Differential expression of the integrin subunits in human fetal membranes

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The distribution of the α_1 , α_3 – α_6 , β_1 , β_3 and β_4 integrin subunits in fetal membranes at term was examined using an indirect immunofluorescence technique and confocal laser scanning microscopy. In the amniotic epithelium, β_4 integrin ($\alpha_6\beta_4$) exhibited distinct basal localization, whereas β_1 integrins ($\alpha_3\beta_1$, $\alpha_5\beta_1$) were localized basolaterally. This finding suggests that integrins, especially $\alpha_6\beta_4$ which is a structural component of the hemidesmosomes, may function as basement membrane receptors. Integrins localized laterally may play a role in cell–cell interactions. β_1 ($\alpha_1\beta_1$, $\alpha_5\beta_1$) integrins are probably involved in cell–matrix interactions in the connective tissue layers which are rich in collagens and fibronectin. Cytotrophoblasts, located predominantly towards the chorionic basement membrane, mainly expressed $\alpha_6\beta_4$, while those located predominantly in the vicinity of decidua expressed $\alpha_5\beta_1$, $\alpha_3\beta_1$ and $\alpha_1\beta_1$. Decidual cells expressed $\alpha_3\beta_1$ and $\alpha_1\beta_1$, whereas α_1 , α_5 , α_6 , β_1 and β_4 were expressed in blood vessels. This pattern of integrin expression reflects the reported difference in composition of the extracellular matrix at these locations and obviates an important role for $\alpha_5\beta_1$ at the chorio–decidual interface. The differential integrin expression at the cell–basement membrane interfaces demonstrated in this study (at amniotic epithelium, cytotrophoblasts, decidual cells and blood vessels) indicated a differential recognition of basement membranes by these cells. β_4 may have a specialized function at the blood vessels, and possibly in cytotrophoblasts, that is distinct from its role in hemidesmosome-mediated attachment. This study suggests that integrins may be involved in cell–matrix and cell–cell adhesions in fetal membranes and may therefore be important for maintaining their normal structural and functional integrity.

Introduction

The normal development, structure and function of fetal membranes (amniochorion and decidua) are very important for normal progress and outcome of human pregnancy (Malak and Bell, 1993). Fetal membrane dysfunction may lead, for example, to their prelabour rupture, a condition that is associated with a high risk of maternal morbidity, pre-term birth and neonatal morbidity and mortality (Alger and Pupkin, 1986).

Fetal membranes are composed of a cellular component and a complex and extensive extracellular matrix (ECM). The cellular component is mainly of epithelial (amniotic epithelium and cytotrophoblasts) or mesenchymal origin (for example, fibroblasts, myofibroblasts, decidual cells). The ECM is composed of collagens (for example, I, III and IV), non-collagenous glycoproteins (for example, fibronectin) and laminin (Aplin and Campbell, 1985; Aplin *et al.*, 1985; Klima and Schmidt, 1988; Malak *et al.*, 1993) and proteoglycans. The ECM plays an important role in regulating cell function, tissue development

during normal intra- and extra-uterine morphogenesis and in reparative processes (Hay, 1973, 1989). In fetal membranes, the ECM has a pivotal role in maintaining their normal structure and function (Artal *et al.*, 1976).

Integrins, a family of transmembrane protein receptors composed of non-covalently linked α and β subunits, play an important role in the interactions between cells and their ECM (Hynes, 1992). There are several integrin subfamilies, each with two to nine α subunits and a commonly shared β subunit (Hynes, 1992). The detection of the α_1 , α_3 or α_5 integrin subunits in colocalization with β_1 implies the presence of the corresponding heterodimer receptors; $\alpha_1\beta_1$, $\alpha_3\beta_1$ and $\alpha_5\beta_1$, respectively (Korhonen *et al.*, 1991; Hynes, 1992). Other α subunits can interact with more than one β subunit, for example, $\alpha_6\beta_1$ and $\alpha_6\beta_4$. Integrins are involved in anchoring cells to the ECM and each $\alpha\beta$ receptor recognizes one or more extracellular ligand (Hynes, 1992). Integrins are also involved in modulating cellular activities by transmitting signals into and out of cells (Hynes, 1992), organization of the cytoskeleton (Akiyama *et al.*, 1989) and ECM (Darribere *et al.*, 1990) and in mediating important cell–cell interactions (Hynes, 1992). It has been reported that integrins may add to the cohesive strength necessary for the stationary epithelial sheet to remain intact

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Table 1. Monoclonal antibodies used in this study

Primary antibody	Specificity	Immunized animal	IgG isotypes	IgG isotype ($\mu\text{g ml}^{-1}$)	Source
102DF5	β_1	Mouse	IgG1	62.0	Ylanne and Virtanen (1989)
90BB10	β_3	Mouse	IgG1	78.0	Ylanne <i>et al.</i> (1988)
3E11	β_4	Mouse	IgG1	2.80	Bioquote, York
TS2/7	α_1	Mouse	IgG1	4.70	Hemler <i>et al.</i> (1984)
P1B5	α_3	Mouse	IgG1	3.70	Bioquote, York
B-5G10	α_4	Mouse	IgG1	4.10	Hemler <i>et al.</i> (1987)
P1D6	α_5	Mouse	IgG3	2.13	Bioquote, York
GoH3	α_6	Rat	IgG2a	ND	Sonnenberg <i>et al.</i> (1987)
INN-hPRL-3	Human prolactin	Mouse	IgG3	4.00	Serotec, Oxford

ND: not determined (used as undiluted culture supernatant).

under the effect of stretch (Larjava, 1991). The fetal membranes, including their epithelial sheets (amniotic and cytotrophoblast), stretch to double their post-delivery surface area to cope with uterine growth (Parry-Jones and Priya, 1976). It is to be anticipated, therefore, that integrins would be essential for maintaining the integrity of the fetal membranes. It has been reported that α_6 and β_4 may be involved in amniotic epithelium and trophoblast interactions with the ECM (Behzad *et al.*, 1991; Aplin, 1993).

To understand the possible role of different integrins in the fetal membranes, we examined the distribution of α_1 , α_3 – α_6 , β_1 , β_3 and β_4 integrin subunits in fetal membranes at term with an indirect immunofluorescence technique and confocal laser scanning microscopy. The latter allows more precise localization of membrane proteins since it produces images in which out-of-focus blur is essentially absent and, therefore, offers improved optical resolution and contrast (White *et al.*, 1987).

Materials and Methods

This study was approved by the Ethical Committee of Leicestershire Health Authority.

Tissues

Fetal membranes (amniochorion and the attached decidua) were obtained immediately after normal term, vaginal deliveries. The membranes were examined to exclude any with extensive tearing, separation of their layers and clinical signs of infection. Two long strips of the fetal membranes extending from the rupture site to the placental edge were obtained. Polymorphonuclear infiltration was used as an indicator of infection (Salafia *et al.*, 1989). Multiple specimens from 10 fetal membranes were included in this study. Specimens, taken from half-way between the rupture site and the site of placental attachment, were washed briefly in Tris-buffered saline, pH 7.6. Strips of the fetal membranes were rolled up with the amnion innermost, immediately frozen in liquid hexane and dry ice mixture (-70°C) and stored at -80°C until used. Additional specimens were fixed in 3% glutaraldehyde.

Antibodies and immunoglobulin

Monoclonal antibodies against integrin subunits were obtained commercially or as gifts (Table 1). Non-specific binding of the integrin antibodies was detected by staining control tissue sections with IgG at a concentration similar to that of IgG of the integrin antibodies. Purified mouse IgG (Sigma, Poole, Dorset) and anti-human prolactin antibody (used as a source for IgG3) were used.

Secondary antibodies (ICN Immunobiologicals, Thame, Bucks) were conjugated to fluorescein isothiocyanate. The working dilution was 1/500. To reduce non-specific binding of the secondary antibodies, normal serum (diluted 1/20) obtained from the animal species used to raise their secondary antibodies was included in the primary antibody preparation. Non-specific binding of the secondary antibodies was detected by staining control tissue sections with Tris-buffered saline instead of the integrin antibodies.

Indirect immunofluorescence microscopy

Cryostat sections were cut at a thickness of 10–12 μm and fixed in acetone at -20°C for 5 min. Autofluorescence was quenched by using 0.25 mol L-lysine l^{-1} at 4°C for 60 min. Tissue sections were incubated with the primary antibodies at 37°C for 60 min and, subsequently, to the appropriate secondary antibodies at 37°C for 30 min. Control sections were treated in the same way, except that primary integrin antibodies were replaced with purified IgG diluted in Tris-buffered saline, antibody against human prolactin or Tris-buffered saline. The tissue sections were examined using a confocal laser (argon ion) scanning microscope (Lasersharp MRC 600, Bio-Rad, Microscience Ltd, Hemel Hempstead, Herts). Images were obtained as single optical sections of the tissue preparation using a single channel fluorescence mode. The signals were collected over three frames using a real-time integration filter (Kalman filter) to remove photon noise.

Sequential cryostat tissue sections were stained with haematoxylin and eosin. Glutaraldehyde-fixed specimens were processed and embedded in Araldite and tissue sections (1 μm thick) were stained with alkaline toluidine blue solution (Malak *et al.*, 1993).

Results

Amniotic epithelium and its basement membrane

The amniotic epithelium was represented by a single layer of apparently simple, non-ciliated cuboidal cells (Fig. 1). The basement membrane appeared as a narrow band lying along the base of the amniotic epithelium.

Integrin subunits β_1 , α_5 and α_3 were basolaterally distributed in the amniotic epithelium (Fig. 2). The expression of α_1 was non-polarized with basolateral and apical localization and was also found in the cytoplasm. β_4 was specifically localized to the basal surface of these cells and although α_6 was also detected at highest intensity at this site, less immunoreactivity was detected in the basal part of their lateral surfaces (Fig. 3). The basal distribution of β_4 and α_6 was punctate.

Connective tissue

The connective tissue layers of the fetal membranes (compact, fibroblast, spongy and reticular layers) were composed mainly of a fibrillar network. The compact layer was acellular while the fibroblast and reticular layers contained many cells and the spongy layer contained very few cells (Fig. 1).

The fibroblast and reticular layers contained cells which expressed mainly β_1 , α_5 and α_1 and to a lesser degree α_3 (Fig. 4). No immunoreactivity related to β_4 or α_6 was detected in this layer (Fig. 5).

Cytotrophoblast and its basement membrane

The thickness of the cytotrophoblast layer was variable. Within the cytotrophoblast, a layer of tightly packed cells was detected that abutted onto the chorionic basement membrane and was of variable thickness. The intercellular spaces surrounding these cells were narrow, whereas the intercellular spaces of the cytotrophoblast located predominantly towards the decidua were wider (Fig. 1). In some sections, degenerate villi were found within the cytotrophoblast layer. These were surrounded by a basement membrane (apparent as a narrow dense eosinophilic band) and cytotrophoblast that were adhering tightly to each other.

The cytotrophoblast located predominately towards the chorionic basement membrane (Figs 4, 5) and that surrounding the degenerate villi (not shown) exhibited an intense pericellular, punctate expression of β_4 and α_6 (especially the basal

Fig. 1. (a) A transmitted light micrograph of a haematoxylin- and eosin-stained section of human fetal membrane. Scale bar represents 30 μm . (b) Diagrammatic representation of the layers of fetal membranes. (c) Toluidine blue-stained section of the cytotrophoblast layer (scale bar = 10 μm) showing the intercellular spaces between the basal and superficial cytotrophoblasts. AE: amniotic epithelium; ABM: amniotic basement membrane; BT: basal cytotrophoblasts (cells predominately located towards the basement membrane); C: compact layer; CBM: chorionic basement membrane; D: decidua; F: fibroblast layer; R: reticular layer; S: spongy layer; ST: superficial cytotrophoblasts (cells predominantly located towards the decidua).

Fig. 2. A confocal indirect immunofluorescence micrograph of the distribution of (a) β_1 , (b) α_5 , (c) α_3 and (d) α_1 integrin subunits in amniotic epithelium of human fetal membranes. Integrin subunits β_1 , α_5 and α_3 , were basolaterally expressed. The expression of α_1 was non-polarized with basolateral and apical localization; it was also found in the cytoplasm. Scale bars represent 5 μm .

surface adjacent to the chorionic basement membrane) (Fig. 5). The punctate expression was less prominent than that in the amniotic epithelium. These cells were less intensely positive for β_1 and α_3 , faintly positive for α_5 and mainly negative for α_1 (Fig. 4). The cytotrophoblast located predominantly towards the decidua expressed β_1 , α_5 , α_3 and α_1 (Fig. 4), but were less intensely positive for β_4 and α_6 (Fig. 5).

Decidua

The decidual cells exhibited less dense eosinophilic cytoplasm and more regular nuclei than did the cytotrophoblast (Fig. 1). Decidual cells expressed β_1 , α_1 and α_3 . They were very faintly positive for α_5 (Fig. 4) and negative for β_4 (Fig. 5). Immunoreactivity related to α_6 was very faint and was detected only in some tissue sections.

Blood vessels of the decidua expressed high intensity β_4 -related immunoreactivity, were less intensively positive for α_1 , α_5 and β_1 , faintly positive for α_6 , and negative for α_3 (not shown).

No convincing immunoreactivity with anti- α_4 and β_3 could be detected in any layers of the fetal membranes. In control tissue sections, no staining was detected except a very faint staining of the amniotic epithelium with IgG1 at concentrations of more than $46 \mu\text{g ml}^{-1}$. The distribution of integrin subunits in the various layers of the fetal membranes is shown (Table 2).

Discussion

In this study, we report the distribution of integrin subunits in human fetal membranes at term by indirect immunofluorescence, using antibodies against these subunits. However, the distribution of integrin heterodimer receptors can be inferred from such studies for two reasons. First, the individual α or β subunits of integrins are not transported to the cell surface (Springer *et al.*, 1987; Heino *et al.*, 1989; Rosa and McEver, 1989) and second, the detection of the α_1 , α_3 or α_5 integrin subunits in colocalization with β_1 implies the presence of the corresponding heterodimer receptors: $\alpha_1\beta_1$, $\alpha_3\beta_1$ and $\alpha_5\beta_1$, respectively (Korhonen *et al.*, 1991; Hynes, 1992). Integrin subunit α_6 may form the heterodimers $\alpha_6\beta_1$ and $\alpha_6\beta_4$ with β_1 and β_4 , respectively. However, immunoprecipitation experiments have suggested that $\alpha_6\beta_4$ is the major α_6 -containing complex on cells that express α_6 , β_1 and β_4 subunits (Hemler *et al.*, 1989). Although the ECM ligands of most integrin receptors have been identified (Hemler *et al.*, 1989), that of $\alpha_6\beta_4$ integrin has yet to be established. However, it may act as a laminin receptor (Lee *et al.*, 1992).

The presence of integrins at cell–basement membrane interfaces and the presence of their ligands in the basement membrane may indicate that they function as basement membrane receptors (Korhonen *et al.*, 1991). Integrin expression was different at the various cell–basement interfaces found in fetal membranes, that is, at the amniotic epithelium ($\alpha_6\beta_4$, $\alpha_5\beta_1$, $\alpha_3\beta_1$), cytotrophoblast ($\alpha_6\beta_4$ and to a lesser extent $\alpha_3\beta_1$),

Fig. 3. A confocal indirect immunofluorescence micrograph of the distribution of (a) β_4 and (b) α_6 integrin subunits in amniotic epithelium of human fetal membranes. β_4 was specifically localized to the basal surface of the epithelium and, although α_6 was also detected at highest intensity at this site, less immunoreactivity was detected in the basal part of the lateral surfaces. Scale bars represent 5 μm . Pseudocolour intensities: white > red > yellow > green > blue (negative staining).

Fig. 4. A confocal indirect immunofluorescence micrograph of the distribution of (a) β_1 , (b) α_5 , (c) α_1 and (d) α_3 integrin subunits in human fetal membranes. β_1 , α_5 , α_1 , and to a lesser extent α_3 , were expressed in the connective tissue layers. The cytotrophoblasts located predominantly towards the basement membrane were positive for β_1 and α_3 and faintly positive for α_5 and α_1 . The cytotrophoblasts located predominantly towards the decidua expressed β_1 , α_5 , α_3 and α_1 . Decidual cells expressed β_1 , α_1 and α_3 and were faintly positive for α_5 . Scale bars represent 50 μm . AE: amniotic epithelium; BT: basal cytotrophoblasts (cells located predominantly towards the basement membrane); CT: connective tissue; D: decidua; DV: degenerate villus; F: fibroblast layer; R: reticular layer; ST: superficial cytotrophoblasts (cells located predominantly towards the decidua).

decidual cells ($\alpha_3\beta_1$, $\alpha_1\beta_1$) and decidual blood vessels ($\alpha_6\beta_4$, $\alpha_5\beta_1$, $\alpha_3\beta_1$). This indicates that these cells may differentially recognize basement membrane components.

The presence of $\alpha_6\beta_4$ in the amniotic epithelium and cytotrophoblast is in agreement with other reports (Behzad

et al., 1991; Aplin, 1993). The punctate expression of $\alpha_6\beta_4$ integrin indicates that it may be localized to well-defined structures. Indeed, $\alpha_6\beta_4$ has been reported to represent one of the two transmembrane proteins present in hemidesmosomes (Stepp *et al.*, 1990). Hemidesmosomes mediate epithelial and

Fig. 5. A confocal indirect immunofluorescence micrograph of the distribution of (a) β_4 and (b) α_6 integrin subunits in human fetal membranes. β_4 and α_6 were negative in connective tissue and decidua. The cytotrophoblasts located predominantly towards the basement membrane exhibited an intense staining for β_4 and α_6 , but cells located predominantly towards the decidua were less intensely positive for β_4 and α_6 . Scale bars = 100 μm . AE: amniotic epithelium; BT: basal cytotrophoblasts (cells located predominantly towards the basement membrane); CT: connective tissue; D: decidua; ST: superficial cytotrophoblasts (cells located predominantly towards the decidua). Pseudocolour intensities: white > red > yellow > green > blue (negative staining).

Table 2. Distribution of integrin subunits in human fetal membranes (amniochorion and decidua)

Source	Integrin subunit							
	α_1	α_3	α_4	α_5	α_6	β_1	β_3	β_4
Amniotic epithelium	+	+	–	+	+	+	–	+
Connective tissue cells	+	–/+	–	+	–	+	–	–
Basal cytotrophoblast	–	–/+	–	–/+	+	–/+	–	+
Superficial cytotrophoblast	+	+	–	+	–/+	+	–	–/+
Decidual cells	+	+	–	–/+	–	+	–	–

+: intense immunoreactivity present; –/+ : less intense or equivocal immunoreactivity; – : no reactivity detected. ^aNon-polarized expression; ^bbasolateral expression (α_6 is mainly basal); ^cbasal expression.

mesenchymal cell attachment to the underlying basal lamina and are found to be numerous along the basal surface of amniotic epithelium (Aplin *et al.*, 1985; Malak *et al.*, 1993). However, the presence of hemidesmosomes at the basal surface of fetal membrane cytotrophoblast is controversial (Wang and Schneider, 1983; Schmidt, 1992) and endothelial cells lack typical hemidesmosomes (Hieda *et al.*, 1992). Therefore, the adhesive function of $\alpha_6\beta_4$ in blood vessels, and possibly in cytotrophoblasts, may be distinct from its role in hemidesmosome-mediated attachment. The exact localization of β_4 in the absence of hemidesmosomes remains to be determined.

The basal localization of β_1 ($\alpha_3\beta_1$, $\alpha_5\beta_1$) in the amniotic epithelium and pericellular localization of β_1 ($\alpha_3\beta_1$, $\alpha_1\beta_1$) integrins in decidual cells suggest that they may be used as

basement membrane receptors at these sites. A similar role was described for β_1 ($\alpha_3\beta_1$, $\alpha_2\beta_1$) integrins in skin (Carter *et al.*, 1990a, b). Recently, $\alpha_5\beta_1$ was found to be localized specifically to the basal membrane of the basal cells of the stratified squamous epithelium of the cornea (Stepp *et al.*, 1993), suggesting that it may have a role in cell–basement membrane adhesion.

β_1 ($\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$) integrins were laterally localized in the amniotic epithelium; however, their ECM ligands (fibronectin, laminin and collagens I and VI) have not been detected in the amniotic cell–cell contact regions. This suggests that β_1 integrins may play a role in cell–cell interactions. Indeed, β_1 ($\alpha_2\beta_1$, $\alpha_3\beta_1$) integrins have been found to be concentrated in cell–cell contacts of keratinocytes, where they perform a functional role in the maintenance of cell–cell interactions

(Larjava *et al.*, 1990; Marchisio *et al.*, 1990; Carter *et al.*, 1990b). The exact nature of this role is not fully characterized but it has been proposed (Larjava *et al.*, 1990) that an unidentified ligand molecule mediates cell–cell adhesion indirectly by bridging integrin receptors located in two adhering cells. Alternatively, it may involve direct association of integrin dimers on adjacent cells (Larjava *et al.*, 1990) or direct interaction of integrins with unidentified membrane co-receptors in adhering cells (Carter *et al.*, 1990b).

Cells of the reticular and fibroblast layers expressed β_1 integrin similar to fibroblasts (Ylanne and Vitranen, 1989), smooth muscle cells (Belkin *et al.*, 1990) and macrophages (Kohn and Klingemann, 1991). However, term placental villous stromal cells do not express β_1 (Korhonen *et al.*, 1991), which may reflect different cellular populations or cell–matrix interactions in these sites. The ECM of the connective tissue contains collagens, laminin and fibronectin (Klima and Schmidt, 1988; Malak *et al.*, 1993) which are known ligands for the β_1 ($\alpha_1\beta_1$, $\alpha_5\beta_1$, $\alpha_3\beta_1$) integrins.

Within the cytotrophoblast layer, two subpopulations with distinctive patterns of integrin expression were detected. This integrin expression may reflect the reported difference in the composition of ECM surrounding these two cytotrophoblast subpopulations (Malak *et al.*, 1993). The cytotrophoblast located predominantly towards the chorionic basement membrane expressed pericellular $\alpha_6\beta_4$ and to a lesser extent $\alpha_3\beta_1$ (collagens, fibronectin and laminin receptors). The ECM surrounding these cells is mainly composed of basal lamina-like material (Malak and Bell, 1994). It is stained positive for laminin, collagen IV and fibronectin (Aplin and Campbell, 1985; Klima and Schmidt, 1988; Malak *et al.*, 1993) but it does not contain fibrillar collagens, for example, collagens I and III (Malak *et al.*, 1993).

In contrast, the cytotrophoblast located predominantly towards the decidua expressed mainly $\alpha_5\beta_1$ (fibronectin receptor), $\alpha_3\beta_1$ and $\alpha_1\beta_1$ (laminin and collagens receptor) and less $\alpha_6\beta_4$. The ECM at this site contains fibrillar collagens (collagen III) (Malak *et al.*, 1993), fibrillin-containing microfibrils and to a lesser extent basal-lamina-like material (Malak and Bell, 1994).

The pericellular expression of $\alpha_6\beta_4$ in cytotrophoblast may be an example of cell–matrix interactions that involve β_4 but are not hemidesmosome mediated. Hemidesmosomes have not been reported to mediate attachment of cytotrophoblasts to the ECM.

The intense expression of $\alpha_5\beta_1$ by the cytotrophoblast located near to the decidua is of interest. Although fibronectin, the $\alpha_5\beta_1$ ligand, is widely distributed in fetal membranes (Aplin and Campbell, 1985; Klima and Schmidt, 1988; Lockwood *et al.*, 1991), $\alpha_5\beta_1$ expression follows most closely the distribution of the oncofetal isoform of fibronectin in the fetal membranes (Feinberg *et al.*, 1991; Lockwood *et al.*, 1991). Oncofetal fibronectin binds $\alpha_4\beta_1$ integrin via its alternatively spliced IIICS region; however, we did not detect α_4 integrin subunit in the fetal membranes. This finding suggests that $\alpha_5\beta_1$ may function as a receptor for oncofetal fibronectin in fetal membranes. The presence of α_5 and absence of α_4 integrin subunits on cytotrophoblasts were also reported in normal human implantation sites (Korhonen *et al.*, 1991; Damsky *et al.*, 1992), where oncofetal fibronectin is localized to the ECM connecting

extravillous cytotrophoblasts and cytotrophoblastic cell columns to the uterine decidua (Feinberg *et al.*, 1991).

This difference in integrin receptors among the cells of the cytotrophoblast supports the previous histological and antigenic evidence for heterogeneity in the cytotrophoblastic layer of the fetal membranes (Bulmer and Johnson, 1985; Yeh *et al.*, 1989; Malak *et al.*, 1993). However, whether the two subpopulations, defined by differential integrin expression, correspond to the cytotrophoblast subpopulations defined histologically or antigenically, remains to be determined. The difference in integrin expression of the polarized cytotrophoblast ($\alpha_6\beta_4^+$, $\alpha_3\beta_1^{+/-}$) and the non-polarized cells in the vicinity of decidua ($\alpha_5\beta_1^+$, $\alpha_1\beta_1^+$, $\alpha_6\beta_4^{+/-}$) is similar to the integrin expression of the polarized and non-polarized cytotrophoblast of the chorionic villi and was used as an indication of cytotrophoblast differentiation by Damsky *et al.* (1992), a process that may also occur in fetal membrane cytotrophoblast.

This study suggests that integrins are involved in cell–matrix and cell–cell interactions in fetal membranes, and may, therefore, play an important role in maintaining the normal structural and functional integrity and stability of these tissues. However, assays of function are needed to establish a complete understanding of the interactions occurring in integrin-mediated cell adhesion.

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