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Expression of cell adhesion in molecules in murine placentas and a placental cell line

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Expression of Cell Adhesion Molecules in Murine Placentas and a Placental Cell Line[1]

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ABSTRACT

Integrins and vascular cell adhesion molecule-1 (VCAM-1) are required for normal placental development. In this study, integrin subunits α4, αv, β1, and β3, and VCAM-1 were investigated for expression in uteroplacental units (gestation day [g.d.] 6 and 8) and placentas (g.d. 10, 12, 14, 16, and 18) of Swiss-Webster mice. All subunits and VCAM-1 mRNA (identified by reverse transcriptase polymerase chain reaction [RT-PCR]) and protein (detected by immunofluorescence) were present in all tissues throughout gestation. VCAM-1 was expressed strongly in the ectoplacental cone and trophoblast giant cells, α4 was expressed strongly by trophoblast giant cells and moderately by spongiotrophoblast and labyrinthine trophoblast, and αv was expressed more strongly in the spongiotrophoblast than in the labyrinthine zone. The β1 was more strongly expressed in the labyrinthine than the spongiotrophoblast zone, while β3 and VCAM-1 were essentially equal in the two zones. Trophoblast-like SM9–1 cells were positive for all of the adhesion molecules when tested by RT-PCR and immunocytochemistry. Adhesion molecule expression in SM9–1 cells was consistent with expression in the labyrinthine zone. Collectively, the results of this study demonstrate that murine placentas contain mRNA and protein for α4, αv, β1, β3, and VCAM-1, and that expression is cell-specific. These results and the identification of an adhesion molecule-expressing trophoblastic cell line should facilitate future studies on the function of adhesion molecules in placental development.

INTRODUCTION

The integrin family of glycoproteins is expressed in the uteri of all species studied to date. Integrin expression patterns are predictably associated with blastocyst development, implantation, and development of the placenta regardless of the type of placentation [1–7]. These cation-dependent heterodimeric proteins, which comprise non-covalently bound α and β subunits, participate in cell-to-cell and cell-to-substrate adhesion [1]. At least 23 heterodimer combinations are known to be formed from the currently identified 17 α and 8 β subunits [8, 9]. The ligand specificity of the integrin heterodimer is determined by the α and β subunit combinations [1].

Variable patterns of expression of certain integrin subunits in the murine uterus during the reproductive cycle and pregnancy suggest that these proteins are directly involved in the process of implantation and normal placental formation and function [10–12]. The developmental regulation of their expression in the mouse blastocyst is also consistent with their potential involvement in implantation [2]. Integrins participate in a number of functions required for placentation, including cell adhesion, migration, and invasion. Additionally, integrins are involved in bidirectional signaling [13]. They are or may be activated in response to an agonist or changes in the cytoskeletal assembly (inside-out signaling) and, once bound to a ligand, can induce a multitude of intracellular events that can lead to changes in cell migration and gene activation (outside-in signaling). Thus, integrins are attractive as potential participants in the complex events of implantation and placentation.

A number of integrin subunits (α1, α2, α4-α7, β1, and β3) have been identified in normal trophoblast cells of preimplantation and implanting mouse embryos [10], and it appears that abnormal expression by integrin subunits α4, αv, and β1 may be associated with implantation failure and abnormal placental development [14]. In integrin subunit α4-null mice, the chorionic and allantoic membranes fail to fuse, and the fetus dies on or about gestation day (g.d.) 9 [15]. Similar defects occur in fusion of the chorioallantoic membrane in vascular cell adhesion molecule-1 (VCAM-1)-deficient mice [16, 17]. VCAM-1 is the counter-receptor for α4β1. Mice carrying mutations in the αv integrin subunit demonstrate defects in blood vessel formation which result in vascular hemorrhage. Although many αv integrin subunit-null mice die immediately after birth, some die during gestation [18], indicating possible placental abnormalities. Mice with a targeted deletion in the β1 integrin subunit, which binds to 11 different α subunits [1], die during the periimplantation stage as a result of inner cell mass failure [19, 20].

Although these membrane-bound proteins have been determined to be critical for proper placental development in the mouse by knock-out technology, their patterns of expression within the placenta as a function of gestation have not been well characterized. Therefore, in the present study, immunohistochemical and reverse transcription-polymerase chain reaction (RT-PCR) were used to identify the spatial and temporal expression of integrin subunits (α4, αv, β1, and β3) and of VCAM-1 protein and mRNA in the murine placenta. Additionally, a cell line derived from g.d. 9 Swiss-Webster mouse placentas was evaluated for integrin subunit and VCAM-1 expression using immunocytochemistry and RT-PCR techniques to establish its potential for use as an in vitro model for studying the regulation and function of trophoblast adhesion molecules.

MATERIALS AND METHODS

Mice and Tissue Collection

A breeding colony of Swiss-Webster mice maintained in accordance with the guidelines set forth by the Animal Use and Care Committee of the University of Kansas Medical Center supplied the tissues for this study. Uteri were collected from pregnant mice (presence of a vaginal plug indicated g.d. 1) on g.d. 6, 8, 10, 12, 14, 16, 18 (3 mice/g.d.).

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TABLE 1. Antibodies against cell adhesion molecules.

Specificity	Source	Species	IIF Conc.†
α4	Chemicon*	Rabbit	1/200
αv	Chemicon	Rabbit	1/200
β1	Chemicon	Rabbit	1/200
β3	Chemicon	Rabbit	1/200
VCAM-1	Chemicon	Rat	1/100

* Chemicon International Inc., Temecula, CA.
† IIF, indirect immunofluorescence; stock concentration is 1 mg/ml.

For immunofluorescent microscopy, uteroplacental units (embryonic and uteroplacental tissues) were cross-sectioned and frozen in Tissue-Tek Optimal Cutting Temperature (OCT) compound (Miles Inc., Elkhart, IN) in liquid nitrogen vapor and stored at −80°C. Additionally, placental samples were flash-frozen in liquid nitrogen and stored at −80°C for analysis by RT-PCR.

Cell Culture

A cell line derived from a g.d. 9 Swiss-Webster mouse placenta (SM9-1) using methods that have been described [21] (the presence of a vaginal plug was considered g.d. 0) was grown in 75-cm² tissue culture flasks in RPMI 1640 containing antibiotics and supplemented with 10% fetal bovine serum and 50 μM mercapto-ethanol (Sigma Chemical Co., St. Louis, MO) at 37°C, 5% CO_2. All experiments were done on low-passage (passages 10–13) placental cell lines. For immunocytochemistry studies, SM9-1 cells were grown on LabTek Chamber Slides (Nunc, Inc., Naperville, IL). For RT-PCR, SM9-1 cells from two 75-cm² flasks, approximately 80% confluent, were harvested by scraping the cells from the flasks, pelleting, and freezing at −80°C for later extraction of poly(A)$^+$ mRNA.

Indirect Immunofluorescence

Sections of implantation sites and placentas (4- to 8-μm-thick sections) from three different mice per g.d. (n = 3) were cut using a cryostat (Reichert-Jung HistoStat; Cambridge Instruments, Buffalo, NY) and mounted on Plus-coated Superfrost slides (Fisher Scientific, Pittsburgh, PA). The tissues and SM9-1 cells adherent to LabTek Chamber Slides were washed in PBS and fixed in −20°C acetone for 10 min, rinsed in PBS with 0.3% Tween-20, and blocked with 1% BSA and 3% goat serum in PBS for 30 min at room temperature. The tissue sections and cells on glass slides were subsequently incubated with a primary antibody (Table 1) or matching control IgG (negative control) for 1 h at 37°C. After several washes in PBS containing 0.3% Tween-20, the tissue sections and cells were incubated with a biotinylated goat secondary antibody against the host species of the primary antibody. Samples were incubated with fluorescein-streptavidin (Sigma Chemical Co.) for 30 min at room temperature. All slides were overlaid with a coverglass and mounting medium containing glycerol-gelatin with 1% phenol (Sigma Chemical Co.). Slides were viewed with a Nikon Axiophot2 equipped with epifluorescence (Nikon Instruments, Garden City, NY), photographed on 1000 Royal Gold or T-Max 3200 film (Eastman Kodak, Rochester, NY), and scored by two independent observers using the scoring system of negative (−), weak (±), moderate (+), or strong (++) staining as described previously [22].

RT-PCR

Poly(A)$^+$ mRNA was isolated from SM9-1 cells and Swiss-Webster mouse uteroplacental units (g.d. 6 and 8) and placentas (g.d. 10, 12, 14, 16, 18) from 3 different mice per g.d. (n = 3) using mRNA isolation kits (FastTrack; Invitrogen, San Diego, CA). With the exception of murine leukemia virus (MuLV) reverse transcriptase (mRT), mRT buffer, and RNasin ribonuclease (RNase) inhibitor, which were purchased from Promega (Madison, WI), all of the components for these procedures were purchased from Perkin-Elmer (Norwick, CT). RT-PCR analysis employed primers and conditions that have been previously described [23–26]. Table 2 gives the primer sequences and cycle conditions. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA signals were used as loading controls. Five hundred nanograms of sample was reverse-transcribed in 20 μl of reaction mixture containing 5 mM $MgCl_2$, 1 M each of dATP, dCTP, dGTP, and dTTP, 10 U/ml RNasin, 10 U/m; mRT, 5 μM oligo $dt_{(16)}$, and buffer (25°C, 10 min; 42°C, 60 min; 99°C, 5 min; 5°C, 5 min; Perkin-Elmer Model 2400 thermocycler). The RT reaction was followed by a PCR reaction conducted in a total volume of 100 μl that contained 2 mM $MgCl_2$, single-strength PCR buffer II, 2.5 U/100 μl AmpliTaq DNA polymerase, and 1 μM each of 5′ and 3′ primers. PCR products and molecular weight markers were separated on 3% agarose gels containing ethidium bromide and were visualized by ultraviolet light.

RESULTS

Uteroplacental units (g.d. 6 and 8) and placentas (10, 12, 14, 16, and 18) were examined for the expression of integrin subunits (α4, αv, β1, and β3) and VCAM-1 proteins. The results are summarized in Table 3.

TABLE 2. PCR primers and cycling conditions.

Protein		Sequence	Temp	Cycle	bp	Ref.
α4	5′	5′-TCCAAAAATCCCCTATCCTCTC-3′	58	30	660	[26]
	3′	5′-AAGCCATCCTGCTGCAAAC-3′				
αv	5′	5′-GTTGGGAGATTAGACAGAGGA-3′	53	25	288	[24]
	3′	5′-CAAAACAGCCAGTAGCAACAA-3′				
β1	5′	5′-TGTTCAGTGCAGAGCCTTCA-3′	53	25	452	[24]
	3′	5′-CCTCATACTTCGGATTGACC-3′				
β3	5′	5′-GGGGACTGCCTGTGTGACTC-3′	58	30	521	[24]
	3′	5′-CTTTTCGGTCGTGGATGGTG-3′				
VCAM-1	5′	5′-CAACGATCTCTGTACATCCC-3′	55	25	839	[25]
	3′	5′-AGAGGCTGTACACTCTGCCT-3′				
G3PDH	5′	5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′	55	25	983	[23]
	3′	5′-CATGTAGGCCATGAGGTCCACCAC-3′				

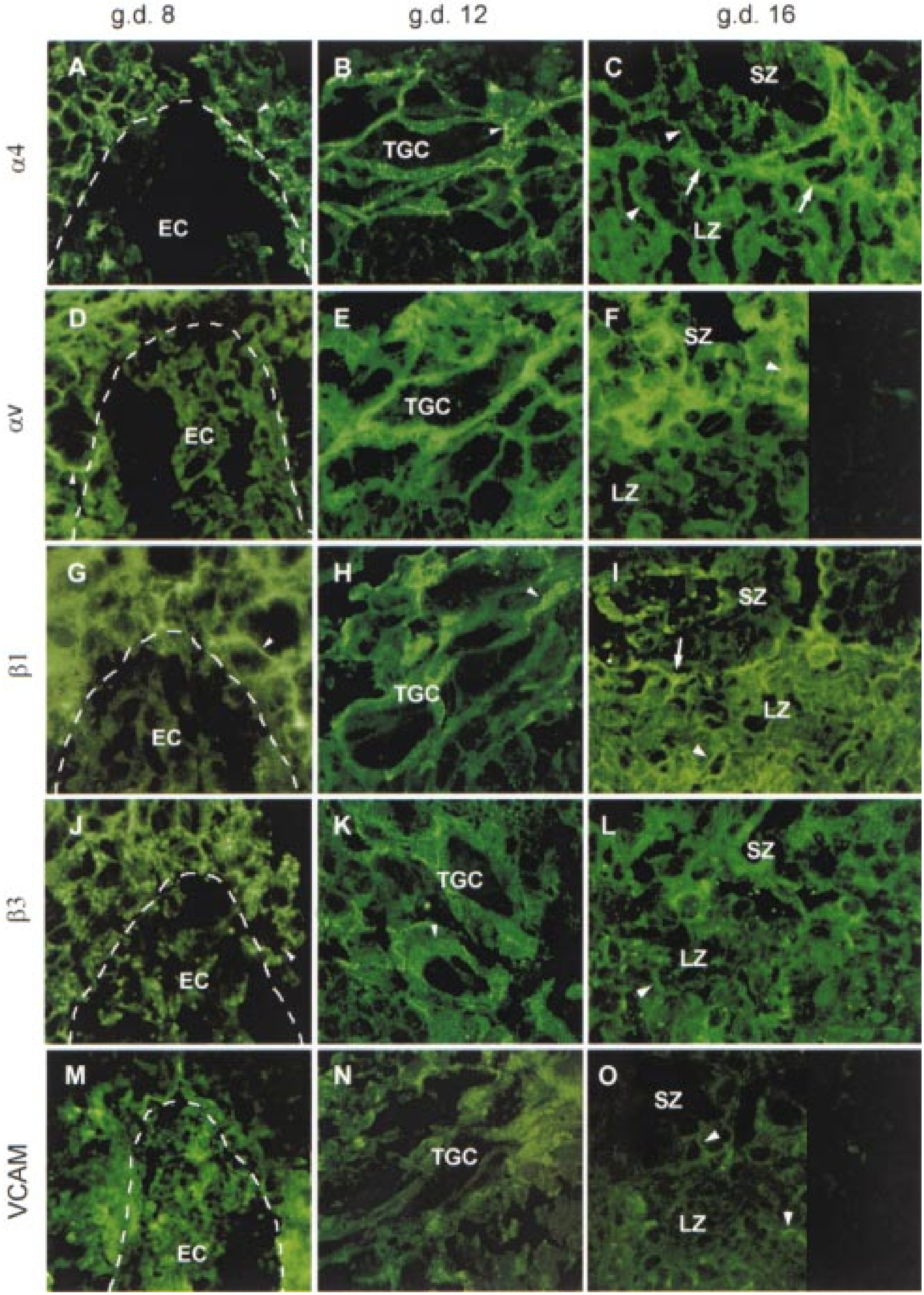
g.d. 8
g.d. 12
g.d. 16
α4
αv
β1
β3
VCAM
A
EC
B
TGC
C
SZ
LZ
D
EC
E
TGC
F
SZ
LZ
G
EC
H
TGC
I
SZ
LZ
J
EC
K
TGC
L
SZ
LZ
M
EC
N
TGC
O
SZ
LZ

TABLE 3. Summary of integrin subunit and VCAM-1 protein expression in trophoblast cells.*
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Gestation day	Cells	Integrin subunits α4	αv	β1	β3	VCAM-1
6	EPC	+	+	+	+	++
	TGC	++	+	+	+	++
8	EPC	+	+	+	+	++
	TGC	++	+	+	+	++
10	SZ	+	++	+	+	±
	LZ	+	+	++	+	±
12	SZ	+	++	+	+	±
	LZ	+	+	++	+	±
14	SZ	+	++	+	+	±
	LZ	+	+	++	+	±
16	SZ	+	++	+	+	±
	LZ	+	+	++	+	±
18	SZ	+	++	+	+	±
	LZ	+	+	++	+	±

* Abbreviations: EPC, ectoplacental cone; TGC, trophoblastic giant cells; LZ, labyrinthine zone; SZ, spongiotrophoblast zone; ++, high expression; +, moderate expression; ±, weak expression.

Placental Expression of Integrin Subunits and VCAM-1 Proteins

In the first series of experiments, the expression of integrin subunits α4, αv, β1, and β3 in uteroplacental units and placentas was investigated using immunofluorescence. All integrin subunits and VCAM-1 exhibited specific patterns of expression that varied as a function of stage of gestation. Although the purpose of this study was to examine adhesion molecules in the placenta, staining in the decidua was also noted.

Figure 1A shows that at g.d. 8, the α4 integrin subunit was expressed in the ectoplacental cone and trophoblast giant cells of the postimplantation conceptus. Trophoblast giant cells throughout gestation demonstrated an intense punctate pattern of expression that appeared to be colocalized with the plasma membrane surface (Fig. 1B). From g.d. 10 through term, staining was detected in all placental zones. The spongiotrophoblast and labyrinthine zones and chorionic plate were intensely labeled. As seen in Figure 1C, the spongiotrophoblast zone had an intense punctate staining pattern at points of cell-to-cell contact and a diffuse staining pattern in the cytoplasm. The cells in the labyrinthine zone expressed a diffuse pattern of staining in the cytoplasm with slightly more intense labeling at points of cell-to-cell contact. Interestingly, a single band of cells located between the labyrinthine and spongiotrophoblast zones expressed a higher intensity of staining than either the cells of the labyrinthine or spongiotrophoblast zones.

Figure 1, D–F, demonstrates the staining pattern of αv in the murine placenta. In early postimplantation placenta, this integrin subunit was localized to the ectoplacental cone and trophoblast giant cells (Fig. 1D). The trophoblast giant cells of later stages (> g.d. 10) had a diffuse staining pattern colocalized to the plasma membrane (Fig. 1E). The spongiotrophoblast and labyrinthine zones and chorionic plate demonstrated staining with gestational variations, although the spongiotrophoblast zone was more intensely labeled than either the labyrinthine zone or the chorionic plate. The spongiotrophoblast zone showed decreased staining intensity in g.d. 16 and 18 placental samples, but staining still remained more intense than in other compartments (Fig. 1F). Staining of the spongiotrophoblast cells for αv highlighted a perinuclear localization and gave a diffuse staining pattern throughout the cytoplasm and on the cell plasma membrane. Staining in the labyrinthine zone was also diffuse.

Expression of the β1 integrin subunit in the developing placenta at g.d. 8 is shown in Figure 1, G–I. The ectoplacental cone and trophoblast giant cells of the early postimplantation conceptus stained strongly for the β1 integrin subunit (Fig. 1G). Figure 1H shows trophoblast giant cells from a g.d. 12 placenta, which exhibited the typically strong staining in this cell type. The stain was localized to the plasma membrane and appeared to exhibit both a diffuse and punctate pattern on the cell surface. The spongiotrophoblast and labyrinthine zones and chorionic plate were strongly stained although there was variability among cells in the spongiotrophoblast zone (Fig. 1I). All cells within the labyrinthine zone displayed a diffuse staining pattern in the cytoplasm and on the cell surface.

The ectoplacental cone and trophoblast giant cells of early postimplantation conceptus (g.d. 8) were positively stained (Fig. 1J) for the β3 integrin subunit. The trophoblast giant cells of the mature placenta (g.d. 12) displayed a fine punctate pattern on plasma membrane (Fig. 1K). The cells of the spongiotrophoblast and labyrinthine zones and chorionic plate were less intensely stained. Although the staining intensities of the spongiotrophoblast and labyrinthine cells were generally similar, compartment-specific patterns were detectable (Fig. 1L). The β3 integrin in the labyrinthine zone was localized predominantly to the cell membrane, whereas the cells of the spongiotrophoblast layer were diffusely stained throughout the cytoplasm. No differences were detected through gestation.

Figure 1M demonstrates that at g.d. 8, the counter receptor for the α4β1 integrin, VCAM-1, was detected on the ectoplacental cone and in trophoblast giant cells of the early postimplantation conceptus. The trophoblast giant cells from the mature placenta (g.d. 12) were weakly positive for VCAM-1 and displayed a diffuse staining pattern on the cell periphery (Fig. 1N). The spongiotrophoblast zone was weakly positive with a diffuse pattern, as illustrated for g.d. 16 in Figure 1O. The labyrinthine zones and chorionic plate contained little VCAM-1 although some cells were more intensely labeled than others. In general, the staining pattern was diffuse in both the labyrinthine and spongiotro-

FIG. 1. Uteroplacental units from g.d. 8 (**A, D, G, J, M**) and placental sections from g.d. 12 (**B, E, H, K, N**) and g.d. 16 (**C, F, I, L, O**) were stained by indirect immunofluorescence. **A**) g.d. 8, anti-α4 integrin. Arrowhead points to trophoblast giant cell. **B**) g.d. 12, anti-α4 integrin. Arrowhead indicates site of cell-to-cell contact. **C**) g.d. 16, anti-α4 integrin. Arrows indicate a band of strongly positive cells at the LZ/SZ junction. Arrowheads point to sites of cell-to-cell contact. **D**) g.d. 8, anti-αv integrin. Arrowhead points to trophoblast giant cell. **E**) g.d. 12, anti-αv integrin. **F**) g.d. 16, anti-αv integrin. Arrowhead indicates point of cell-to-cell contact. **G**) g.d. 8, anti-β1 integrin. Arrowhead points to trophoblast giant cell. **H**) g.d. 12, anti-β1 integrin arrow indicates a point of contact between neighboring cells. **I**) g.d. 16, anti-β1 integrin. Arrow indicates a site of cell-to-cell contact. **J**) g.d. 8, anti-β3 integrin. Arrowhead points to trophoblast giant cell. **K**) g.d. 12, anti-β3 integrin. Arrowhead indicates a site of cell-to-cell contact. **L**) g.d. 16, anti-β3 integrin. Arrowhead indicates a site of cell-to-cell contact. **M**) g.d. 8, anti-VCAM-1. Arrowhead points to trophoblast giant cell. **N**) g.d. 12, anti-VCAM-1. **O**) g.d. 16, anti-VCAM-1. Arrowheads indicate sites of cell-to-cell contact. EC, Ectoplacental cone; TGC, trophoblast giant cells; LZ, labyrinthine zone; SZ, spongiotrophoblast zone. Matching IgG controls were used for every tissue; examples are shown as insets in **F** and **O**. Dashed line indicated approximate boundary of ectoplacental cone. Original magnification: ×20 for **A, D, G, J, M**; ×40 for **B, E, H, K, N**; ×20 for **C, F, I, L, O**.

TABLE 4. Notable features of the integrin subunits and VCAM-1.

Adhesion molecule	Feature
α4 integrin	- strong at points of cell-to-cell contact - intense at inter-zone borders, especially trophoblast giant cells - little cytoplasmic staining
αv integrin	- strongest in spongiotrophoblast zone; decreased there on g.d. 16, 18 - diffuse staining in cells - present at points of cell-to-cell contact
β1 integrin	- strongest in labyrinthine zone - variable staining in the spongiotrophoblast zone - strong at points of cell-to-cell contact
β3 integrin	- staining was diffuse and localized to the cell periphery - present at points of cell-to-cell contact in trophoblast giant cells - no differences in staining intensities between zones
VCAM-1	- strongest in g.d. 6 and 8 ectoplacental cone and trophoblast giant cells - present at points of cell-to-cell contact - weak in spongiotrophoblast and labyrinthine zones and trophoblast giant cells of the defined placenta

phoblast compartments, with some staining localized to points of cell-to-cell contacts.

The notable staining features for the integrin subunits α4, αv, β1, and β3 and VCAM-1 are summarized in Table 4.

Placental Expression of Integrin Subunits and VCAM-1 mRNA

The above experiments indicated that placental cells express integrin subunit and VCAM-1 proteins. Thus, the next set of experiments was conducted to determine whether these proteins are transcribed within the placenta. Poly(A)$^+$ mRNA was isolated from uteroplacental units (g.d. 6, 8) and placentas (g.d. 10, 12, 14, 16, and 18). Messenger RNA for the specific integrin subunits α4, αv, β1, and β3 and VCAM-1 was detected in all uteroplacental units and placentas tested. Figure 2 shows semiquantitative RT-PCR analysis of g.d. 8 uteroplacental units and g.d. 16 placentas. Integrin subunit and VCAM-1 mRNA were present throughout gestation, indicating that these proteins were transcribed within the placenta. Strikingly, levels for β3 mRNA were low when compared to G3PDH mRNA levels even though the protein was easily detected by indirect immunofluorescence (Fig. 1, J–L). Conversely, VCAM-1 mRNA was detected at a higher concentration than G3PDH mRNA, but the protein was weakly expressed in the mature placenta. These data suggest differential regulation at the levels of transcription and translation.

Expression of Integrin Subunits and VCAM-1 in a Murine Trophoblastic Cell Line

Having determined that protein and message for the integrin subunits and VCAM-1 were present in the developing placenta, the next set of experiments was conducted to investigate the presence of the same panel of integrin subunits and VCAM-1 proteins and mRNA in the murine trophoblastic cell line SM9-1. As demonstrated in Figure 3, the pattern and intensity of integrin subunits and VCAM-1 expression in SM9-1 cells paralleled expression of the same proteins in placentas. Figure 3A shows the staining pattern of the α4 integrin subunit as detected by indirect immunofluorescence. SM9-1 cells exhibited a strong perinuclear

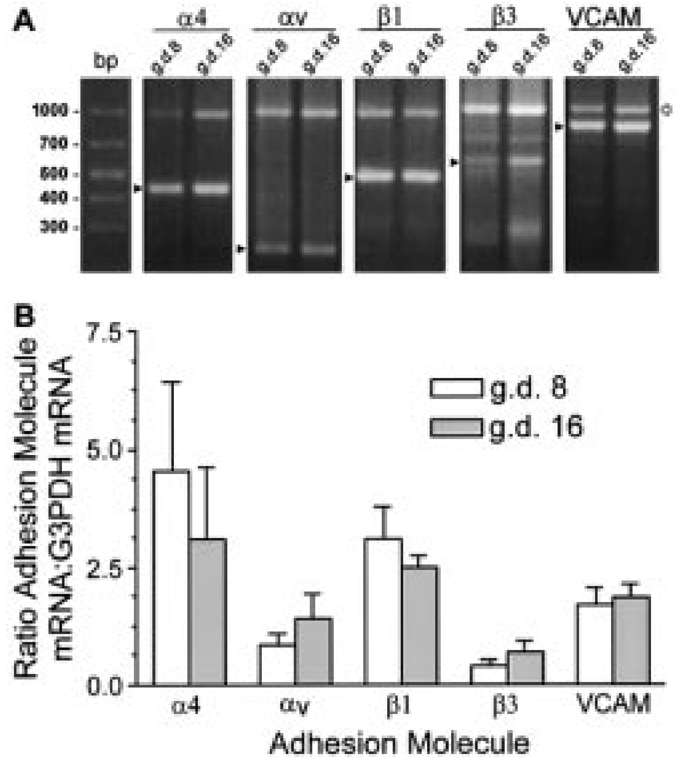

FIG. 2. Identification of α4, αv, β1, β3, VCAM-1, and G3PDH mRNA in poly(A)$^+$ mRNA isolated from g.d. 8 and 16 placental samples. **A**) The expected amplicons (arrowheads) for each integrin subunit and VCAM-1 were present in RNA from isolated placental tissue. G3PDH (open arrow) was used as a loading control in all lanes. **B**) The ratio of adhesion molecule mRNA:G3PDH mRNA obtained by scanning densitometry (n = 3).

staining pattern and a distinct but diffuse pattern along cell-cell borders. As seen in Figure 3B, the αv integrin subunit was detected in a perinuclear arrangement and was found on cell-cell borders. The antibody to the αv subunit revealed a distinct staining pattern characterized by radial streaks near the leading edge of the cell. By contrast, the stain for the β1 subunit was very intense and localized to the perinuclear region and cell-cell contacts (Fig. 3C). The staining for β3 was less intense, but this protein was also localized to sites of cell-cell contact with some intracellular staining (Fig. 3D). Similarly, as seen in Figure 3E, the staining for VCAM-1 was not intense, although protein was detected throughout the cytoplasm and at sites of cell-cell adhesion.

Poly(A)$^+$ mRNA was isolated from cultured SM9-1 cells and analyzed for the presence of the same panel of integrin subunits and VCAM-1 transcripts. Figure 4 demonstrates the presence of specific message from each of the integrin subunit genes and the VCAM-1 gene in the SM9-1 cell line. Each primer set produced an amplicon of the expected size for each integrin subunit and VCAM-1 (see Table 2 and Fig. 2). Similar to observations on placental mRNA, higher intensity signals were observed for the α4, αv, and β1 integrin subunits and VCAM-1 transcripts than for the β3 integrin subunit.

DISCUSSION

Although integrins and VCAM-1 are known to be vital to placental formation and function [1], little is known about the expression of these proteins during development. The first goal of this investigation was to identify the nor-

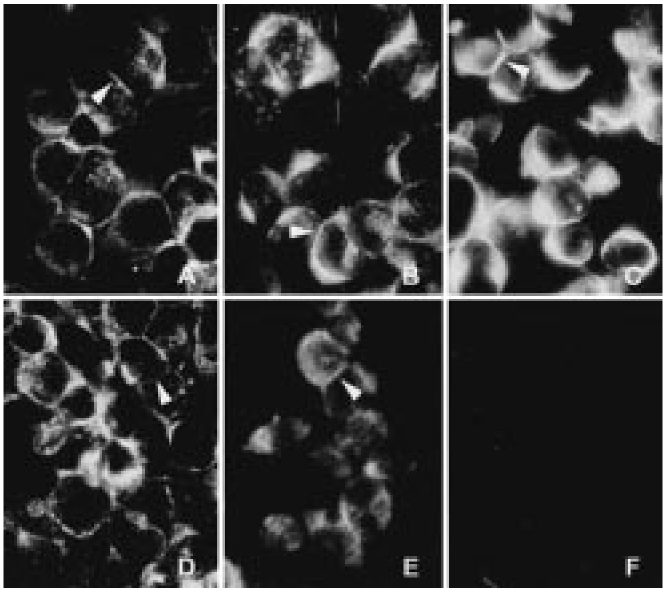

FIG. 3. Expression of integrin subunits and VCAM-1 protein in the placental cell line SM9-1. SM9-1 cells were stained by indirect immunofluorescence for integrin subunits α4 (**A**), αv (**B**), β1 (**C**) and β3 (**D**); VCAM-1 (**E**); and matching control (**F**). Arrowheads indicate points of cell-to-cell contact between neighboring cells. Original magnification ×40.

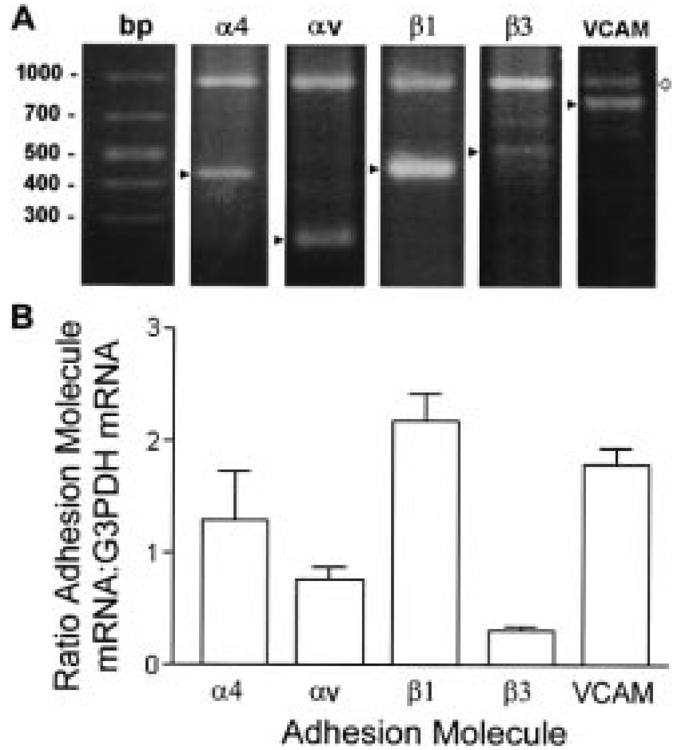

FIG. 4. Identification of α4, αv, β1, β3, VCAM-1, and G3PDH mRNA in poly(A)$^+$ mRNA from the placental cell line, SM9-1. **A**) The expected amplicons (arrowheads) for each integrin subunit and VCAM-1 were present in RNA from SM9-1 cells. Open arrow marks G3PDH which was used a loading control in all lanes. **B**) The ratio of adhesion molecule mRNA:G3PDH mRNA obtained by scanning densitometry (n = 3).

mal patterns of expression of a selected group of integrin subunits and VCAM-1 as a function of gestation. We established 1) that proteins for integrin subunits (α4, αv, β1, and β3) and VCAM-1 are expressed within the developing murine placenta, and 2) that mRNAs for the specific integrin subunits and VCAM-1 are present in uteroplacental units and placentas. The second goal was to determine whether the placental cell line SM9-1 expressed proteins and transcripts for the selected panel of integrin subunits and VCAM-1. These experiments showed that the SM9-1 placental cell line expressed mRNA for the same adhesion molecules that were identified in the placenta.

Transcription of the α4 integrin gene and expression of the protein as well as its corresponding dimer, β1, within the developing placenta are not surprising. Both proteins are required for normal implantation and placental development, as demonstrated in mice with null mutations for these proteins [14]. The current study showed that the protein for α4 integrin subunit was present throughout the placenta with no zonal preference. Although the precise role and regulation of the α4 integrin subunit in placental development is unknown, function-perturbing antibodies to the α4β1 integrin injected into rats and rabbits result in allograft rejections [27, 28], indicating that α4β1 may be involved in creating an immune-privileged site.

The expression of β1 integrin subunit mRNA and protein in placental development is more complex. If β1 formed heterodimers only with α4, then the localization of the stains would be identical, but this is not the case. Our observation that β1 integrin staining was more intense than α4 integrin staining in the labyrinthine zone suggested that it may bind to a different α integrin subunit(s). The β1 subunit is the most versatile of the integrin subunits and can bind 12 different α subunits [1], including the two α subunits examined in this study. It is probably because of this multifaceted nature of β1 that mice null for the β1 subunit die around g.d. 5 [18].

The expression patterns of αv and β3 mRNA and proteins in mouse placentas reported here confirm and extend observations made by others [10] which showed that αv and β3 integrin subunit proteins are constitutively expressed on the trophoblast through g.d. 8. While the protein for β3 integrin subunit was expressed equally in both the spongiotrophoblast and labyrinthine layers, the spongiotrophoblast cells expressed a higher level of αv integrin subunit protein than the labyrinthine layer. The data from this study showed that the intensity of staining of αv was stronger than that of the β3 integrin in the spongiotrophoblast zone, suggesting that αv may dimerize with another integrin subunit (i.e., β1, β5, β6) in the zone and may reflect differences in the invasive character of these cell types. The αv family of integrins is very important in angiogenesis, neovascularization, and tumor migration [29]. Because angiogenesis and neovascularization are critical for normal placental function, and the transfer of nutrients and other important factors to the growing fetus, aberrant expression of αv could result in conditions detrimental to fetal growth and development.

The transcription of VCAM-1 mRNA in the uteroplacental units, and expression of VCAM-1 protein in the ectoplacental cone and trophoblast giant cells in early gestation suggest that this protein is important for early placental formation, an idea that is consistent with previous observations on the function of VCAM-1 in early placental formation using targeted disruption of the VCAM-1 gene [16]. VCAM-1 is the counter receptor for the α4β1 integrin [1] and is expressed on a variety of cell types, in which its primary function is to mediate cell-to-cell adhesion [17]. Thus, VCAM-1 may act in conjunction with α4β1 to create an immune-privileged site and/or to aid in cell-to-cell interactions in the formation of a normal placenta.

The placental cell line SM9-1 was examined for protein and mRNA expression of the adhesion molecules. As in the placenta, the SM9-1 cells contained mRNA encoding the integrin subunits α4, αv, β1, and β3, and VCAM-1. The protein expression of adhesion molecules was similar if not identical to expression in the labyrinthine zone. For example, the SM9-1 cells were moderately stained for the αv integrin subunit and intensely stained for β1 integrin subunit. Since VCAM-1 and the integrin subunits α4 and β3 showed no zonal differences in staining in the placenta, they could not be used to differentiate between the spongiotrophoblast and labyrinthine zones. Thus, the SM9-1 placental cell line may comprise a valuable model system to study the regulation and function of trophoblast adhesion molecules.

The involvement of integrins in normal placental development is unquestioned, as demonstrated in studies with mutant mice. This study demonstrates for the first time that the murine placenta contains mRNA and protein for integrin subunits α4, αv, β1, and β3 and VCAM-1 that vary during gestation. The characterization of integrin expression in the normal developing placenta should aid future studies designed to investigate paracrine factors that modulate placental adhesion molecules and interactions between adhesion molecules and cytokines such as tumor necrosis factor α [30] and Fas ligand [31]. Identifying patterns of expression in the trophoblast cell line lays the groundwork for future in vitro studies on the regulation of adhesion molecules.

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