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# Induction of Mouse β Integrin Expression Following Transfection With Human α4 Chain

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**Abstract** We report here an analysis of the expression and function of the α chain of human VLA-4 in stable mouse L cell transfectants and the requirement for the β chain in these processes. L cells were transfected with human α4 cDNA or α4 and human β1 cDNA. Unexpectedly, human α4 cDNA, when transfected alone, could induce de novo surface expression of host β7 and increased expression of host β1. Induction of mouse β7 and β1 surface expression was not due to de novo gene activation, but instead represented α4/β intracellular subunit association and transport to the cell surface. Transfection with human β1 prevented surface expression of mouse β integrins. Whereas human α4 and human β1 subunits associated very tightly in anti-α4 immunoprecipitates, human α4 and mouse β subunits were only partially associated. Furthermore, binding of human/mouse chimeric receptors to recombinant VCAM, a major ligand for α4β7 and α4β1, was very poor, whereas human α4/human β1 receptors bound strongly to VCAM. One α4 transfectant, which exhibited a tight human α4/mouse β1 association, could be induced, but only after PMA activation, to bind strongly to VCAM. These results indicate that α4 subunits have specific affinity for β7 and β1 integrins and require β subunits for surface expression as well as high affinity ligand binding activity. Our results indicate that a tight association between the α4 and β subunit appears to be critical for ligand binding, consistent with a direct as well as regulatory role for the β subunit in ligand binding. Furthermore, these studies demonstrate that expression of foreign recombinant proteins can alter host cell protein expression resulting in de novo surface protein expression.



**Key words:** β1 integrin, β7 integrin, α/β integrin subunit association, VLA-4/VCAM adhesion, integrin surface expression

Integrin receptors play an essential role in cell-matrix and cell-cell interactions. Integrin/receptor ligand interactions are involved in the development of the immune response, cell migration, and tumor metastasis [Hemler, 1990; Abelda and Buck, 1990; Rouslahti, 1991]. Integrins are heterodimeric molecules made up of noncovalently associated α and β subunits. The α4 subunit, which is highly expressed on lymphocytes, monocytes, and eosinophils, can associate with either the β1 or the β7 subunit. Both α4β1 and α4β7 are expressed on activated T and B lymphocytes and have been implicated in the development and progression of a number of autoimmune diseases [Yednock et al., 1992; Yang et al., 1993; Abraham et al., 1994; Burkly et al., 1994]. The α4β1 integrin binds to the alternatively spliced connecting segment 1 (CS1) of fibronectin [Wayner et al., 1989; Guan and Hynes, 1990] and vascular cell adhesion molecule (VCAM), a cellular ligand induced by proinflammatory cytokines on endothelial cells and on some stromal cells [Elices et al., 1990; Marlor et al., 1992; Rosen et al., 1992]. The α4β7 integrin binds to mucosal addressin cell adhesion molecule (MadCAM) as well as to CS1-fibronectin and VCAM [Berlin et al., 1993; Strauch et al., 1994]. Although cellular α4 expression is relatively restricted, β1 expression in association with other α subunits is widespread.

To elucidate the function of the α4 integrin, we have transfected murine L cells with human α4 cDNA and generated clones of stable transfectants, which expressed high levels of surface α4. We asked whether human α4 expression re-

Abbreviations: VLA-4, very late activation antigen-4; VCAM, vascular cell adhesion molecule; PMA, phorbol myristate acetate; PCR, polymerase chain reaction; FACS, fluorescence activated cell sorting.

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quired cotransfection with human β1 cDNA and whether expressed α4 integrins were functional. We found not only that human α4 was readily expressed in L cells even in the absence of human β1, but also that α4 transfection induced the surface expression of mouse β1 and β7. Optimal ligand-binding, however, required human β1 expression.

## MATERIALS AND METHODS

### Cell Culture

L cells (ATCC 929) were maintained in Dulbecco's modified Eagle's medium with 4.5 g/L glucose, 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin G, and 100 μg/ml streptomycin. Jurkats were maintained in RPMI 1640 medium in 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin G, and 100 μg/ml streptomycin. All tissue culture components were from Biowhittaker, Walkersville, MD.

Transfectants were selected and maintained in L cell media supplemented with G418 at 0.4 mg/ml (Gibco BRL, Gaithersburg, MD).

### Antibodies

C212.7 is an anti-α4 antibody, generated by immunization of Balb/c mice with a human T cell line [Aune and Pogue, 1989; Blue et al., 1993]. A human anti-CD29 (anti-β1) was obtained from AMAC. Anti-mouse VLA5 (α5), anti-mouse β7, anti-mouse β1, and anti-mouse VCAM were obtained from Pharmingen (San Diego, CA). Goat anti-mouse Ig-FITC was obtained from TAGO Inc. (Burlingame, CA) and goat anti-rat Ig G $F(ab')^2$-FITC was obtained from Jackson Immuno Research (West Grove, PA).

### PCR Amplification and Cloning

Total RNA was isolated from Jurkat cells as described by Chomczynski and Sacchi [Chomczynski and Sacchi, 1987]. α4 and β1 cDNAs were amplified from Jurkat RNA by RT-PCR (Perkin Elmer, Oak Brook, IL) using oligo dT as a primer. Primers for PCR were designed to amplify the α4 cDNA in three separate segments (A, B and C) and the β1 cDNA in two separate overlapping segments (D and E) (Fig. 1). The primers were designed according to the sequence published in Genbank (accession nos. X15356 and X07979). Primers either spanned unique restriction sites or were modified at the 5′ end so the PCR products could be directionally cloned into pBluescript II $SK^-$ (Stratagene, La Jolla, CA). Amplifications were performed on a Perkin Elmer Thermal Cycler 9600 using the RT-PCR kit (Perkin Elmer). Reactions were heated to 95°C (5 min) before the addition of AmpliTaq, and then subjected to 30 cycles of PCR at 95°C (30 sec), 55°C (15 sec), and 72°C (1 min). The PCR products were purified using QIAquick-spin PCR purification kit (Qiagen, Chatsworth, CA) and digested with the appropriate restriction enzymes (New England Biolabs, Beverly, MA). Fragments were gel purified using QIAEX (Qiagen) and ligated into digested, gel purified, and CIP treated pBluescript. The PCR products A, B, and C were ligated into pBluescript at XhoI-HindIII, HindIII-PstI, and PstI-BamHI sites, respectively. Fragments D and E were ligated into pBluescript at BamHI-XhoI restriction sites. Ligation products were transformed into competent DH5α cells (Gibco BRL). Plasmid DNA from ampicillin resistant colonies was prepared by Magic Minipreps (Promega, Madison, WI) and digested with the appropriate restriction enzymes to identify inserts of the correct size. Positive clones were sequenced with Sequenase (U.S. Biochemical, Cleveland, OH) and those with the correct sequence were spliced together to generate both full-length α4 and β1 cDNAs.

In order to construct full-length α4 cDNA, fragment B was excised from pBluescript-B with PstI and HindIII and ligated into the construct pBluescript-C, which had also been digested with PstI and HindIII. Subsequently, fragment A was excised from pBluescript-A with XhoI and HindIII and ligated into the construct pBluescript-BC, which had also been digested with XhoI and HindIII. The resulting construct, pBluescript-ABC, contained the full-length human α4 cDNA.

To generate full-length β1 cDNA, fragment E was excised from pBluescript-E with Nsi-XhoI and ligated into pBluescript-D also digested with Nsi-XhoI. The resulting construct, pBluescript-DE, contained the full-length human β1 cDNA.

Full-length α4 was subcloned into the eukaryotic expression vectors, pcDNAI/NEO (Invitrogen, San Diego, CA) and pcDNA3 (Invitrogen) at the XhoI-XbaI sites. Full length β1 was subcloned into pcDNA3 at the BamHI-XhoI sites.

### Transfections

L cells were transfected with either 10 μg of linearized pcDNA3-β1 or pcDNA3-α4 using Lipo-

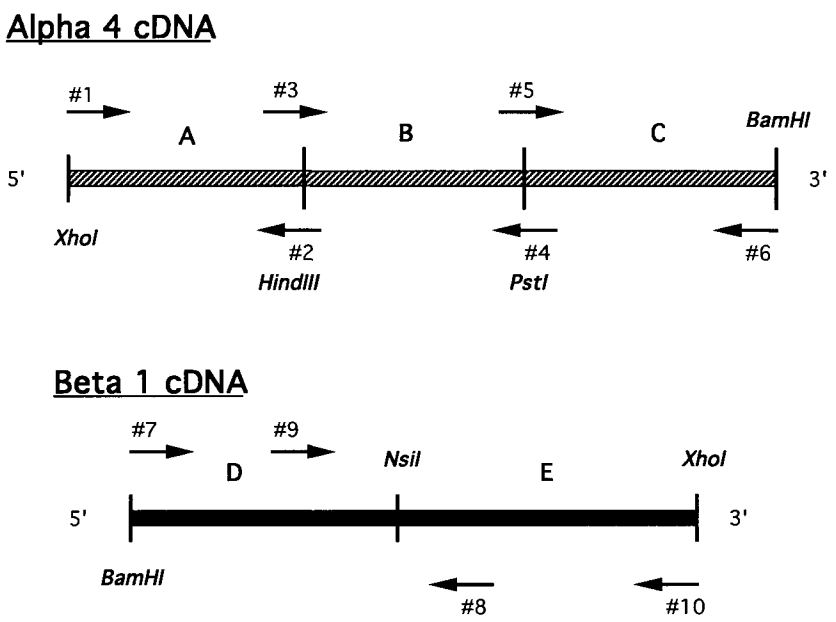


**Fig. 1.** Cloning of α4 and β1 cDNA. α4 cDNA fragments A, B, and C were generated using the indicated primers: #1 = CGC TCT CGA GTG AAT GTT CCC CAC CGA GAG C. #2 = GTA CGA TCC AAG CTT TTT ACC TTT C. #3 = GGT AAA AAG CTT GGA TCG TAC. #4 = GCC AAT ACT GCA GTC AAG TTG TAC. #5 = GTA CAA CTT GAC TGC AGT ATT GGC. #6 = CTC TGG ATC CGA AAG AAG TCC TTA ATC ATC ATT GC. β1 cDNA fragments were constructed using the following primers: #7 = TGG GGA TCC GAC GCC GCG CGG AA. #8 = TGC CTC GAG TTT GGC ATT CAC ATT CAC AGA. #9 = TGG GGA TCC TTT ACC AAA TGA TGG ACA ATG. #10 = GTC CCT CGA GAA AAG GTC AAA AAG GCA CAA T. Fragments were ligated as described in Materials and Methods. Locations of restriction sites are as indicated.

fectAMINE (Gibco BRL) according to the manufacturer's protocol. Transfectants were selected for neomycin resistance 72 h posttransfection by growth in the presence of G418 at 0.4 mg/ml (Gibco BRL). Stable G418-resistant transfectants expressing human α4 or β1 were established by cell sorting with a monoclonal antibody against α4 or β1, respectively.

To establish clones which would express both the α and β subunits of VLA-4, a β1 clone (β1.8) was selected and cotransfected with the construct pcDNA3-α4. Double transfectants were established by cell sorting for the expression of α4 subunit. Clones expressing both α4 and β1 were established after single cell sorting with C212.7.

### Northern Analyses

Total RNA (10 μg), isolated using Trizol (Gibco BRL) was separated on a 1% agarose/formaldehyde gel, transferred to Nytran membrane (Schleicher and Schuell, Keene, NH) and hybridized according to the manufacturer's protocol. The cDNA probes were obtained by RT-PCR from purified mouse T cell (7 day ConA activated) total RNA. PCR products were digested with restriction enzymes to confirm their identity. The β1 cDNA was generated with primers β1 11 (5'ACA GAA TCA AAG AAC AGT CCT 3') and β1 12 (5' AAA ATA AGC TCA ACC CAA AAG 3'). The β7 cDNA was generated with primers β7 13 (5'GGA TAC TGC AAA TGC AAC CG3') and β7 14 (5'GAT TCC ACT CCC TTC TCT TGG3'). Probes were labeled using a random primed DNA labeling kit (Boeringer Mannheim Biochemicals, Indianapolis, IN).

### Immunoprecipitation

Confluent transfectants in T75 flasks were washed in biotin buffer (PBS containing 14 mM glucose) and then incubated at room temperature for 30 min in 5 ml of biotin buffer containing 0.4 mg of Sulpho-NHS-Biotin (Pierce, Rockford, IL). The reaction was quenched with 4 mM glycine for 10 min at room temperature. Cells were then removed with 0.02% EDTA (Sigma, St. Louis, MO). Cells were lysed in lysis buffer (50 mM Tris, pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1% NP-40, 50 nM AEBSF [aminoethyl-benzenesulfonylfluoride], 2 μM Leupeptin, and 10 nM E-64). The samples were centrifuged at 14,000 rpm for 15 min and pre-

cleared with GammaBind G Sepharose (Pharmacia, Piscataway, NJ). The lysates were incubated with the desired mAb for 18 h at 4°C, GammaBind added for 1 h, and the pellets washed 3 times in wash buffer (50 mM Tris, pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% NP-40, and 0.05% Tween-20) and 1 time in wash buffer without detergent. Control samples were incubated with Gamma Bind Sepharose only and processed as above. Immunoprecipitates were run out on 4–12% Tris-Glycine gels (Novex, San Diego, CA) and electroblotted onto nitrocellulose (Schleicher and Schuell). The blot was incubated at room temperature for 1 h in 10 mM Tris, pH 8.0, 150 mM NaCl, 5% Nonfat dry milk. The biotinylated proteins were visualized with Streptavidin-POD (Boehringer Mannheim Biochemical) and the chemiluminescence ECL kit (Amersham, Arlington Heights, IL). Western blotting was carried out as specified by Amersham.

### FACS Analysis and Cell Sorting

Cells were washed in PBS w/o $Mg^{2+}$ and $Ca^{2+}$, removed from tissue culture flasks by treatment with 0.02% EDTA (Sigma), and stained with the appropriate monoclonal antibodies. Approximately $1 \times 10^6$ cells were incubated with the primary antibody for 30 min at 4°C. The cells were then washed 3 times in PBS containing 2% FBS and incubated with the respective secondary antibody conjugated to fluorescein for 30 min at 4°C. Cells were washed as above and were either sorted on a FACStar Plus or analyzed on a FACScan (Becton Dickinson, San Jose, CA).

### Cell Permeabilization

Washed cells were incubated for 10 min on ice with 0.5% formaldehyde in PBS. After washing in PBS containing 1 mM $Ca^{2+}$ and 1 mM $Mg^{2+}$, cells were permeabilized with 0.05% saponin (Sigma) for 10 min at room temperature. Subsequently, cells were washed in PBS and incubated in PBS containing 0.1% BSA and 0.1% Tween 20. The latter buffer was used for permeabilized cells throughout the staining procedure. Cells were resuspended in PBS for FACS analysis.

### Adhesion Assay

Recombinant VCAM1-3 (first 3 domains of VCAM) [Webb et al., 1993] at 5 μg/ml was immobilized on 96 well Immulon 2 plates in 0.05 M $Na_2CO_3$ buffer, pH 9, overnight at 4°C. Nonspecific sites were blocked with 1% BSA for 1 h at room temperature and the plate was washed 2 times with PBS. It has been shown previously that the 3 domain form supports adhesion of VLA-4 positive cells nearly as well as full-length VCAM [Webb et al., 1993]. Transfectants were pelleted and incubated with 6-carboxy-fluorescein-diacetate (6-CFD) in PBS for 15–20 min at room temperature. Subsequently, 6-CFD-labeled cells were washed 3 times and added to wells. After a 20 min incubation at room temperature, the plate was inverted, wells were refilled with PBS and centrifuged inverted. Fluorescence was read at 485 and 530 nm on a Cyto Fluor™ 2300 fluorimeter (Millipore, Bedford, MA).

## RESULTS

### α4 Transfection Induces β7 and Increases β1 Surface Expression

L cells are fibroblast-like murine cells that do not express cell surface β7 integrin (Fig. 2c; see also Fig. 4B) and only low levels of surface β1. Human α4 was transfected into L cells and stable transfectants were established as described in Materials and Methods. When transfectants were stained with various monoclonal antibodies, it was found that cells transfected with α4 cDNA expressed substantial amounts of cell surface mouse β7 (Fig. 2). Interestingly, when cells were transfected first with human β1 cDNA and then with α4 cDNA, no induction of murine β7 expression was observed (Fig. 2I).

α4 transfectants were selected for high expression of α4 by several rounds of cell sorting with anti-α4 prior to single cell cloning. Cell populations, following each round of cell sorting, exhibited increased β7 expression, suggesting that β7 expression was linked to α4 expression. To show that induction of β7 surface expression was indeed associated with high α4 expression and did not represent expression by only a few selected clones, unsorted transfected L cell populations were stained simultaneously for human α4 (FL1) and mouse β7 (FL2) (Fig. 3d). Unselected cell populations were also stained for α4 and mouse β1 (FL2) or with Ab against α4 and combined Ab against β7 and β1 (Fig. 3e,f). Figure 3 clearly shows that high α4 surface expression is associated with high mouse β7 and β1 expression. These results indicate that expression of human α4 in L cells results in de novo surface expression of mouse β7 and increased surface expression of mouse β1 integrin.

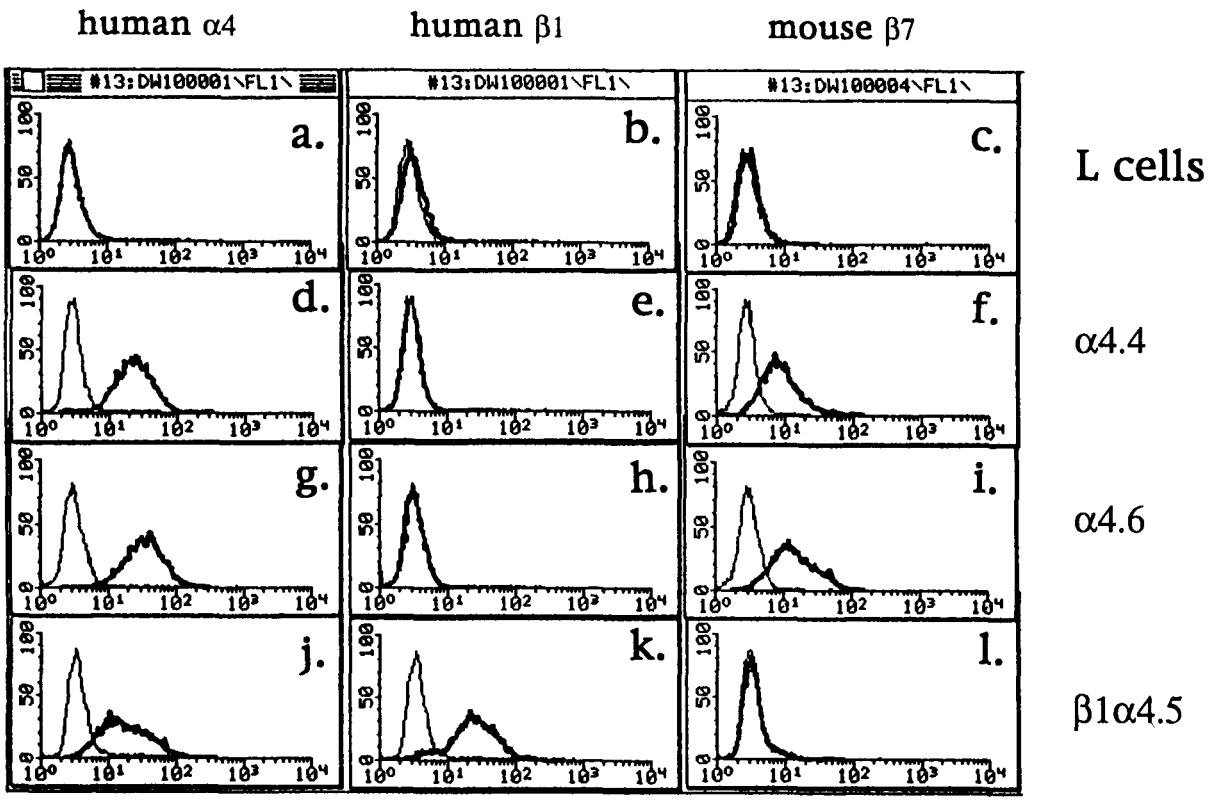


**Fig. 2.** Mouse β7 expression by L cells following transfection with human α4. L cells and the indicated cloned transfectants were stained with antibody to human α4 (**a,d,g,j**); antibody to human β1 (**b,e,h,k**); and antibody to mouse β7 (**c,f,i,l**). *Thin lines* = control Ab; *thick lines* = anti-human α4, anti-human β1, or anti-mouse β7.

### Coprecipitation of Mouse β Integrins With the Human α4 Subunit

L cell transfectants were surface-labeled with biotin (see Materials and Methods) and immunoprecipitated to determine whether human α4 subunits associate with transfected human β1 and possibly even with mouse β integrins. Figure 4A shows immunoprecipitates from two double (human α4, human β1) transfectants. Anti-α4 immunoprecipitates show that α4 is present in an uncleaved form (150 Kd) and also cleaved forms (80, 65 Kd) and that α4 is also associated with a band of about 120 Kd. The 120 Kd band corresponds to the protein precipitated by the anti-β1 Ab. The anti-β1 immunoprecipitates also contain cleaved and uncleaved α4. Immunoprecipitation of untransfected surface-labeled L cell lysate showed no reactivity with human anti-α4 and human anti-β1 (Fig. 4B). Absence of β7 and α5 expression (Fig. 4B) was confirmed by FACS analysis (Fig. 2 and data not shown).

All α4 transfectants expressed both the uncleaved and cleaved forms of α4 (Fig. 4A,C,D, and E). Although many of the α4 transfectants expressed substantial amounts of surface mouse β7 as detected by FACS analysis (Fig. 2) and immunoprecipitation (Fig. 4C and E), very little or reduced amounts of α4 were associated with β7 in anti-β7 immunoprecipitates (Fig. 4C and E). Also a reduced amount of β7 appeared to be associated with human α4 in anti-α4 immunoprecipitates (Fig. 4C and E, clone α4.5). The band associated with α4 in Fig. 4E (clone α4.4) most likely represents mouse β1 protein, based on FACS data (not shown). The α4.5 clone was one of the few clones which exhibited significant β7–α4 association (Fig. 4E, right lanes). These results indicate that mouse β7 can associate with human α4, but the interaction is weaker than the one between human α4 and human β1 (Fig. 4A). The clone α4.16 (Fig. 4D) differed from the other α4 clones in that α4 was strongly associated with a 120 Kd band, which was not human β1 or mouse β7 (Fig. 4D). Surface staining (see Fig. 8A) suggested that this band was mouse β1. The commercially available anti-mouse β1 Ab was a dissociating antibody and

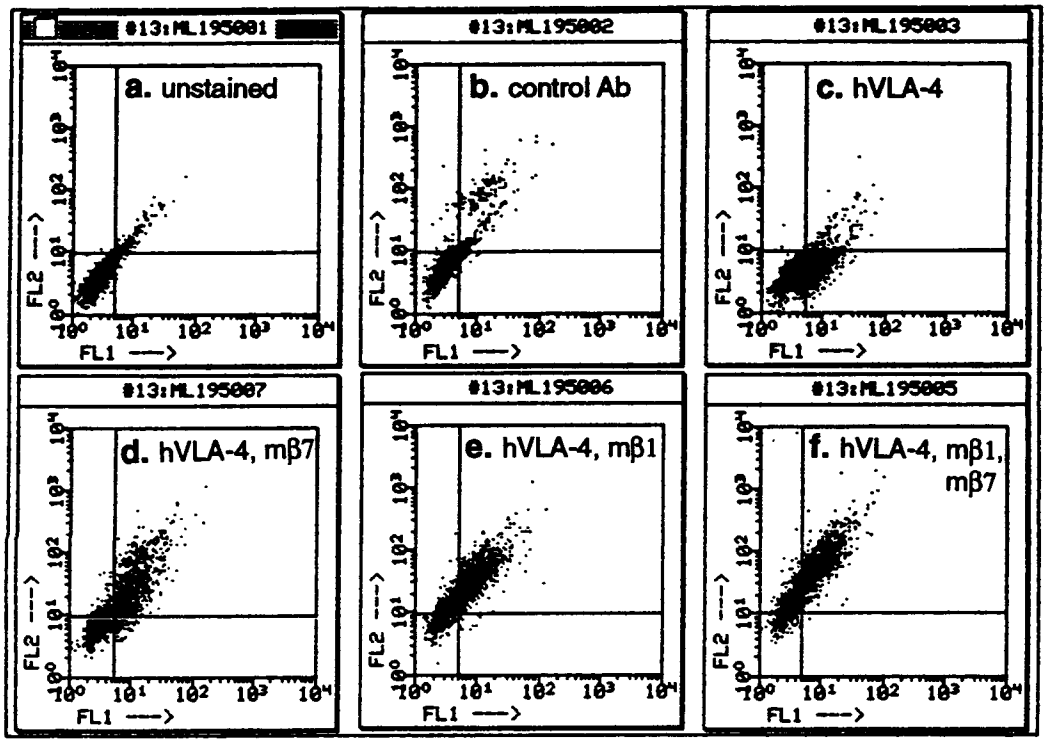


Fig. 3. Correlation between α4 expression and mouse β integrins. Unsorted L cells transfected with α4 cDNA were left unstained (**a**), treated with CD4-FITC control Ab (**b**), anti-human VLA-4-FITC (**c**), anti-human VLA-4-FITC and anti-mouse β7 (**d**), anti-human VLA-4-FITC and anti-mouse β1 (**e**), anti-human VLA-4-FITC and anti-mouse β1, anti-mouse β7 (**f**).

also cross-reactive with human β1 (data not shown) and therefore could not be used to examine α4/β1 association directly. However, displacement of the 120 Kd band (Fig. 4D) in anti-α4 immunoprecipitations, using anti-α4 Ab coupled to Cyanogen Bromide-activated Sepharose in the presence, but not absence of anti-mouse β1 Ab (data not shown), demonstrated that this band was indeed mouse β1.

### Function of Transfected Human α4

α4-transfected clones were tested for adhesion to recombinant immobilized VCAM. The results showed that only the cells expressing both human α4 and human β1 (clones β1α4.5 and β1α4.12) adhered well to VCAM (Fig. 5). Adhesion by all cells transfected with α4 in the absence of β1 was minimal ($<15\%$ cells adhering). This difference was not due to higher α4 surface expression (Fig. 2), because clones were selected with similar α4 expression. Even double transfectants (such as β1α4.12) with α4 expression lower than that of some single transfectants, adhered strongly to VCAM (Fig. 5). These results indicate that human β1 contributes significantly to ligand binding.

Adhesion to VCAM was also carried out in the presence of PMA, a VLA-4 agonist. PMA has been shown to enhance ligand binding by converting an inactivated VLA-4 conformation to an activated conformation. This process is believed to involve inside-out signaling by the β1 subunit [Shimizu et al., 1990; Wilkins et al., 1991]. Most of the cells transfected with α4 only did not exhibit enhanced VCAM binding after PMA treatment. One exception was clone α4.16 (Fig. 6). After PMA treatment, α4.16 bound to VCAM as well as transfectants expressing both human subunits (Fig. 6B). The α4.16 clone exhibited an α4 tightly associated with murine β1 as shown by immunoprecipitation (Fig. 4D). These results indicate that murine β1 can transduce the appropriate signal following PMA treatment and that the human α4, mouse β1 chimera is functional. It also suggests that a strong α,β association may be required for efficient adhesion.

### Are Mouse β1 Integrins Induced by Transfection With Human α4?

To determine if induction of murine β integrin surface expression following transfection of

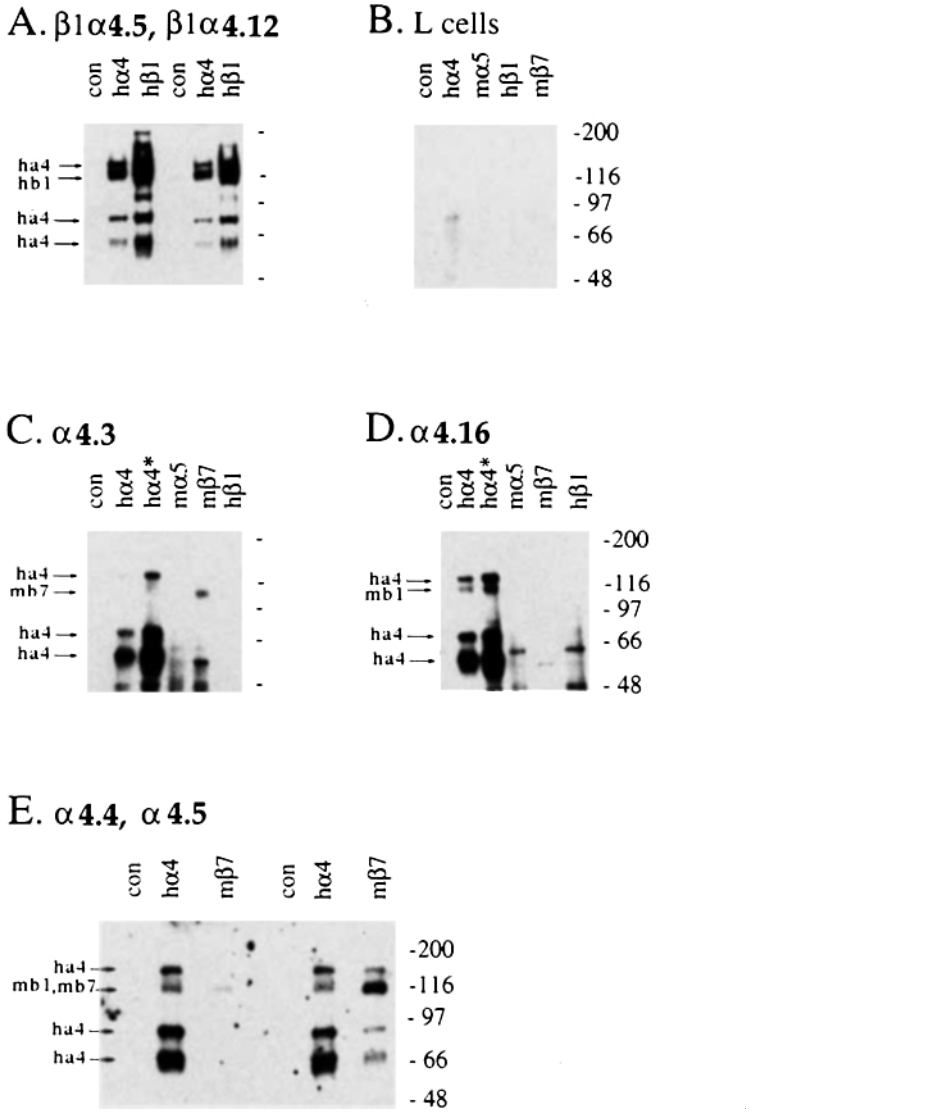


**Fig. 4.** Association of α4 and β integrin subunits. **A:** Surface-biotinylated double transfectants (β1α4.5, left, and β1α4.12, right) were lysed and immunoprecipitated with anti-human α4 (hα4), anti-human β1 (hβ1), or control beads (con). **B:** L cell lysates were immunoprecipitated as in A as well as with anti-mouse α5 (mα5) and anti-mouse β7 (mβ7). **C** and **D:** Single α4 transfectants were immunoprecipitated as in B except that two different anti-human α4 monoclonal antibodies were used (hα4: C212.7, hα4*: CIII372.2A). **E:** α4 transfectants α4.4 (left) and α4.5 (right). Positions of human and mouse integrin bands are indicated by arrows.

L cells with human α4 cDNA involves increased transcriptional activity, mRNA from L cells and various α4 transfectants were probed for murine β1 and β7 mRNA (Fig. 7). Surprisingly, all clones and untransfected L cells, which do not express cell surface β7, expressed mRNA for both mouse β7 (Fig. 7A) and mouse β1 (Fig. 7B). Two bands can be seen in the Northern blot probed for mouse β7 mRNA, suggesting the presence of alternatively spliced β7 mRNA [Erle et al., 1991]. Therefore, our results suggest that transfection of human α4 did not significantly affect mRNA levels of β integrins.

To determine whether α4 transfection affects mouse β protein production, murine β1 and β7 expression on intact (Fig. 8A) and β1 and β7 protein levels in permeabilized cells (Fig. 8B) were analyzed. The results showed no detectable β7 surface expression on mouse L cells (Fig. 8A, panel b), whereas similar or higher levels of murine β7 were found in permeabilized L cells than in α4 transfectants (Fig. 8B, panel b). This suggests that L cell β7 is present inside the cell and becomes surface-borne when an α4 integrin subunit is available. Very little murine surface β1 was expressed on L cells (Fig. 8A, panel a),

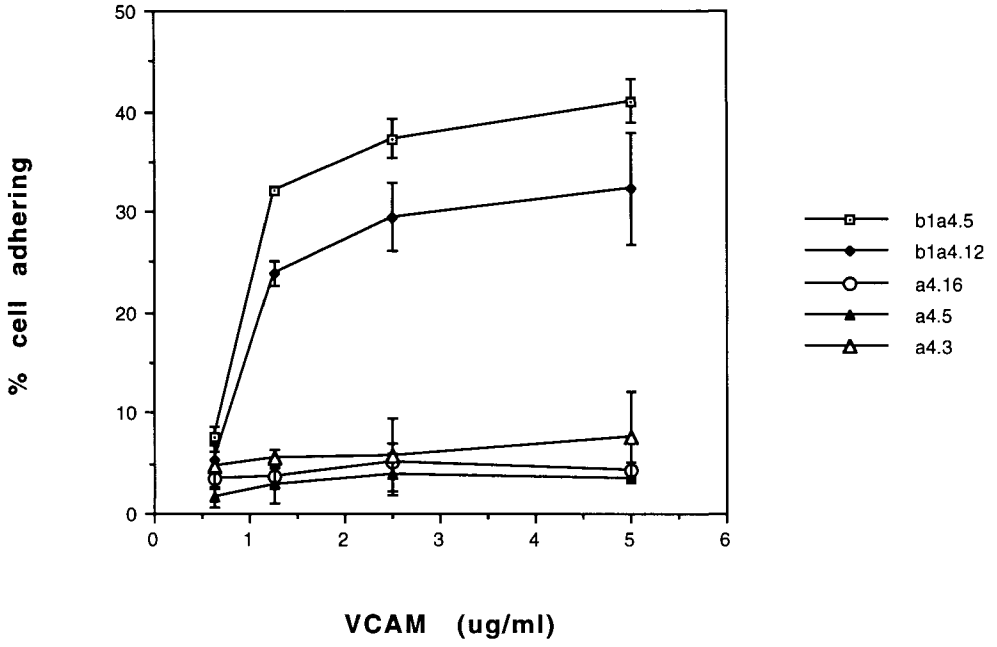


Fig. 5. Adhesion of α4 cell transfectants to recombinant VCAM. Double (b1a4.5, b1a4.12) and single (a4.16, a4.5, and a4.3) α4 transfectants were assayed for adhesion to immobilized VCAM1-3.

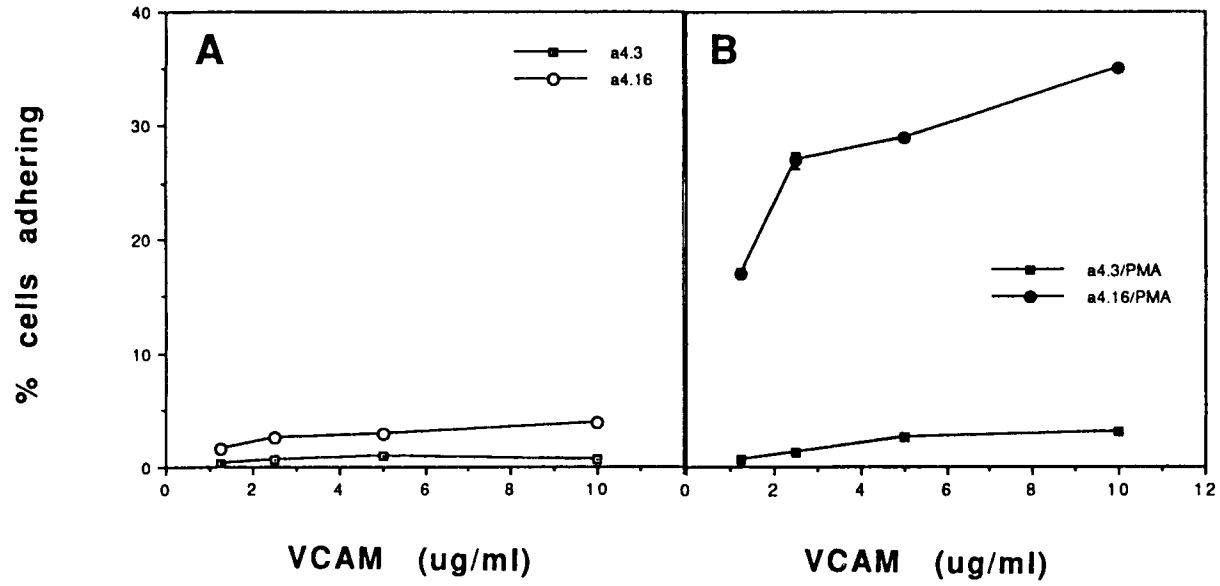


Fig. 6. Adhesion of α4 transfectants to VCAM in the presence of PMA. α4 transfectants were assayed for adhesion in the absence (A) or presence (B) of PMA.

but substantial levels of β1 were detected in permeabilized L cells (Fig. 8B, panel a). Again similar or lower levels of β1 were detected in all permeabilized α4 transfectants except for α4.16 (Fig. 8B, panel e). In the α4.16 clone, β1 expression slightly exceeded that found in L cells. This α4 transfectant also expressed high surface murine β1 (Fig. 8A, panel e). It should be noted that quantitative comparison of β integrin surface expression (Fig. 8A) and β integrin expression in permeabilized cells (Fig. 8B) cannot be done because of different FACS methodologies. Surface expression is assayed on viable unmodified cells, whereas antigen expression on permeabilized cells is carried out on fixed cells, resulting in different fluorescence properties of these cells.

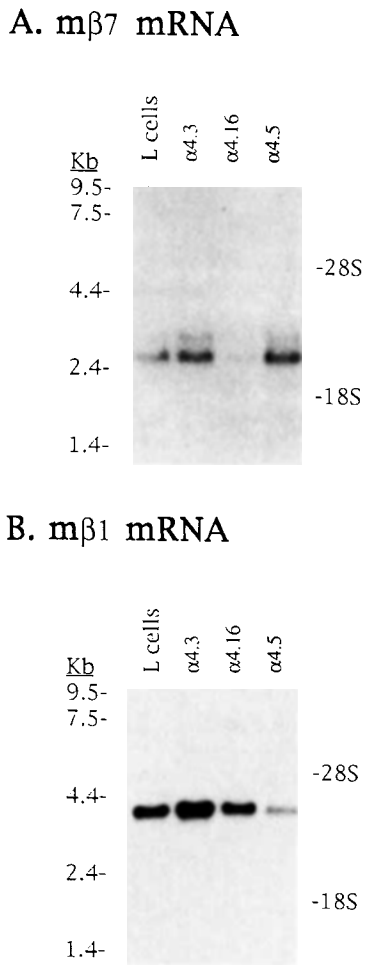


**Fig. 7.** Northern blot analysis of mouse β1 and β7 mRNA. Total RNA (10 μg/sample) obtained from untreated L cells and L cell clones transfected with human α4 cDNA (α4.3, α4.16, α4.5) was probed for mouse β7 mRNA **(A)** or mouse β1 mRNA **(B)**.

Regardless, our results demonstrate that L cells contain intracellular pools of β1 and β7 protein and that transfection with the human α4 subunit induces surface expression of murine β integrins. Dependence of surface expression of integrins on prior assembly of α,β subunits has been demonstrated previously [Cheresh and Spiro, 1987; O'Toole et al., 1989; Duperray et al., 1989].

## DISCUSSION

We have demonstrated the induction of mouse β7 and β1 surface expression following transfection with the human α4 subunit. De novo murine β7 and increased murine β1 surface expression correlated with degree of human α4 expression on cloned transfectants as well as on unselected transfected L cell populations. Partial physical association of human α4 with murine β7 or β1 could be demonstrated by immunoprecipitation. However, only human α4 paired with human β1 could fully function in ligand binding. Our results indicate that transport and surface expression of α integrins requires association with β subunits. Furthermore, our results indicate that (1) the strength of α4/β associations are critical for ligand binding and (2) the β subunit, which lacks ligand binding specificity, is required for ligand binding.

Our results also demonstrate that expression of a recombinant protein can affect host protein expression and transport. The α4 subunit is known to associate with two different β subunits, β1 and β7. Although the β1 subunit associates with numerous α integrin subunits, β7 is only known to associate with α4 and αe, a protein highly homologous to α4 [Shaw et al., 1994]. Expression of recombinant human α4 by L cells caused the association and transport to the surface of α4 with the murine β7 (Fig. 4E, Fig. 2) and β1 (Fig. 4D, Fig. 8) homologs. Recently, it was reported that transfection of CHO cells with murine α4 increased endogenous hamster β1 subunit expression [Jaspers et al., 1994]. Interestingly, when L cells were transfected with human β1 prior to transfection with the α4 subunit, human α4 did not induce β7 surface expression (Fig. 2, clone β1α4.5). Since β7 appears to be synthesized and accumulates in L cells (Figs. 7, 8B), these results suggest that α4 associates with murine β7 and induces β7 cell surface expression only when its human counterpart is not available. The importance of α/β integrin associations is demonstrated by genetic diseases such as Glanzmann's thrombasthenia and leukocyte deficiency disease (LAD), where a defect in the β subunit in some cases prevents α/β association and surface expression and leads to severe pathology [Kishimoto et al., 1989].

Our data indicate that the association of the β subunit with α4 is not only required for transport to the cell surface, but also for function. L cells, transfected with both human β1 and human α4, adhered well to recombinant VCAM (Fig. 5) and alternatively spliced CS-1-fibronectin (data not shown). Both subunits were tightly associated as shown by the immunoprecipitations in Figure 4A. Even L cell clones with low levels of human α4 and human β1 expression adhered much better to VCAM than L cells expressing high levels of human α4 in the absence of human β1. In the cells transfected with

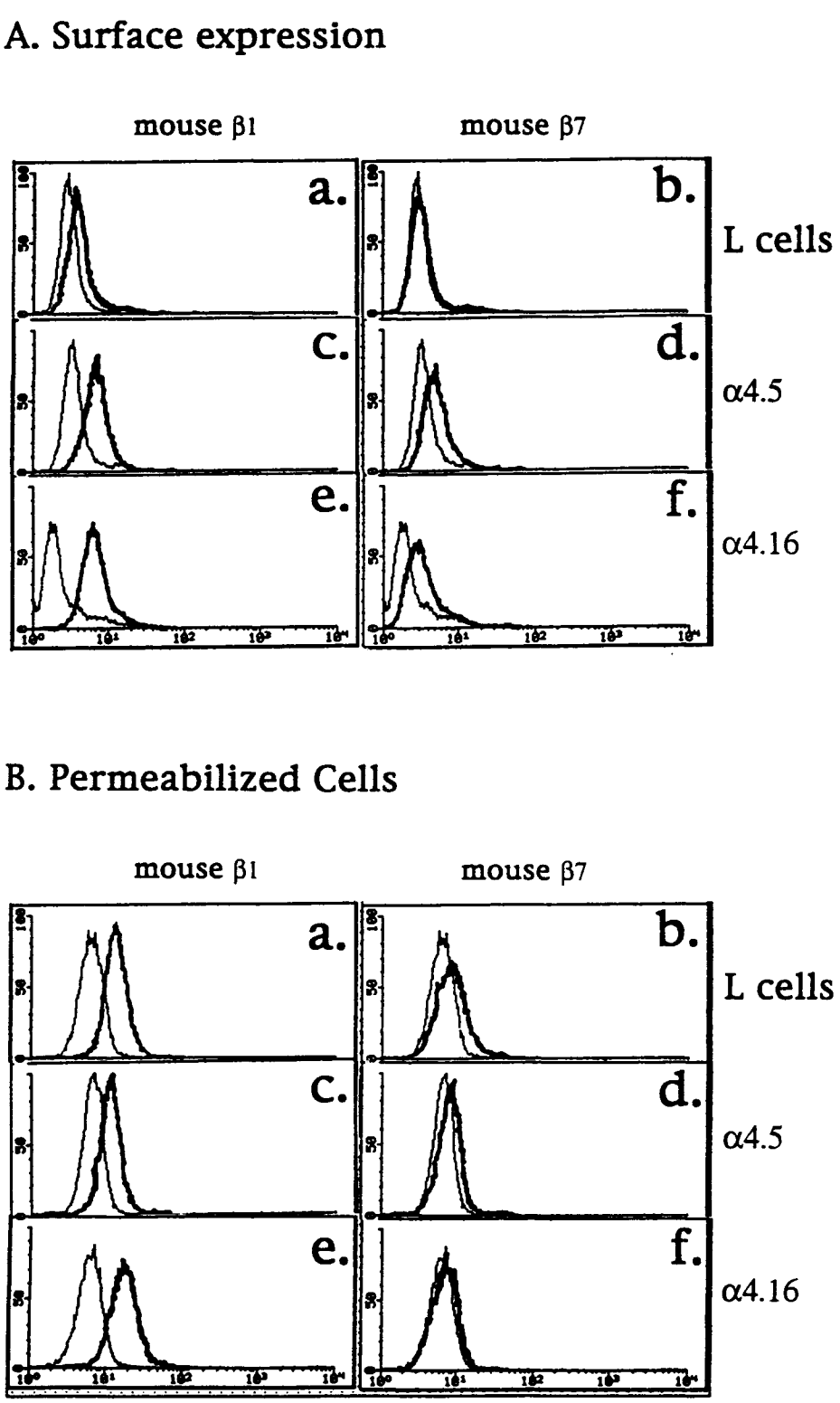


**Fig. 8.** Staining of intact and permeabilized α4 transfectants. L cells were stained with anti-mouse β1 (**a**) and anti-mouse β7 (**b**). Mouse β1 (**c,e**) and mouse β7 (**d,f**) expression by α4 transfectants. Surface expression (**A**), permeabilized cells (**B**). *Thin lines* represent control staining with anti-mouse VCAM Ab.

α4 only, only partial association of α4 with murine β7/β1 could be detected by immunoprecipitation (Fig. 4C, E). A relatively tight association was only observed in clone α4.16 (Fig. 4D). Interestingly, this also was the only α4 clone, which could be induced by PMA to bind to recombinant VCAM (Fig. 6B). It has been shown previously that murine VLA-4 can bind to both human and mouse recombinant VCAM [Romanic and Madri, 1994] (data not shown). Our results suggest that the nature of the association between the α and β subunits may be important for ligand binding function and that the β subunit is critical for ligand binding. The β subunit may be essential for controlling the three-dimensional structure of the α integrin subunit. Alternatively, although ligand specificity appears to be confined to the α integrin subunit, the β subunit may provide essential ligand contact points [Loftus et al., 1994]. Evidence for involvement of β integrins in ligand binding also comes from studies with sequence-specific Ab [Andrieux et al., 1991; Calvette et al., 1991; Wang et al., 1992] and mutational studies [Bajt and Loftus, 1994]. Although murine β1 and β7 are highly homologous to their human counterparts, clearly the differences affect α,β association and function.

One of the initial questions we addressed was: Is the α subunit limiting for integrin surface expression and can the β subunit be expressed without an α subunit? Prior to generating α4 transfectants, we transfected L cells with human β1 cDNA and established stable β1 L cell clones. Surface staining of these clones revealed mouse VLA-5 (α5) surface expression, which was not detectable on the parental L cells (Fig. 4B and data not shown). These results indicate that transfection with human β1 induced the surface expression of the mouse α5 subunit. Therefore, neither α or β integrin subunits alone can be transported to the surface without an α counterpart subunit. Because the strength of association between α and β subunits may be weak in chimeras, α or β subunits may not appear to be associated in immunoprecipitations. The latter may account for the apparent expression of sole integrin subunits [Crowe et al., 1994]. Dependence of surface expression or secretion (truncated subunit) of other α integrin subunits on the expression of the β subunit has been shown previously [Cheresh and Spiro, 1987; O'Toole et al., 1989; Duperray et al., 1989; Briesewitz et al., 1993]. However, in the case of secreted integrins, only α secretion, but not β secretion, was dependent on the expression of the partner subunit [Briesewitz et al., 1993].

In conclusion, our results demonstrate that cell surface expression of α4 requires either β7 or β1 and can result in de novo β7 surface expression. Ligand binding function was dependent on the β subunit, which has been implicated in cellular signaling as well as stabilizing α subunit conformation [Guan et al., 1991; Chen et al., 1994; Lukashev et al., 1994]. Our results indicate that the β subunit plays an essential role in ligand binding and that the strength of association between the α4 and β subunit may regulate ligand binding.

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