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Permalink
https://escholarship.org/uc/item/6px2z314

Journal
The Journal of cell biology, 139(2)

ISSN
0021-9525

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Publication Date
1997-10-01

DOI
10.1083/jcb.139.2.563

Peer reviewed
Presentation of Integrins on Leukocyte Microvilli: A Role for the Extracellular Domain in Determining Membrane Localization

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Abstract. Adhesion of blood leukocytes to the endothelium involves multiple steps including initial attachment (tethering), rolling, and firm arrest. Presentation of adhesion molecules on leukocyte microvilli can substantially enhance tethering. Localization of L-selectin to microvilli and of CD44 to the planar cell body have been shown to depend upon their transmembrane and cytoplasmic domains. We investigated the role of leukocyte integrin transmembrane and cytoplasmic domains in initiating adhesion under flow and in microvillous localization. Integrins α4β7, αLβ2, and αMβ2 were heterologously expressed in K562 cells. α4β7 initiated adhesion under flow and localized to microvilli, whereas β2 integrins did not initiate adhesion and localized to the cell body. Chimeric integrins were produced by replacing the α4β7 cytoplasmic and/or transmembrane domains with the homologous domains of αLβ2 or αMβ2. Unexpectedly, these chimeras efficiently mediated adhesion to the α4β7 ligand mucosal addressin cell adhesion molecule–1 under flow and localized to microvilli. Therefore, differences between the transmembrane and cytoplasmic domains of α4 and β2 integrins do not account for differences in ability to support attachment under flow or in membrane localization. Integrins α4β1, α5β1, α6β1, αvβ3, and αEβ7 also localized to microvilli. Transmembrane proteins known or suspected to associate with extracellular domains of microvillous integrins, including tetraspans and CD47, were concentrated on microvilli as well. These findings suggest that interactions between the extracellular domains of integrins and associated proteins could direct the assembly of multimolecular complexes on leukocyte microvilli.

Leukocyte recruitment to tissues from blood involves a number of steps. Adhesions between leukocytes and the vascular endothelium (Springer, 1994; Butcher and Picker, 1996). In some cases, initial binding of leukocyte adhesion molecules to their endothelial ligands can lead to the transient arrest, or tethering, of the leukocyte followed by leukocyte rolling. Rolling cells can then be “activated” via incompletely understood mechanisms, which lead to an increase in the activity of certain adhesion molecules and the arrest of the leukocyte on the luminal surface of the endothelium. In other cases, leukocytes may arrest immediately, without rolling. After arrest, leukocytes can extravasate into the underlying tissue. Different leukocyte adhesion molecules can be expressed on different steps in this process (von Andrian et al., 1991). L-selectin and the E- and P-selectin ligands are expressed on some leukocytes and mediate initial adhesion (tethering and rolling), but do not support firm arrest. In contrast, the leukocyte β2 integrins αLβ2 (LFA-1, CD11a/CD18) and αMβ2 (Mac-1, CD11b/CD18) mediate firm arrest but not initial adhesion. Another integrin subfamily, the α4 integrins α4β1 (VLA-4, CD49d/CD29) and α4β7 (LPAM-1), can support both initial adhesion and firm arrest (Sriramarao et al., 1994; Alon et al., 1995; Berlin et al., 1995).

Presentation of certain leukocyte adhesion molecules on microvilli substantially enhances the ability of these molecules to support tethering and rolling on endothelial ligands. The importance of receptor distribution was highlighted by studies of the adhesion molecules L-selectin and CD44 (von Andrian et al., 1995). L-selectin is located primarily on microvilli, whereas CD44 is concentrated on the planar cell body. L-selectin–CD44 chimeras were used to examine the role of cytoplasmic and transmembrane domains in receptor localization and the ability to roll on ligands. A chimera comprising the L-selectin extracellular domain fused to the CD44 transmembrane and cytoplasmic domains (L/CD44) localized to the cell body, and a CD44 extracellular, L-selectin transmembrane and cytoplasmic domain chimera (CD44/L) localized to microvilli. Although replacement of the transmembrane and cytoplasmic domains of L-selectin or CD44 did not alter their ability to adhere under static (no flow) conditions, it affected adhe-
sion under flow. L-selectin (on microvilli) supported initial attachment better than the L/CD44 chimera (cell body), whereas CD44/L (microvilli) supported initial attachment better than CD44 (cell body). These results indicate that the transmembrane and/or cytoplasmic domains account for the differences in localization of L-selectin and CD44, and strongly suggest that microvillous localization is important for optimal initial adhesion under flow.

Available evidence about the distribution of integrins on the leukocyte surface is also consistent with a role for microvillous presentation in initial adhesion under flow. Integrins αLβ2 and αMβ2 are concentrated on the planar cell body and do not support initial adhesion, whereas α4β1 and α4β7 localize primarily to microvilli and do support tethering and rolling (Erlandsen et al., 1993; Berlin et al., 1995). The mechanism underlying the differential topography of these integrins on nonadherent leukocytes is not known. However, studies of other integrins have established a central role for the cytoplasmic domains of integrin β subunits in localization of integrins on membranes of adherent cells (LaFlamme et al., 1992, 1994; Briesewitz et al., 1993; Sastry and Horwitz, 1993; Ylanne et al., 1993; Pasqualini and Hemler, 1994). Interactions with several cytoskeletal proteins such as talin and α-actin (demonstrated in vitro) are suggestive of links to microfilament fibers that may regulate protein localization. In addition, several transmembrane proteins, such as tetraspan proteins (including CD9, CD53, CD63, CD81, and CD82) (Slupsky et al., 1989; Rubinstein et al., 1994; Berditchevski et al., 1995, 1996, 1997), CD32 (FcγRIIA) (Worth et al., 1996), and CD47 (integrin-associated protein) (Lindberg et al., 1993), have been shown to associate with integrins. These interactions are known or suspected to involve the extracellular domains of these proteins, and their role (if any) in integrin localization is unknown.

We sought to examine the role of leukocyte integrin transmembrane and cytoplasmic domains in microvillous localization and in initial adhesion under flow. Here we report that replacement of α4β7 transmembrane and cytoplasmic domains with the homologous domains of β2 integrins does not alter membrane localization or initiation of adhesion under flow. This unexpected result suggests that differences in localization of leukocyte integrins to microvilli are determined by the extracellular domain.

**Materials and Methods**

**Cell Lines**

K562 human erythroleukemia cells (CC-243; American Type Culture Collection, Rockville, MD) were maintained in growth medium: RPMI 1640 supplemented with 10% FBS, penicillin (50 IU/ml), streptomycin (50 µg/ml), and glutamine (2 mM). Stably transfected K562 lines expressing human integrin α4 (K562-α4β1) and α4β7 (K562-α4β7) were described previously (Tidswell et al., 1997). Additional K562 integrin transfectants were provided by other investigators: K562-αLβ2 and K562-αMβ2 (I. Graham, Washington University, St. Louis, MO) (Graham et al., 1994); K562-α6β1 (A. Sonnenberg, The Netherlands Cancer Institute, Amsterdam, The Netherlands) (Hogervorst et al., 1993); K562-αVβ3 (S. Blystone, Washington University) (Blystone et al., 1994).

**cDNAs**

The pCDM8-α4 cDNA plasmid (Kamata et al., 1995) was a gift from Y. Takada (Scripps Research Institute, La Jolla, CA). The cloning of the β7 cDNA has been previously described (Erle et al., 1991). Integrin β2 cDNA (Hickstein et al., 1988) was provided by D. Hickstein (University of Washington, Seattle, WA). Integrin αL (Larson et al., 1989) and αM (Corbi et al., 1988) cDNAs were provided by T. Springer (Center for Blood Research, Boston, MA). eCα DNA (Shaw et al., 1994) was a gift from G. Russell and M. Brenner (Harvard Medical School, Boston, MA). Chimeric integrin subunits were constructed using splice overlap extension PCR (Horton et al., 1989). The amino acid splice sites for each construct are shown in Fig. 1. Chimeric α subunits were subcloned into pCDM8 (Invitrogen, San Diego, CA) and chimeric β subunits were subcloned into pCEP4 (Invitrogen). The integrity of the constructs was confirmed by DNA sequencing.

**K562 Cells in log phase growth were washed twice in electroporation buffer (HBSS, 20 mM Hepes, 6 mM dextrose). 8 × 10^6 cells were resuspended in 0.2 ml buffer and transferred to 2 mm electroporation cuvettes (BTX, San Diego, CA). Stable transfections were performed using the pCDM8-α4 and pCEP-β7 constructs (25 µg each) plus 5 µg pkB-neo (Stratagene, La Jolla, CA) at 900 µFarad, 200 V, 13 Ω (Electro Cell Manipulator 600; BTX). Samples were left at room temperature for 10 min before and after electroporation. Cells were then placed in 20 ml growth medium and maintained at 37°C in a 5% CO₂ incubator. After 48 h, transfectants were selected using growth medium containing 500 µg/ml each of hygromycin B (Calbiochem-Novabiochem Corp., La Jolla, CA) and G418 (GIBCO BRL, Gaithersburg, MD). Stably transfected clones were obtained by limiting dilution and analyzed by flow cytometry as previously described (Tidswell et al., 1997). Transfectants were maintained in selection medium.

**Antibodies**

Fib 504 (anti-integrin β7) was a gift of E.C. Butcher (Stanford University, Palo Alto, CA) (Andrew et al., 1994). HP1/2 was used to detect the integrin α4 subunit (Pulido et al., 1991). 7E4 (anti-β2) and GoH3 (anti-α6) were purchased from Immunotech (Westbrook, ME). L230 (anti-αv) and B11G2 (anti-α5) were provided by D. Sheppard and C. Damsky (University of California, San Francisco, CA). Antibodies against the integrin-associated proteins CD53 (clone H29; PharMingen, San Diego, CA), CD63 (MAb1787, Chemicon International, Inc., Temecula, CA), and CD32 (clone IV.3; Medarex, East Annandale, NJ) were obtained from commercial sources. The anti-CD47 antibody B6H12 was a gift from E. Brown (Washington University). Hybridoma supernatants or purified IgG preparations were used for flow cytometry analysis and immunoelectron microscopy. Gold-conjugated secondary antibodies (6- or 12-nm particles) were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA).

**Immunoelectron Microscopy**

Transfectants were immunolabeled in suspension. Cells were prefixed for 20 min in 0.2% paraformaldehyde/PBS at 4°C. After washing twice with PBS containing 10% goat serum, cells were incubated with primary antibody diluted in PBS containing 10% goat serum. After a 30-min incubation at 4°C and a wash with PBS, gold-conjugated secondary antibody in PBS was added. Cells were incubated for 30 min at 4°C, washed twice with PBS, and fixed in 0.1 M sodium cacodylate buffer, pH 7.4, with 3% glutaraldehyde. Before embedding, cells were rinsed in 0.1 M cacodylate buffer and postfixed in 1% osmium tetroxide/0.1 M cacodylate buffer. After rinsing, cells were dehydrated through a graded series of acetone washes, and infiltrated and embedded in Spurr’s epoxy resin (Ted Pella, Inc., Redding, CA). Sections (70-nm thick) were stained with uranyl acetate and lead citrate, and examined with a CM120 Philips electron microscope (Philips Electron Optics, Inc., Mahwah, NJ). 50–100 cells were examined and representative cells were photographed. Each experiment was repeated at least twice. Colloidal gold distribution on immunolabeled cells was determined by analysis of electron micrographs (×19,500–×40,000). Gold particles associated with cell body or microvilli were counted from 3–11 micrographs that represented different individual cells.

**Static Adhesion Assay**

Static adhesion assays were performed as previously described (Tidswell et al., 1997). Briefly, 21-well glass slides (Structure Probe, West Chester, PA) were coated with mucosal addressin cell adhesion molecule–1 (Mad-
CAM–1IgG fusion protein (4 ng/well) or intercellular adhesion molecule–1 (ICAM-1)–IgG fusion protein (0.23 μg/well) overnight at 4°C. MADCAM–1IgG (Tidswell et al., 1997) was a gift of M. Briskin (Leukocyte site Inc., Boston, MA). ICAM–1IgG (Piali et al., 1995) was a gift of B. Imhof (Centre Medicale Universitaire, Geneva, Switzerland). After blocking with 4% BSA for 2 h, 4 × 10^5 cells were resuspended in 15 μl of 10 mM Hepes, 150 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, with or without 1 mM MnCl2, and added to the wells. Cells were allowed to adhere for 90 min at room temperature. After washing, cells were fixed with 3% glutaraldehyde and stained with 0.5% crystal violet. Adherent cells were counted using a microscope. Assays were performed in triplicate.

**Adhesion Under Flow**

Capillary tubes (100-μl capacity; Drummond, Broomall, PA) were coated at 4°C overnight with 20 μl of solution containing MADCAM-IgG (0.2 μg/ml) or ICAM–1IgG (0.7 mg/ml) and blocked with 4% BSA for 2 h at 37°C. K562 transfectants were washed with HBSS containing 1 mM EDTA, and resuspended at 5 × 10^5 cells/ml in HBSS/10 mM Hepes containing 1 mM each of Ca^2+ and Mg^2+ . To measure adhesion under flow, cells were perfused through the coated capillary tube using a syringe pump (74000 series; Cole-Parmer Instrument Co., Vernon Hills, IL) at a flow rate of 0.67 ml/min. Calculated shear stress was 1.0 dyne/cm^2 according to Poussille’s law of dynamic shear (Berlin et al., 1995). Results were captured using a TMS microscope (Nikon, Garden City, NJ), CCD video camera (Sony, Park Ridge, NJ), and time lapse SVMS videocassette recorder (Panasonic, Secaucus, NJ). After 2 min of flow, five randomly chosen fields from the coated region were analyzed for 5 s each. All adherent cells (rolling or arrested) were counted. Cells did not adhere to areas coated with 4% BSA alone (control).

**Results**

**Generation of Transfectants Expressing Chimeric and Mutant α4β7 Constructs**

To examine the role of cytoplasmic and transmembrane domains of α4β7 in adhesion and membrane localization, we expressed chimeric integrin heterodimers (Fig. 1). Each construct included the extracellular domains of α4 and β7. In one construct, designated α4β7(αLβ2c), most of the cytoplasmic domains of α4 and β7 were replaced with homologous regions of αL and β2. In a second construct, α4β7(αMβ2tc), all of the transmembrane and cytoplasmic domains of α4 and β7 were replaced with homologous domains of αM and β2. The integrin α and β subunit cDNAs were cotransfected into K562 human erythroleukemia cells, which do not normally express αL, αL, αM, β2, or β7. The levels of protein expression on the transfectants K562-α4β7, K562-α4β7(αLβ2c), and K562-α4β7(αMβ2tc) were determined to be similar by flow cytometry (Fig. 2, A–C). Two truncated cDNAs, αΔ4 (truncated after amino acids GFFKR) and βΔ7 (truncated after amino acids VLAYR), were also produced. The αΔ4 was expressed in combination with β7 on transfected K562 cells (K562-αΔ4β7), although at levels somewhat below those seen with other constructs (data not shown). We were unable to detect expression of βΔ7 on cells cotransfected with α4, despite a previous report that the homologous truncation mutant of mouse β7 was expressed on transfected cells (Crowe et al., 1994). We were able to document heterologous expression of other wild-type integrins, including αLβ2, αMβ2, α6β1, αvβ3, and αEB7, on appropriate K562 transfectants by flow cytometry (Fig. 2, D–H).

**Adhesion of Transfectants to MAADCA-M1 under Static Conditions**

We analyzed the ability of integrin-transfected K562 cells to adhere to MADCAM-1, an α4β7 ligand, and ICAM-1, an αLβ2 ligand, under static (no flow) conditions (Fig. 3). After transfection with α4 cDNA alone, K562 cells express α4β1 but not α4β7 (Tidswell et al., 1997). These cells failed to adhere to MADCAM-1. In contrast, cells transfected with both α4 and β7 (K562-α4β7) adhered efficiently to MADCAM-1. As previously reported, adhesion was increased in the presence of Mn^2+. The chimeric transfectants, K562-α4β7(αLβ2c) and K562-α4β7(αMβ2tc), also adhered to MADCAM-1, and the extent of adhesion was very similar for chimeric and wild-type α4β7 transfectants. As expected, K562-α4β7 cells did not adhere to ICAM-1, whereas K562-αLβ2 cells did.

**Adhesion of Transfectants to MADCA-M1 under Flow**

We next examined the ability of the wild-type and chimeric integrins to initiate adhesion under flow (Fig. 4). K562-α4β7 cells adhered to MADCAM-1 under flow. This adhesion was dependent upon both α4β7 and MADCAM-1 because K562 cells transfected with α4 alone (K562-α4β1) did not adhere to MADCAM-1, and K562-α4β7 cells did not adhere to capillary tubes coated with other ligands, such as ICAM-1. The chimeric integrins, α4β7(αLβ2c) and α4β7(αMβ2tc), both supported adhesion to MADCAM-1 under flow. Wild-type α4β7 and the chimeric integrins were very similar in their ability to initiate adhesion under flow. We also examined the resistance to detachment from MADCAM-1 at increasing shear stress conditions (up to 10 dynes/cm^2). We did not find differences between wild-type and chimeric transfectants in this assay (data not shown). At a shear stress of 1 dyne/cm^2, most cells remained adherent during the time interval of cell counts. Transfectants expressing a truncated α4 subunit (K562-α4Δβ7) also adhered to MADCAM-1 under flow, although at a somewhat lower rate (perhaps related to lower levels
of expression of this construct, data not shown). As expected from previous reports (von Andrian et al., 1991), K562-αLβ2 cells were unable to initiate adhesion to their ligand, ICAM-1, under flow (Fig. 4).

Membrane Localization of Wild-type and Chimeric α4β7, αLβ2, and αMβ2 Integrins on Transfected K562 Cells

Previous reports demonstrated that integrin α4β7 localizes primarily to microvilli of mouse TK-1 lymphoma cells, whereas integrins αLβ2 and αMβ2 localize primarily to the cell body of TK-1 cells and human neutrophils respectively (Erlandsen et al., 1993; Berlin et al., 1995). We began by using immunoelectron microscopy to determine whether these integrins would localize similarly in transfected K562 cells. K562 cells had numerous microvillous projections. α4β7 was found primarily on microvilli (Fig. 5 A). In contrast, αLβ2 and αMβ2 integrins were located primarily on the cell body (Fig. 5, B–D). Both α4β7(αLβ2c) and α4β7(αMβ2tc) localized to microvillous projections (Fig. 5, E and F). A quantitative analysis of the distributions of these integrins is shown in Table I. The α4Δβ7 construct was also concentrated on microvilli (not shown). These results indicate that the transmembrane and cytoplasmic domains are not responsible for the differential localization of α4β7 versus αLβ2 and αMβ2.

Presentation of Other Leukocyte Integrins on Microvilli

In addition to α4β7, αLβ2, and αMβ2, leukocytes express other integrins that play roles in adhesion to endothelial cells, to other cells, and to extracellular matrix proteins. One of these, αEβ7, can initiate adhesion to vascular cell adhesion molecule–1 (VCAM-1) under flow and has been reported to be expressed on lymphocyte microvilli (Alon et al., 1995; Berlin et al., 1995). We confirmed that αEβ7 was also expressed preferentially on microvilli of K562-αEβ7 transfectants (Table I). We found that the T cell integrin αEβ7 (Cepek et al., 1994), which can mediate adhesion to epithelium but has no established role in endothelial adhesion, was also localized to microvilli of K562-αEβ7 cells (Fig. 6 A, and Table I). Integrin α6β1, a laminin receptor which is expressed on monocytes and some lymphocytes, was concentrated on microvilli of K562 transfectants (Fig. 6 B, and Table I). The vitronectin receptor, integrin αvβ3, is also a receptor for the endothelial cell ligand platelet/endothelial cell adhesion molecule–1 (Piali et al., 1995) and is expressed on monocytes and other cells. We found that αvβ3 localized predominantly to microvilli (Fig. 6 C, and Table I). The fibronectin receptor, integrin α5β1, is constitutively expressed on K562 cells and was also expressed predominantly on microvilli (not shown).
Localization of Transmembrane Proteins Known to Associate with Integrins

Integrins have been shown to associate with a variety of intracellular and transmembrane proteins. Some of these interactions occur within the cell and are mediated by integrin cytoplasmic domains, whereas others are known or suspected to be extracellular. Since our results suggested that extracellular (and not transmembrane or cytoplasmic) domains determine integrin localization, we performed immunoelectron microscopy to localize several cell surface proteins known or suspected to interact with integrin extracellular domains. Several members of the tetraspan family of transmembrane proteins have been shown to associate with α4β7, αLβ2, αMβ2, and some other integrins (Berditchevski et al., 1996; Mannion et al., 1996). These associations are likely to involve the extracellular domains of tetraspans and integrins (see Discussion). Several tetraspans are constitutively expressed on K562 cells. CD53 (82 ± 5% on microvilli, Fig. 7 A), CD63 (92 ± 4% on microvilli, Fig. 7 B), and CD81 and CD82 (data not shown) are all localized predominantly to microvilli. Another transmembrane protein, CD47 (integrin-associated protein), has been shown to associate with αvβ3 via its extracellular domain. CD47, like αvβ3, was distributed mostly on microvilli (Fig. 7 C). CD32 is a transmembrane protein that has been reported to associate with αMβ2 (which is on the cell body; Fig. 5 C) and possibly with the tetraspan CD82 (found on microvilli, see above) (Lebel-Binay et al., 1995). CD32 was expressed on both microvilli (62 ± 15%) and the cell body (38 ± 15%) (Fig. 7 D).

Discussion

In this study, we examined the role of leukocyte integrin cytoplasmic and transmembrane domains in adhesion under flow and microvillous localization. We began by confirming that previously described differences in leukocyte integrin adhesive activity and membrane localization were also seen in K562 cell integrin transfectants. As expected, α4β7 was able to initiate adhesion under flow and localized...
Table I. Integrin Distribution on Transfected K562 Cells

| Integrin    | Percent on microvilli | Percent on body | Gold particles counted |
|-------------|-----------------------|-----------------|-----------------------|
| α4β7        | 89 ± 9                | 11 ± 9          | 1,288                 |
| α4β1        | 81 ± 7                | 19 ± 7          | 348                   |
| αLβ2        | 31 ± 14               | 69 ± 14         | 99                    |
| αMβ2        | 22 ± 13               | 78 ± 13         | 236                   |
| α4β7(αLβ2c) | 90 ± 9                | 10 ± 9          | 260                   |
| α4β7(αMβ2c) | 87 ± 6                | 13 ± 6          | 386                   |
| αEβ7        | 83 ± 6                | 17 ± 6          | 149                   |
| α6β1        | 77 ± 9                | 23 ± 9          | 401                   |
| αvβ3        | 80 ± 10               | 20 ± 10         | 2,741                 |

Transfected K562 cells expressing various integrins were analyzed by immunoelectron microscopy after staining with appropriate anti-integrin antibodies. For each integrin, all gold particles in at least three photomicrographs were counted. Percentages are presented as mean ± SD.

to microvilli, whereas αLβ2 and αMβ2 mediated adhesion only under static conditions and localized to the planar cell body. Two chimeras, α4β7(αLβ2c) and α4β7(αMβ2c), were expressed to examine the roles of the extracellular, transmembrane, and cytoplasmic domains in adhesion and membrane localization. Both chimeras were as efficient in initiating adhesion under flow as the wild-type integrin α4β7. The chimeras were found predominantly on microvilli, indicating that the extracellular domain (and not the transmembrane or cytoplasmic domains) determined membrane localization of α4β7. We have so far been unable to determine whether β2 integrin localization to the cell body is also independent of the transmembrane and cytoplasmic domains. We attempted to address this issue by expressing a chimeric integrin composed of the extracellular domain of αMβ2 and the transmembrane and cytoplasmic domains of α4β7, but have not yet been successful in these experiments. In addition to α4β7, several other integrins (α4β1, α5β1, α6β1, αvβ3, and αEβ7) were localized to microvilli. Transmembrane proteins known or suspected to interact with integrin extracellular domains, including tetraspan family members and CD47, were also found to be concentrated on microvilli.

The ability of leukocyte adhesion molecules to support initial adhesion under flow is influenced by several factors including affinity, avidity, and accessibility to endothelial ligands. Previous studies of nonintegrin adhesion molecules indicate that cytoplasmic domains can have dramatic effects upon initiating adhesion under flow without altering static adhesion. Experiments involving the use of L-selectin–CD44 chimeras suggest that cytoplasmic domains may influence adhesion under flow by targeting these receptors to the microvillous or cell body (see Introduction). However, it is clear that the cytoplasmic domain of L-selectin also has effects on initiation of adhesion that are independent of receptor positioning. A truncation mutant of L-selectin lacking the 11 COOH-terminal amino acid residues of the cytoplasmic domain was localized to microvilli and retained the ability to bind ligand, but was unable to support rolling on endothelium (Kansas et al., 1993; Pavalco et al., 1995). This mutant lost the ability to associate with the cytoskeletal proteins α-actinin and vinculin, suggesting that interactions between adhesion molecule cytoplasmic domains and cytoskeletal proteins can be important in regulation of initial adhesive interactions under flow.

Figure 6. Localization of αEβ7, α6β1, and αvβ3 integrins by immunoelectron microscopy. K562 transfectants were stained for protein expression using 12-nm gold particles (arrows) as described in Materials and Methods. Staining of K562-αEβ7 with Fib 504 (anti-β7, A), K562-α6β1 with anti-α6 (GoH3, B), and K562-αvβ3 with L230 (anti-αv, C) revealed that each of these integrins was found primarily on microvilli. Bar, 0.5 μm.
found that replacement of the cytoplasmic and/or trans-
membrane domains of α4β7 (which does support adhesion
under flow) with the homologous domains of αLβ2 or
αMβ2 (which do not) did not affect adhesion to the α4β7
ligand MAAdCAM-1 under either static or flow conditions.
This indicates that the cytoplasmic domains cannot ac-
count for the differences in ability of these integrins
to support adhesion under flow. We also found that truncation
of the α4 subunit after the conserved GFFKR motif
had little if any effect on initiation of α4β7-mediated adhe-
sion to MADCAM-1. Others have previously shown that
the same truncation of α4 did not affect the ability of α4β1
to initiate adhesion to its ligand, VCAM-1 (Kassner et al.,
1995). The α4 truncation was reported to decrease the
cell’s resistance to detachment from VCAM-1 in the face
of increasing shear force, suggesting that the α subunit cy-
toplasmic domain plays a role in strengthening adhesion.
We were unable to detect any difference in resistance to
detachment between wild-type and chimeric α4β7 inte-
grins, suggesting that α4β7, αLβ2, and αMβ2 integrin cy-
toplasmic domains are similar in their ability to mediate
adhesion strengthening.

Many of the adhesion molecules that initiate adhesion
to endothelium under flow are concentrated on leukocyte
microvilli. These include L-selectin (Picker et al., 1991; Er-
landsen et al., 1993; Pavalko et al., 1995), P-selectin glyco-
protein ligand–1 (Moore et al., 1995; Bruehl et al., 1997),
and the integrins α4β7 and α4β1 (Berlin et al., 1995; and
this report). Other adhesion molecules, including CD44
and the integrins αLβ2 and αMβ2, are found predomi-
nantly on the cell body (Erlandsen et al., 1993; Berlin et al., 1995; von Andrian et al., 1995; and this report). Little information is available about the mechanisms that lead to the selective display of certain adhesion molecules on microvilli. It seems likely that interactions between the cytoplasmic domains of adhesion molecules and specific cytoskeletal elements can play an important role. In support of this concept, the localization of L-selectin–CD44 chimeras was shown to be determined by the cytoplasmic and/or transmembrane domains, and not by the extracellular domains (see Introduction). Concentration of integrins in other structures, such as focal adhesions and hemidesmosomes, is known to depend upon the β subunit cytoplasmic domain. We were surprised to find that our analysis of integrin chimeras did not demonstrate a role for the cytoplasmic or transmembrane domains of either the α or β subunit in determining membrane localization. Replacement of the αβ7 cytoplasmic and/or transmembrane domains with homologous domains of αLβ2 or αMβ2 did not interfere with microvillous localization. Put another way, replacement of the extracellular domain of the αLβ2 or αMβ2 integrins with the extracellular domain of αβ7 resulted in a shift from cell body to microvillous localization. These results indicate an important role for the extracellular domain in directing localization of integrins to the microvillus versus the cell body.

Integrin extracellular domains can interact with other cell surface proteins. For example, the transmembrane protein CD47 interacts with αvβ3 and this interaction depends upon the extracellular domain of CD47 (Lindberg et al., 1996). Several members of the tetraspan family of transmembrane proteins, including CD53, CD63, CD81, and CD82, have been shown to coprecipitate with some integrins, including α4β1, α6β1, and αβ7, but not with αLβ2 or some other integrins (Berditchevski et al., 1996; Manion et al., 1996). These integrins are likely to involve the integrin extracellular domain, since mutations of the α subunit extracellular domain substantially reduce association whereas alterations of the α subunit cytoplasmic domain have no effect. We found that CD47, CD53, CD63, CD81, and CD82, all known to associate with integrins that we localized to microvilli, were themselves concentrated on microvilli. Our data are consistent with the hypothesis that interactions with tetraspans and CD47 help target certain integrins to microvilli. The widespread expression of tetraspans and CD47 on leukocytes and other cells makes this hypothesis difficult to test directly. At least one integrin that we localized to microvilli, α5β1, apparently does not associate with tetraspans or CD47, suggesting that other interactions also are important (Berditchevski et al., 1996). This hypothesis assumes that α4β7 and other microvillous integrins are actively concentrated on microvilli, but αLβ2 and αMβ2 are not. An alternative explanation of our results is that microvillous expression is the “default pathway” for integrins, and that the extracellular domains of αLβ2 and αMβ2 prevent these integrins from being displayed on microvilli. This could be mediated by interactions between β2 integrins and associated cell surface proteins. Although αMβ2 has been shown to associate with CD32, the pattern of expression of CD32 (on both cell body and microvilli) suggests that this interaction is not responsible for the concentration of αMβ2 on the cell body.

We found that many integrins and integrin-associated proteins were preferentially expressed on microvilli. Some of these integrins, including αβ7 and αβ1, play important roles in mediating leukocyte adhesion under flow. Other microvillous integrins, such as αβ1, and αβ7, mediate adhesion to extracellular matrix proteins or epithelial cells, but have no known role in initiating leukocyte–endothelial interactions. This suggests that the assembly of multimolecular complexes containing integrins and integrin-associated proteins on microvilli may have other important roles in adhesion and signaling.

We thank K.L. McDonald and P. Sicurella (Robert D. Ogg Electron Microscope Laboratory, University of California, Berkeley, CA) and G. Antipa and G. Lum (San Francisco State University, San Francisco, CA) for their expert advice and courtesy in allowing us to use their electron microscope facilities. We thank S. Wu for optimizing the static adhesion assay protocol and are grateful to R. Pytel and D. Sheppard for critically reviewing this manuscript.

This study was supported by National Institutes of Health grants HL50024 (to D.J. Erle) and HL03230 (to M. Tidswell). M. Abi Abitorabi was supported by National Institutes of Health training grant HL07155 and National Research Service Award 1F32HL09364.

Received for publication 28 May 1997 and in revised form 24 July 1997.

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