	Human trophoblast	Porcine trophoblast	Human uterine epithelium	Porcine uterine epithelium
α1β1	Ln, Col, Pe	+			+	+		+
α2β1	Col	+					+	
α3β1	Fn, Col, Ln		+		+	+	+	+
α4β1	Fn, VCAM-1		+	+		+	+[a,b]	+[a]
α5β1	Fn, Vn	+			+	+		+[a]
α6β1	Ln	+	+		+		+	
α7β1	Ln	+						
α9β1	Tn						+	
αvβ1	Fn, Vn			+	+	+[c]	+[c]	+
αvβ3	Fn, Vn, Os, vWf, Fib, BSP1, Pe, PECAM-1	+	+	+	+	+[c]	+[a]	+
αvβ5	Fn, Vn, Os		+		+		+	
αvβ6	Fn, Tn				+		+	
αIIbβ3	Fn, Fib, vWf, Vn	+						

Note. Ligands: BSP1, bone sialoprotein 1; Col, collagens; Fib, fibrinogen; Fn, fibronectin; Ln, laminins, Os, osteopontin; Pe, perlecan; PECAM-1, platelet endothelial cell adhesion molecule; Tn, tenascin; VCAM, vascular cell adhesion molecule-1; vWf, von Willebrand factor.
[a] Regulated during the estrous or menstrual cycle.
[b] Glandular uterine epithelium only.
[c] Only individual integrin subunits have been detected.

luminal epithelium has been postulated (39). Although expression of this heterodimer has not been explored in the uterus by using an antibody that recognizes the heterodimer as opposed to the individual subunits, the two subunits are present at the time corresponding to the window of implantation. The αvβ1 integrin can bind to the RGD amino acid sequence that is found in fibronectin (with high affinity) and vitronectin (with low affinity). Evaluating only the subunits of this heterodimer is problematic since both αv and β1 subunits can bind to multiple subunits. Thus, an increase in the level of either the αv or the β1 subunit may be the result of another heterodimer (αvβ3 or α9β1, respectively) increasing rather than the αvβ1 heterodimer.

The heterodimer αvβ3 has been implicated in implantation in humans (40). The maximal expression of αvβ3 on the human uterine luminal epithelium coincides with the rise in progesterone during the window of implantation. The αvβ3 integrin is the most versatile integrin, being capable of binding to a wide variety of extracellular matrix (ECM) components, including vitronectin, fibronectin, oncofetal fibronectin, and osteopontin. It is noteworthy that α4β1 has been detected during the implantation window in human endometrium. However, this integrin is expressed in the glandular epithelium and not the luminal epithelium, which reduces the likelihood that it is uniquely involved in the early events of implantation. Other integrin heterodimers expressed by the endometrium are either constitutively expressed and not localized to the apical surface of the uterine epithelium (α3β1 and α6β4), are expressed in nonepithelial cells (α6β1), or are downregulated during the midsecretory stage (α2β1 and αvβ6).

Little information is available on integrin expression by human trophoblast cells at the time of implantation. Since murine implantation is also invasive, some insights may be gained from the examination of the mouse trophectoderm. During the initial stages of apposition and adhesion, the murine trophectoderm expresses integrins α5β1 and αvβ3 (12). However, as the mouse conceptus begins to invade beyond the uterine epithelium, the trophectoderm switches integrin expression to include integrins α1β1, α6β1, and α7β1. These receptors can bind to laminin and collagen, presumably support invasion of the epithelium, and facilitate migration into the stroma. The availability of ligands for integrin-binding on both the conceptus and uterine epithelium must be considered. Three scenarios exist for trophoblast-uterine epithelium interactions: (i) integrins expressed on the trophectoderm bind to ligands on the uterine epithelium; (ii) integrins expressed on the apical surface of the uterine epithelium bind to ligands on the trophectoderm; or (iii) integrins are expressed on both cell types and bind to extracellular matrix components found in the intercellular space.

The third explanation appears to be the most reasonable. In humans, oncofetal fibronectin, referred to as trophoblastic glue, is present on the invading human trophoblast (41), and osteopontin is present on the apical surface of the uterine epithelium. Both are ligands for the integrins expressed on the uterine epithelium and trophectoderm at the time of implantation. Osteopontin and αvβ3 have been reported to be co-expressed on uterine epithelia, decidualizing stroma cells and cytotrophoblast cells of the baboon (37). Additionally, the attachment and outgrowth of murine trophoblasts to fibronectin and vitronectin can be blocked with RGD-containing peptides *in vitro* (42, 43). These data support the theory that integrins present on the cell surface of the uterine epithelium and trophoblast bind to ECM components secreted by both the trophoblast and uterine epithelium and act as a tether between the two cell types.

Noninvasive implantation. In porcine implantation, which is noninvasive, the uterine luminal epithelium expresses integrin subunits α1, α3, α4, α5, αv, β1, and β3 as detected by indirect immunofluorescence microscopy (36). The expression of subunits α4, α5, and β1 on the uterine luminal epithelium is related to the stage of the estrous cycle with the highest level of expression corresponding to the porcine implantation window (Days 10–15 of the estrous cycle). Two integrin subunits, αv and β3, are constitutively expressed at high levels on the uterine luminal epithelium throughout the estrous cycle. Integrin subunits α1 and α3 exhibit low expression in the uterine epithelium, and their expression is restricted to the basal aspect of the uterine epithelium, which is consistent with a role in cellular attachment to the basement membrane.

Analysis of implantation sites indicates that the subunits α4, α5, αv, β1, and β3 are present on both the porcine trophectoderm and uterine epithelium at sites of attachment. The known heterodimer combinations from these five subunits include α4β1, α5β1, αvβ1, and αvβ3. These receptors, like those found in the human luminal epithelium at the time of implantation, are all members of the fibronectin/vitronectin family of receptors. In human trophoblasts, α5β1 interacts with fibronectin and restrains invasion (44). The function of α5β1 in the porcine trophoblast may be similar. The porcine uterine epithelium expresses vitronectin and oncofetal fibronectin, and the conceptus expresses fibronectin, oncofetal fibronectin, and vitronectin (36, 45), which are ligands for the integrins expressed at the contacting surfaces of the luminal epithelium and trophectoderm during implantation. The porcine implantation offers an interesting model to study the attachment stage of implantation as the underlying epithelial cells are not destroyed by the subsequent invasion of the trophoblast through the uterine epithelium as is the case in other species (46).

In summary, it appears that the expression of integrins and their ligands are important elements of attachment and adhesion of the conceptus to the uterine epithelium, regardless of the mode of implantation. Integrins expressed on the conceptus appear to be largely conserved among species examined to date with the same integrins being expressed on uterine epithelium. As described elsewhere, the induction of new integrin receptor populations (specific to colla-

gen and laminin) occurs later in conceptuses in species that exhibit invasive implantation.

Integrins and Placental Development. Subsequent to the attachment of trophectoderm to the uterine luminal epithelium, the trophoblast cells of the conceptus begin to invade the uterus and form the functional placenta. The formation of the placenta establishes the fetal-maternal interface by either direct interaction with the maternal blood supply (hemochorial) or close association with the uterine blood vessels (endotheliochorial). Direct interaction does not occur in animals that express an epitheliochorial type of placenta (i.e., pigs, horses and ruminants) (47).

Trophoblast cells invade through the uterine epithelium by fusing with the underlying epithelium (rabbits), intruding between uterine epithelial cells (carnivores), or disassociating and phagocytosing the uterine epithelium from the basal lamina (rats and mice) (14). It is unknown which form of invasion human trophoblasts use, but it is generally believed that they displace and phagocytose the uterine epithelium. Invading trophoblast cells secrete metalloproteinases that facilitate their migration between the uterine epithelial cells. The trophoblast cells subsequently undergo rapid proliferation. Integrins in the developing placenta may work to develop and maintain the architecture of the placenta, create new vasculature (angiogenesis), aid in migration of the trophoblast cells, and possibly activate specific signal transduction pathways that promote the survival of the developing fetus (48).

During the development of the human placenta, trophoblast cells differentiate into two distinct pathways forming syncytiotrophoblast and cytotrophoblast. Precursor cytotrophoblast cells fuse to form multinucleated syncytiotrophoblast, which encases the chorionic villi. Chorionic villi emerge from the chorionic plate's large stem villi (or primary villi) and branch into increasingly smaller villi forming secondary and tertiary (or definitive) villi. These villi are termed free or floating villi and are in direct contact with the maternal blood circulation where they perform nutrient and gas exchange for the fetus (49). Some of these villi form columns of aggregated cytotrophoblast cells called anchoring or attached villi that invade the uterine wall, infiltrate the decidua, and replace endothelial cells in maternal spiral arteries. Cytotrophoblasts may migrate as far as the myometrial layer (50).

As cytotrophoblast cells migrate into decidua, their cell adhesion molecule profiles change. The cytotrophoblasts proximal to the anchoring villi express $\alpha 6\beta 4$. This integrin binds to hemidesmosomes and is a marker for normal epithelium. These cells also express integrins $\alpha v\beta 5$ and $\alpha v\beta 6$, which may impede cell migration (51). As the cytotrophoblasts form cell columns that become anchoring villi, integrins $\alpha 6\beta 4$, $\alpha v\beta 5$, and $\alpha v\beta 6$ are no longer expressed. At the terminal end of the trophoblastic column where the cells fan into the decidua, called the cytotrophoblastic shell, the cells express the $\alpha v\beta 3$ integrin that may facilitate invasion. The integrin, $\alpha 5\beta 1$, is expressed in the distal cell column as well. However, experiments by Damsky *et al.* (52) demonstrated that $\alpha 5\beta 1$ may act to control the rate of migration and invasion into the maternal system. Finally, as the cytotrophoblast cells reach the uterine interstitium, they express $\alpha 1\beta 1$, a laminin/collagen receptor (51–53). By the time the cytotrophoblast cells invade the maternal vasculature, they express a cell adhesion profile similar to the endothelium, which includes the display of vascular cell adhesion molecule-1 (VCAM-1) and platelet/endothelial cell adhesion molecule-1 (PECAM-1), but not E-cadherin or E-selectin (54).

The trophoblast cells of the murine placenta are not as invasive as those of the human placenta, although both placentas are hemochorial. During early gestation (gestation Days 5–8), the murine trophoblast cells invade the uterine epithelium and differentiate to form trophoblast giant cells and the ectoplacental cone. By gestation Day 12, the placenta is completely formed with four distinct layers (i.e., the outermost trophoblast giant cells, spongiotrophoblast zone, labyrinthine zone, and innermost chorioallantoic plate). In the mouse, integrin subunits $\alpha 4$, αv, $\beta 1$, and $\beta 3$ are present throughout placental development, but display spatial differences as detected by indirect immunofluorescence and mRNA analysis (55). All subunits ($\alpha 4$, αv, $\beta 1$, and $\beta 3$) are expressed by the trophoblast giant cells throughout gestation. Subunits $\alpha 4$ and $\beta 3$ show no compartmental differences, whereas αv is more strongly expressed in the spongiotrophoblast zone, and $\beta 1$ is more strongly expressed in the labyrinthine zone. The disparity between the integrin subunits $\beta 3$ and αv in the spongiotrophoblast zone suggests that αv may form heterodimers with other β subunits (i.e., $\beta 5$ or $\beta 6$), which would result in a less invasive phenotype.

In summary, these data indicate that integrins are associated with trophoblast invasion and aid in the development of the placenta. Some integrins, including $\alpha 6\beta 4$, $\alpha v\beta 5$, and $\alpha v\beta 6$, are expressed by cytotrophoblast cells in anchoring villi where they may limit migration. As cytotrophoblast cells migrate into the decidua, they express $\alpha v\beta 3$ and $\alpha 5\beta 1$. The ratio of $\alpha v\beta 3$ to $\alpha 5\beta 1$ may be important since $\alpha v\beta 3$ may promote invasion whereas $\alpha 5\beta 1$ may have a role in limiting invasion into the vasculature.

Regulation of Placental Integrin Expression

Placental Cytokines. Cytokines are small (about 15–30 kDa), short-lived peptides involved in autocrine and paracrine regulation within a local environment. The uterus and placenta manufacture a number of cytokines that possess the ability to modulate placental growth and development (56). There has been little research conducted on the direct effect of cytokines on integrin regulation during implantation and placental development. However, data from knockout mice, experiments on other cell types, and the known intimate association between some cytokines and integrins strongly suggest an important role in placental development. In the following section, uterine and placenta-derived cytokines that are likely to control the ex-

pression of integrins during implantation and placentation are discussed.

Tumor necrosis factor-α. Tumor necrosis factor-α (TNF-α) is a highly conserved, pro-inflammatory cytokine that has been detected in many tissues, including the cycling and pregnant uterus of humans, rats, and mice (57). Mammalian placentas are also major sources of TNF-α (58–60). TNF-α transduces signals through two receptors, TNF receptor-1 (TNF-R1), which is associated with induction of apoptosis and stimulation of a protective NF-κB pathway, and TNF-R2, whose functions are less well understood. One role of TNF-α may be to stimulate apoptosis in trophoblast cells through TNF-R1, which could have a major influence on placental modeling and remodeling (61). However, through the NF-κB pathway, TNF-α acting through TNF-R1 could alter expression of multiple genes (62, 63). Messenger RNA for TNF-R2 is found in trophoblast cells and decidua and is developmentally regulated during gestation, with high levels expressed early (gestation Days 8/9) and again late in placental development (64). The functions of TNF-R2 remain unclear at present but may include stimulation of a cell proliferation-promoting cascade (65).

One potential pathway by which TNF-α may regulate the development of the placenta involves modulation of adhesion molecules, including integrins and VCAM-1, a member of the IgG superfamily of adhesion molecules. Many integrin-induced effects may be mediated by TNF-α, which has been shown to modulate the expression of various integrin subunits in *in vitro* experiments with endothelial (66) and metastatic cells (67, 68), and to upregulate rapidly the expression of VCAM-1 in endothelial cells (69). Additionally, integrins may promote or inhibit cell death and modulate the production of inflammatory mediators, including TNF-α (70). Mice lacking TNF-α/TNF-β have disorganized spleens due to abnormal expression of adhesion molecules (71). Similarly, TNF-α/TNF-$\beta^{-/-}$ mice form a smaller, less intricate labyrinthine region within their placentas, and the overall size of the placenta is reduced (72). Experiments on human trophectoderm showed that although TNF-α does not alter β1 expression, cell migration is significantly reduced (73), indicating that there may be a switch from one β1 heterodimer to another. Given these observations, it seems reasonable to postulate that TNF-α may play a role in the expression of adhesion molecules during placental development.

Interferon-γ. Interferon-γ (IFN-γ) is another powerful pro-inflammatory cytokine that is present in the female reproductive tract (74, 75) and is produced locally. Immunoreactive IFN-γ has been identified in human (76) and murine trophoblast cells (77). Additionally, IFN-γ has been localized to the cells surrounding the implantation site, maternal blood spaces, uterine natural killer cells, and giant trophoblast cells in the mouse placenta (75). The actions of IFN-γ may be indirect. Interferon-γ upregulates TNF-R expression three- to five-fold, which may enhance the actions of TNF-α (78). TNF-α could, in turn, alter the expression of placental adhesion molecules. Although deletion of the IFN-γ gene has not been associated with fertility impairment (79), other interferons such as IFN-α, IFN-β, or IFN-τ might compensate when IFN-α is absent as these cytokines have overlapping functions.

Studies to date indicate that the ability of IFN-γ to modulate the adherence of placental cells may be dependent on the subpopulation of the placental cell and its specific pattern of integrin expression. For instance, pretreatment of human syncytiotrophoblast with IFN-γ allows the adhesion of lymphocytic MOLT-4 cells to cell layers (80), yet murine trophoblastic outgrowth is inhibited by IFN-γ (81). In non-placental cells, IFN-γ has been shown to upregulate some integrins and downregulate others depending on cell type. Integrin subunits α2, α5, α6, and β1 were upregulated in thymocytes (82), whereas in endothelial cells, the activation of αvβ3 integrins was reduced in response to IFN-γ (83). Additionally, integrin activation can also alter the production of IFN-γ; in cytotoxic T cells, blocking the binding of the α6 integrins with specific antibodies reduced the production of IFN-γ (84). Clearly important relationships exist between placental structure and IFN-γ regulation of integrin expression deserving of further investigation.

Interleukins. Interleukins (IL) comprise a group of cytokines that participate in and also regulate pro- and anti-inflammatory immune responses. Interleukins 1β, 2, 4, 6, and 10 have all been identified in the uterus and/or the placenta in mice and humans (56). Interleukins 1β, 2, and 6 are considered pro-inflammatory cytokines and are typically present in the early and late stages of gestation (56). Interleukin 6 is also regarded as a transition cytokine, associated with pro- and anti-inflammatory responses, and has been detected throughout the various stages of pregnancy. Interleukins 4 and 10 are anti-inflammatory cytokines that increase as pregnancy progresses (85). The actions of some interleukins may overlap with TNF-α and/or IFN-γ and may compensate for the loss of TNF-α or IFN-γ when these genes are deleted from the mouse genome. Although the precise role of the interleukins is unknown, interleukins such as IL-4 and IL-10, the Th2-type cytokines, predominate in pregnancy and are believed to downregulate maternal immune responses during pregnancy (77).

Interleukins modulate the ability of trophoblast cells to attach to each other or various substrates. For example, IL-1 inhibits the ability of murine blastocysts to attach to fibronectin-coated Petri dishes, but enhances blastocyst outgrowth from adherent blastocysts (81). Additionally, lymphocytic MOLT-4 cells adhere to IL-1β treated human syncytiotrophoblasts cells (80). The ability of placental interleukins to modulate cell adhesion molecule expression on trophoblast cells has not been studied; however, interleukins have been shown to modulate the expression of adhesion molecules in other cell systems. IL-1β upregulated integrin subunits α2, α5, and α6 in human thymocytes *in vitro* (82), and α1, α5, and β1 in human dermal fibroblasts, and the porcine cutaneous wound model (86). IL-4 upregu-

lated the expression of αv and β3 integrins in murine bone marrow macrophages (87). Integrin subunits α5 and β1 were upregulated in rabbit corneal epithelium by IL-6 (88). It is likely that placental interleukins modulate the expression of adhesion molecules in the developing placenta and the migration of trophoblast cells. However, more experiments are needed to elucidate these interactions.

Leukemia inhibitory factor. Leukemia inhibitory factor (LIF) is a heavily glycosylated 58-kDa protein that is produced by various fibroblast cell lines, stimulated T-lymphocytes, and activated monocytes (89). LIF is also produced by murine blastocysts (90) and uteri (91) where it may regulate the growth, differentiation, and migration of the blastocyst at the time of implantation. In human uteri, LIF expression is localized to endometrial glands during the secretory/postovulatory phase but is not present in the endometrium during the proliferative/preovulatory phase (92). The LIF receptor (LIF receptor-β) is expressed during the proliferative and secretory phases of the cycle and is restricted to the luminal epithelium (92). Additionally, normal implantation is disrupted in LIF-receptor mutant animals, which leads to poor intrauterine nutrition but allows the fetus to grow to term (93).

Even in noninvasive modes of implantation, LIF appears to have a central role. This cytokine has been implicated in the elongation of the porcine trophoblast cells just prior to implantation (94). Thus the uterine expression of LIF in humans, mice, and pigs may have a role in regulating embryo implantation, possibly through an autocrine/paracrine interaction between LIF and its receptor on the luminal epithelium and/or trophoblast. Additionally, deletion of the LIF genome in the mouse has profound effects on reproduction. Embryos of an $LIF^{-/-}$ mouse fail to implant in a mother also deficient in LIF. However, $LIF^{-/-}$ embryos are rescued when placed into a normal mouse uterus (95).

Both integrins and LIF are expressed by the uterus and blastocyst at the time of implantation, which suggests a possible link between the two proteins. LIF can upregulate the integrin αvβ1 in human tumor cells (96) and increase tumor cell binding affinity to fibronectin (97). Both αvβ1 and fibronectin have been implicated in the adhesion of the trophoblast to the uterine epithelium(see 33,39). Additionally, LIF is upregulated in response to IL-1, TGF-β and TNF-α in human bone marrow stromal cells (98), suggesting that other cytokines present in the female reproductive tract may act in cooperation to induce the production of LIF at the precise time needed for implantation. It is evident that an interaction exists between LIF and integrins and that these interactions may be critical to normal implantation and placental development.

Colony stimulating factor-1. Colony stimulating factor-1 (CSF-1), also known as macrophage colony stimulating factor (M-CSF), is a homodimeric glycoprotein produced by a wide range of cell types, including monocytes, granulocytes, endothelial cells, epithelial cells, and fibroblasts. The cytokine is a hematopoietic growth factor that regulates proliferation, differentiation, and viability of bone marrow cells into the mononuclear phagocytic lineage (99). This growth factor has also been detected in uterine and placental tissues of humans (100), mice (101) and pigs (102). Mice lacking CSF-1 (the osteopetrotic *op/op* mice) are infertile when mated with homozygous males. However, when mated with heterozygous males (+/*op*), fertility is partially recovered (103). The availability of CSF-1 and the negative effect of CSF-1 deficiency on pregnancy suggest that this cytokine is important for successful reproduction.

Only recently have studies been conducted on the ability of CSF-1 to modulate cell adhesion molecule expression. When human trophoblast cells were treated with CSF-1 *in vitro*, there was a dose-dependent increase in the α5 integrin subunit and its ligand, fibronectin, as detected by flow cytometry (104). This study indicated the potential of CSF-1 to act in an autocrine/paracrine manner to regulate placental development and invasion. Another placental integrin, αvβ3, can be upregulated by CSF-1, albeit in osteoclasts, where it increases cell spreading (105). Synthesis of the placental cytokines, IL-1, IFN-γ, and TNF-α, is induced by CSF-1, which could lead to modulation of cell adhesion molecules. However, the interaction between CSF-1 and other placental cytokines on integrin expression remains unclear.

Female Steroid Hormones. As detailed above, some integrins on the uterine epithelia of women and pigs display temporal changes in expression. These changes coincide with changes in systemic steroid hormone concentrations (36, 38). Thus, steroidal hormones appear to facilitate implantation by synchronizing uterine receptivity with arrival of the blastocyst in the uterus.

In a study by Bowen *et al.* (36), pigs were ovariectomized and subsequently treated with exogenous estrogen, progesterone, the combination of estrogen and progesterone, or the vehicle alone. Progesterone alone, or in combination with estrogen, dramatically increased the level of expression of α4 and α5 integrin subunits and, to a lesser extent, the β1 subunit as detected by immunocytochemistry of the uterine epithelia. Estrogen-treated uteri were similar to the vehicle control displaying lower levels of α4, α5, and β1. The integrin subunits αv and β3, which have been implicated in implantation, were not affected by steroid treatment and continued to be highly expressed in the endometrium. *In vitro*, porcine uterine epithelial cells respond to steroid treatment only under conditions leading to a polarized phenotype (106). In humans, using an endometrial adenocarcinoma cell line that expresses similar markers to normal endometrial epithelial cells, estrogen alone or in combination with progesterone downregulated the integrin, αvβ3, as measured by flow cytometry and Northern blot analysis (107). As mentioned above, the integrin αvβ3 in uterine epithelial cells is believed to be important to human endometrial receptivity.

Integrin Null Mutations or "Knockouts"

In recent years, null mutation or "knockout" mice lacking specific integrins have been developed using embryonic stem cells and transgenic technology (108, 109). At this time, 19 of the known 26 subunits have been deleted. Of these, four result in embryonic lethality: β1-null mice die just after implantation, α4-null mice die from direct placental abnormalities, and α5- and αv-null mice die *in utero* with probable placental abnormalities. None of the integrin knockout experiments were conducted to establish roles for the proteins in placental development. Furthermore, the placenta was rarely examined for morphological abnormalities.

Integrin α4-Null Mice. The integrin subunit that has the most direct effect on the development of the placenta as illustrated by gene deletion is the subunit α4. Since the α4 integrin subunit is predominantly expressed on circulating blood cells, it was expected that the null mutation would result in dysfunctional leukocyte-endothelial interactions. Instead, the allantoic membrane failed to fuse with the chorionic membrane, which resulted in embryonic death at Day 11. Other defects included impaired development of the epicardium and the coronary vessels, leading to cardiac hemorrhage (110). Gene deletion studies of the α4β1 coreceptor, VCAM-1, have the same phenotype as the α4-null mice, suggesting that α4 interacts with VCAM-1 instead of fibronectin, the alternate α4 ligand, during the formation of the chorioallantoic membrane (111, 112).

Integrin β1-Null Mice. Deletion of the β1 subunit had catastrophic consequences on the developing conceptus, resulting in inner cell mass failure and embryonic death by gestation Day 5 (113, 114). These results were anticipated since β1 binds to as many as 12 different α subunits. However, the blastocyst was still able to attach to the uterine epithelium and initiate invasion prior to embryonic death. The inner cell mass died before the trophoblast, indicating that the requirement for β1 was in the survival of the inner cell mass (ICM) rather than the trophectoderm. Shortly after the formation of the inner cell mass, the proamniotic cavity is formed, which results in the formation of the outer endodermal cell layer and inner ectodermal cell layer separated by a basement membrane. The formation of these layers did not occur in the β1-null mouse, indicating the importance of integrin-extracellular matrix interactions in fetal development (115).

Integrin αv-Null Mice. The αv subunit binds to multiple β subunits, and its elimination was therefore expected to have profound effects on embryogenesis. This was indeed the case. Most fetuses died around embryonic Day 9.5. Those that survived until birth (nearly 20% of the fetuses) died from hemorrhage in the brain and intestine (116). Some placental defects were observed in this knockout model including a compaction of the labyrinthine zone and a decrease in the blood lacunae. The family of αv heterodimers is involved in angiogenesis and as such would play a very important role in placental development. Interestingly, mice in which other β subunits (i.e., β3, β5, and β6) are deleted are viable and display only minor defects (108, 109). These results indicated that the β subunits may be able to substitute for one another in the formation of the placenta, and embryo defects cause fetal death at a later stage.

Integrin α5-Null Mice. Analyses of α5-null mice indicated severe developmental defects. The structures of the embryonic and extraembryonic vasculature were abnormal, and the notochord and somites distal to somite 10 failed to form, resulting in death around embryonic Day 8.5 (117). However, other integrins were able to compensate for the loss of α5β1. Embryonic stem cells from α5-null mice upregulated the αvβ1 integrin and bound directly to fibronectin (118). These data suggested that the α5-null placenta is able to function at a level commensurate with fetal development, but as with the αv subunit, the normal architecture of the placenta was not examined, so this point has not been documented experimentally.

Integrins and Reproductive Dysfunction

Unexplained Infertility. Some women have recurrent unexplained infertility resulting in reproductive failure. There are some differences in the endometrium between the normal fertile woman and those displaying unexplained infertility. Both endometrial stromal leukocyte populations and glycoconjugate profiles of endometrial epithelial cells differ during the peri-implantation phase of the menstrual cycle between normal fertile women and those with unexplained infertility (119, 120).

As mentioned above, integrin subunits α4 and β3 are specifically co-expressed in the glandular and luminal epithelium of the uterus, respectively, only during the times of maximal uterine receptivity in women. Lack of expression of either one of these two integrin subunits has been associated with unexplained infertility (121).

Endometriosis. In some cases of endometriosis, ectopic growth of endometrial cells is evident, and integrins may be responsible for adhesion of the endometrial cells to the peritoneal wall. Viable endometrial tissue fragments are present in peritoneal fluid during the early follicular phase of women with patent tubes (122). Endometrial tissue found within the peritoneum and cells that have the potential to form endometriosis express the integrins α2β1, α3β1, α4β1, α5β1, and α6β1 (123, 124). Cells in the peritoneal fluid frequently express α4β1 in women with endometriosis, but this integrin is absent in normal women (124). Additionally, the expression of αvβ3 is significantly less in women with endometriosis than in either fertile controls or women with infertility problems not related to endometriosis (121).

Luteal Phase Deficiency. Asynchrony between the timing of progesterone-induced uterine differentiation and the arrival of the embryo into the uterus is known as Luteal Phase Deficiency (40). As described above, the expression

and regulation of αvβ3 in human uterine epithelial cells appears to be correlated with the rise and fall of progesterone. Lessey *et al.* (15) showed that there was a consistent loss of β3 integrin subunit expression in endometrial biopsies that demonstrated histologic delay in endometrial maturation. The close correlation between the downregulation of progesterone receptor levels and the expression of epithelial integrins suggests that this event may be critical for implantation.

Preeclampsia. Abnormal placentation can result in the loss of the fetus and/or severe complications for the mother. One of the most common and well-characterized syndromes associated with abnormal placentation is termed preeclampsia. Here, trophoblast invasion is limited, blood vessels are not modified, and maternal blood pressure rises. Normal placentation involves the invasion of the cytotrophoblast into the uterine lining and the sequential expression of specific integrin patterns, from a cell-adhesion molecule phenotype that is characteristic of a static epithelial layer to an invasive phenotype and finally a phenotype that mimics that of the maternal endothelium. The cytotrophoblast cells from preeclamptic placenta do not switch cell adhesion molecule expression as the cells begin to migrate into the maternal tissue (51). The upregulated expression of integrin subunits α1β1 or αvβ3 (and cell adhesion molecules, VCAM and VE-cadherin) in the cytotrophoblast cells does not occur (53). It is not known whether the failure to switch cell adhesion molecules is a cause or an effect of preeclampsia.

Conclusions

It has become increasingly clear over the past few years that appropriate expression of integrins is critical to successful reproduction. In addition to their role in cell-to-cell and cell-to-matrix interactions, integrins are also critical for cell survival, progression through the cell cycle, cellular differentiation, establishment of cell polarity, regulation of gene expression, proinflammatory and anti-inflammatory cytokines, and modulation of cell death. The multidimensional nature of integrins as well as the redundancy in integrin expression on reproductive cell types, and their ability to bind to more than one ligand is likely to make them important participants in the process of reproduction.

It is important to understand that multiple members of the integrin family are expressed on cells of the reproductive system. Of the 23 known integrin heterodimers, at least 8 can be expressed simultaneously on certain reproductive cells such as the uterine epithelium or the trophoblast cells. One somewhat surprising feature that emerges from this information is that the integrins appear to be able to compensate for the loss of one of the subunits. This is best exemplified in the work with the knockout mice where there is a compensation for the loss of one integrin subunit by the upregulation of other subunits. For example, αvβ3 is a very important integrin throughout various steps of reproduction, but the loss of β3 *via* gene mutation does not halt the process of reproduction. Rather, other integrin subunits (namely, β1 and β5 subunits) are upregulated. Another reasonable explanation for the expression of many integrins on the same cell is that this permits rapid responses to the changing environmental conditions that characterize developing tissues, which include alterations in substrates and matrix as well as soluble regulatory molecules.

It is clear that much remains to be learned about the roles of integrins in reproduction and that developing this information base is of the utmost importance. Because these versatile polypeptides function principally as adhesion receptors, and their display is tightly controlled during development, aberrant expression could be one explanation for impaired fertility and impaired reproductive success.

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