Identification of a Sequence within the Integrin β6 Subunit Cytoplasmic Domain That Is Required to Support the Specific Effect of αvβ6 on Proliferation in Three-dimensional Culture*

(Rceived for publication, June 24, 1996)

Rashmi B. Dixit, Aileen Chen, John Chen, and Dean Sheppard

From the Lung Biology Center, Department of Medicine, University of California, San Francisco, California 94143

The integrin αvβ6 augments the proliferation of epithelial cells in collagen gels and in vivo. This effect depends on the presence of a unique carboxyl-terminal region of the β6 subunit cytoplasmic domain. In the present study, we have utilized deletional and alanine substitution mutagenesis within this region to map the amino acids responsible for αvβ6-mediated proliferation in more detail. Replacement or deletion of any of six amino acids (glutamic acid 778, lysine 779, lysine 781, valine 782, aspartic acid 783, and leucine 784) largely abolished the proliferative effects of αvβ6, but none of the mutants examined interfered with αvβ6-mediated cell adhesion or with localization of αvβ6 to focal adhesions. These findings suggest that residues contained within the sequence EKXKVDL are critical for the effects of αvβ6 on proliferation in collagen gels and that pathways initiated by interaction with this sequence are distinct from those required for integrin-mediated cell attachment or focal adhesion formation.

The integrin αvβ6 is a receptor for the extracellular matrix proteins fibronectin and tenasin (1–3). This integrin is restricted in its distribution to epithelial cells and is principally expressed during development, in response to injury and/or inflammation, and in epithelial neoplasms (4, 5). In an effort to identify the functional significance of αvβ6, we have previously examined the effects of heterologous expression of this integrin on cell behavior. When αvβ6 was expressed in the human embryonic kidney cell line, 293, or the colon carcinoma cell line, SW480, the receptor was shown to mediate cell attachment to appropriate ligands and to localize to focal adhesions, properties shared by other members of the integrin family (6). In addition, αvβ6 was uniquely capable of enhancing the ability of transfected cells to proliferate in a three-dimensional culture system and in vivo in nude mice (6).

Most integrin β subunit cytoplasmic domains contain a highly conserved region of 48 amino acids containing subdomains that have been shown to be critical for localization to focal adhesions and for interaction with cytoplasmic signaling proteins such as the focal adhesion kinase and Paxillin (7–10). In addition to containing this highly conserved region, the β6 cytoplasmic domain also contains a completely unique 11-amino acid carboxyl-terminal extension. In a previous study, we found that this unique carboxyl-terminal extension was not required for localization of αvβ6 to focal adhesions nor for αvβ6-mediated cell adhesion to fibronectin (6). However, deletion of this region completely abrogated αvβ6-mediated proliferation both in three-dimensional culture and in vivo (6).

In the present study, we have utilized smaller deletions and saturation alanine substitution mutagenesis within this region to map the amino acids responsible for this specific effect of αvβ6 in more detail.

MATERIALS AND METHODS

Cell Lines, Antibodies, and Reagents—The human colon carcinoma cell line SW480 was obtained from American Type Culture Collection (Rockville, MD) and maintained in Dulbecco’s modified Eagle’s medium (DMEM; Biowhittaker, Walkersville, MD). Monoclonal anti-αvβ6 (E7P6) and anti-αv (L230) antibodies were prepared as described previously (3). Mouse monoclonal antibodies P1H5, directed against α2, P3D10, directed against α5, P5D2, directed against β1 and P1F6 against β5, were gifts from Elizabeth Wayner (University of Minnesota, Minneapolis, MN). Mouse monoclonal anti-α6, J1B5, and anti-α3, P1B5, and Genetecin (neomycin analogue G418) were purchased from Life Technologies, Inc. Enzymes EcoRI, XhoI, XhoI, T4 DNA polymerase, and T4 DNA ligase were obtained from Promega Corp. (Madison, WI). Type I collagen, collagenase, and bovine serum albumin were purchased from Sigma. Fibronectin was prepared from human plasma as described by Englavail and Rouslahiti (11).

Preparation of Expression Plasmids—A cDNA containing the entire coding region of human β6 in the mammalian expression vector pcDNAIneo (Invitrogen, San Diego, CA) was constructed as described previously (3). To construct truncated versions of pcDNAIneoβ6 that lacked selected portions of the cytoplasmic domain, cDNA fragments were amplified by polymerase chain reaction using a 5’ upstream primer corresponding to nucleotides 2050–2070 and 3’ downstream primers at the following positions: 1) nucleotides 2558–2578 to prepare pcDNAIneoβ6T-1; 2) nucleotides 2559–2578 to prepare pcDNAIneoβ6T-2; and 3) nucleotides 2550–2569 to prepare pcDNAIneoβ6T-3. The full-length plasmid, pcDNAIneoβ6, was used as a template. The 3’ primers introduced stop codons to replace amino acids Cys788, Ser785, and Val783, respectively, and each primer included an XhoI recognition sequence to facilitate ligation into the unique XhoI site in the pcDNAIneo polylinker. Each polymerase chain reaction fragment included a unique BstEI site in β6 (nucleotide 2067). The polymerase chain reaction products were digested with XhoI and BstElI and ligated into pcDNAIneoβ6 that had been digested with the same enzymes. The authenticity of each mutant clone was confirmed by dideoxy sequencing using Sequenase 2.0 (U. S. Biochemical Corp.).

For site-directed mutagenesis, full-length β6 cDNA was ligated into the mutagenesis plasmid pAlter-1 (Promega) between Smal and XhoI sites in the poly linker. Nine alanine substitution mutations were generated with the Altered Sites In Vitro Mutagenesis System utilizing nine distinct mutant oligonucleotides designed to individually replace each of amino acids 778–786 in the β6 cytoplasmic domain with alanine. pAlter-1 contains two antibiotic resistance genes encoding resist-

* This work was supported in part by National Institutes of Health Grants HL/A 132589, HL47412, and HL53949. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† These two authors made equal contributions to this manuscript.

‡ Present address: University of California, Irvine Medical School, Irvine, California.

¶ To whom correspondence should be addressed: Lung Biology Center, UCSF, Box 0854, San Francisco, CA 94143. Tel.: 415-206-5901; Fax: 415-206-4123; E-mail: deans@itsa.ucsf.edu.

1 The abbreviations used are: DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline.

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 271, No. 42, Issue of October 18, pp. 25976–25980, 1996
Printed in U.S.A.
**Integrin-induced Proliferation**

Cells were incubated in the presence or the absence of PBS, and blocked with 1% bovine serum albumin in serum-free DMEM. The 500 plates with 10 mM EDTA, washed, and blocked with normal goat serum E7P6, respectively. Briefly, cells were harvested from tissue culture with 1% formaldehyde and 0.5% crystal violet in 20% methanol, and the 3 individual colonies were harvested and further subcloned by limiting dilution. Stable transfectants were screened for β6 expression by flow cytometry.

**Flow Cytometry**—Expression of the integrin subunits α2, α3, α5, α6, β1, β5, and αvβ6 was analyzed for all stable transfectants using the monoclonal antibodies P1H5, P1B5, P3D10, J1B5, P5D2, P1F6, and E7P6, respectively. Briefly, cells were harvested from tissue culture plates with 10 ml EDTA, washed, and blocked with normal goat serum (Vector Laboratories, Burlingame, CA) at 4 °C for 10 min. Washed with phosphate-buffered saline (PBS), and incubated with the primary antibody (listed above) for 20 min at 4 °C. The cells were washed and then incubated with goat anti-mouse secondary antibody conjugated to phycoerythrin (Chemicon, San Diego, CA) for 20 min at 4 °C, washed twice with PBS, and resuspended in PBS for analysis with FACSort (Becton Dickinson, Rutherford, NJ).

**Cell Proliferation in Collagen Gels**—Proliferation assays were performed as described previously (6). Bilayer collagen gels comprising of a 500-μl cell-free underlayer and a 500-μl cell-containing upper layer were prepared in 24-well tissue culture plates. Type 1 collagen (3.9 mg/ml) was dissolved in 40 mM acetic acid for 48 h. To this solution an equal volume of a 2 × concentrate of DMEM supplemented with 10% fetal bovine serum, penicillin, streptomycin, and 1 mg/ml G418 was added. The pH was adjusted to 7.4 with 1 M NaOH. 500 μl of this was plated and allowed to gel at 37 °C. 10^6 cells were suspended in 500 μl of the DMEM/collagen mixture and overlaid on this layer. After gelation, 500 μl of culture medium containing 5% fetal bovine serum, 1 mg/ml G418 was added to each well. In all experiments, duplicate gels were prepared and were incubated at 37 °C in 5% CO2 for 7 days. At the end of each 3-day period, the cells were harvested by dissolving the collagen matrix with collagenase (15 mg/ml). Total number of cells harvested was determined by counting cells in a hemocytometer.

**Cell Adhesion Assays**—Cell adhesion assays were performed as described previously (1). Briefly, wells of non-tissue culture treated polystyrene 96-well flat bottom microtiter plates (Linbro/Titertek, Flow Laboratories, McLean, VA) were coated with 100 μl of 10 μg/ml fibronectin or 1% bovine serum albumin for 1 h at 37 °C, washed with PBS, and blocked with 1% bovine serum albumin in serum-free DMEM. Cells were incubated in the presence or the absence of the β1-blocking antibody P5D2 for 15 min at 4 °C, and 50,000 cells/well were then plated onto the matrix-coated wells. The plates were centrifuged (top side up) at 10 × g for 5 min and incubated for 1 h at 37 °C in humidified 5% CO2. Nonadherent cells were removed by centrifuging the plates top side down at 10 × g for 5 min. The attached cells were fixed and stained with 1% formaldehyde and 0.5% crystal violet in 20% methanol, and the excess dye was washed off with PBS. The cells were solubilized in 50 μl of 1% Triton X-100 and quantified by measuring the absorbance at 595 nm in a Microplate Reader (Bio-Rad). The data were expressed as the means of the absorbance of triplicate wells. Measurements of absorbance were determined to be linearly related to input cell number over the range of 10,000–70,000 cells/well.

**Immunofluorescence**—Cells were harvested by EDTA treatment before reaching 75% confluence. 3–5 × 10^4 cells were plated on 6-mm diameter wells of 10-well slides (Structure Probe, West Chester, PA) that were previously coated with 10 μg/ml fibronectin and blocked with 2% bovine serum albumin in PBS. The slides were incubated for 4 h at 37 °C in 5% CO2 and washed twice with PBS, and the cells were fixed with 2% paraformaldehyde (Fisher) in PBS for 10 min and permeabilized for 20 min in 0.1% Triton X-100 in PBS. The cell monolayers were blocked with 2% bovine serum albumin and processed for immunofluorescence microscopy. The slides were incubated with anti-human αvβ6 antibody (E7P6, 1:10), with biotinylated sheep anti-mouse IgG (1:50), and with streptavidin-fluorescein (1:100) for 60, 45, and 30 min, respectively. For vinculin staining, the fixed cells were incubated for 1 h with primary mouse anti-vinculin and then for 30 min with fluorescein isothiocyanate-coupled goat anti-mouse IgG (1:50). Stained cells were washed with PBS between incubations. Slides were briefly rinsed in distilled water and mounted with coverslips using Fluoromount G (Fisher).

**RESULTS**

**Deletions within the Carboxyl-terminal 11 Amino Acids Are WellExpressed on the Cell Surface and Do Not Impair αvβ6-mediated Cell Adhesion or Localization of the Receptor to Focal Adhesions**—We have previously reported that heterologous expression of the integrin αvβ6 augments proliferation of SW480 cells in three-dimensional collagen gels and that a unique region of the β6 cytoplasmic domain is required for this effect (6). This unique region corresponding to the carboxy-terminal 11 amino acids (Fig. 1) was not required for cell attachment or for localization of αvβ6 to focal adhesions. To determine the amino acids within this unique region that are responsible for this proliferative effect, we first generated stably transfected SW480 cells expressing truncated β6 lacking the carboxy-terminal one, four, or seven amino acids, respectively (Fig. 1). As shown in Table I, each of these truncation mutants was well expressed on the cell surface, as detected by flow cytometry using the αvβ6 complex-specific monoclonal antibody, E7P6 (3). To determine the ability of each truncation mutant to support αvβ6-mediated cell attachment, adhesion assays were performed for each transfectant on fibronectin-coated 96-well plates in the presence of the β1-blocking antibody, P5D2 (Fig. 2). As expected, mock transfectants demonstrated only minimal β1-independent adhesion to fibronectin, whereas each of the deletion mutants adhered as well as the wild type β6 transfectants. Each of the deletion mutants also localized to focal adhesions as determined by fluorescence microscopy (data not shown). These findings are consistent with our previously reported observations that the carboxy-terminal 11 amino acids were not required for αvβ6-mediated cell adhesion or localization of αvβ6 to focal adhesions (6, 13) and suggest that the truncation mutations we generated did not grossly alter the conformation of the conserved membrane-proximal 48 amino acids of the β6 cytoplasmic domain. To assess the ability of each of the cytoplasmic domain trun-
as well as cells transfected with full-length SW480 cells stably transfected with this construct prolifera
carboxyl-terminal cysteine (T-1) had no effect on proliferation. were plated in collagen gels for 7 days (Fig. 3). Deletion of the
were expressed on the cell surface as determined by flow cy-
expression of other integrins and proliferation in three-dimen-
sional culture, mock, \( \beta 6 \), \( \beta 6T-1 \), \( \beta 6T-4 \), and \( \beta 6T-7 \) transfectants were plated in collagen gels for 7 days (Fig. 3). Deletion of the carboxyl-terminal cysteine (T-1) had no effect on proliferation. SW480 cells stably transfected with this construct proliferated as well as cells transfected with full-length \( \beta 6 \), whereas mock-transfected cells did not proliferate. Although deletion of the carboxyl-terminal four amino acids (T-4) reduced proliferation by approximately 50%, SW480 cells transfected with this construct retained considerable capacity to proliferate in this culture system. These data suggest that the carboxyl-terminal four amino acids are not absolutely required to produce this effect. In contrast, deletion of the carboxyl-terminal seven amino acids essentially abrogated \( \alpha v \beta 6 \)-mediated proliferation, suggesting that one or more critical amino acids is included in the region including valine 782, aspartic acid 783, and leucine 784.

We analyzed at least two clones for each mutation and obtained similar results with each clone, excluding clonal variation as an explanation for any of the observations made. Furthermore, each clone was analyzed for their expression of other integrins on the cell surface (Table I). There was considerable variation among clones in the expression of \( \alpha 2 \), \( \alpha 3 \), \( \alpha 5 \), \( \alpha 6 \), \( \alpha v \), \( \beta 1 \), \( \beta 5 \) and \( \alpha v \beta 6 \). However, there was no consistent relationship between expression of other integrins and proliferation in three-dimen-
sional culture.

Effects of Alanine Substitution Mutations—To further delineate the critical amino acids required for this unique effect of the \( \beta 6 \) cytoplasmic domain, SW480 cells were stably transfected with expression plasmids encoding mutant forms of \( \beta 6 \) in which each of amino acids Glu778 through Thr786 in the \( \beta 6 \) cytoplasmic domain were replaced by alanine (Fig. 1). As shown in Table II, all of the alanine substitution mutations were expressed on the cell surface as determined by flow cy-

tometry, but there was considerable variation in the level of \( \alpha v \beta 6 \) expressed. Despite this range of expression levels, each of the alanine substitution mutants could mediate \( \beta 1 \)-independent cell adhesion to fibronectin (Fig. 4) and localized to focal adhesions in cells plated on fibronectin (data not shown). These data suggest that the alanine substitutions tested, like the deletion mutations described above, did not produce gross changes in the conformation of the conserved membrane-prox-

![FIG. 2. Effect of \( \beta 6 \) cytoplasmic domain deletions on the adhesion of stably transfected SW480 cells to fibronectin. Mock- and \( \beta 6 \)-transfected SW480 cells (full-length \( \beta 6 \) and deletion mutants) were allowed to adhere to fibronectin-coated wells in the presence of the \( \beta 1 \)-blocking antibody P5D2. Nonadherent cells were removed by centrifugation, and the attached cells were stained with crystal violet. Cell adhesion was quantitated by measuring the absorbance at 595 nm in a Microplate Reader. The bars represent mean values from nine data points obtained from triplicate wells in three separate experiments. The lines above the bars represent standard errors.](Image 341x502 to 521x636)

![FIG. 3. Effect of deletions of the carboxyl-terminal extension of \( \beta 6 \) on the ability of heterologously transfected SW480 cells to proliferate in three-dimensional collagen gels. 1 \( \times 10^6 \) cells of each of the transfectants were cultured in collagen gels for 7 days, at which time the gels were dissolved with collagenase, and the cell number was determined with a hemocytometer. The bars represent means of duplicate wells from five experiments, and the lines above each bar represent standard errors.](Image 87x486 to 259x636)

TABLE I

|            | \( \alpha 2 \) | \( \alpha 3 \) | \( \alpha 5 \) | \( \alpha 6 \) | \( \alpha v \) | \( \beta 1 \) | \( \beta 5 \) | \( \alpha v \beta 6 \) |
|------------|---------------|---------------|---------------|---------------|---------------|--------------|--------------|----------------|
| Mock       | 312           | 878           | 166           | 128           | 148           | 1383         | 838          | 6             |
| \( \beta 6 \) | 287           | 837           | 382           | 281           | 201           | 1745         | 683          | 404           |
| T-1        | 218           | 837           | 323           | 134           | 459           | 1423         | 764          | 615           |
| T-4        | 522           | 1143          | 496           | 183           | 521           | 2224         | 623          | 508           |
| T-7        | 112           | 558           | 88            | 73            | 807           | 1111         | 242          | 755           |
The points obtained from triplicate wells, in three separate experiments. 

...of any of the integrin subunits and the magnitude of the pro-

...failure to detect any correlation between the level of expression 

...of the transfectants by flow cytometry (Table II). As with the 

...acid carboxyl-terminal extension of... 

...in the present study on either of these end points. In contrast, 

...effect of any of the deletion or substitution mutants examined 

...mal 48 amino acids are sufficient, as confirmed by the lack of 

...that regions within the membrane-proximal 48 amino acids of the 

...that induced by full-length ß6). The carboxyl-terminal 11 

...alanine substitution mutants as measured by mean fluorescence intensity.

| Expression of various integrin subunits by ß6 alanine substitution mutants as measured by mean fluorescence intensity |
|---------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|
|       | a2    | a3    | a5    | a6    | a7    | ß1    | ß5    | ßvß6  |
| Mock  | 312   | 878   | 166   | 128   | 148   | 1383  | 838   | 6      |
| ß6    | 287   | 837   | 382   | 281   | 201   | 1745  | 683   | 404    |
| E778A | 280   | 877   | 159   | 186   | 162   | 1332  | 593   | 198    |
| K779A | 326   | 1124  | 119   | 63    | 404   | 1408  | 894   | 161    |
| Q780A | 256   | 955   | 108   | 64    | 287   | 1310  | 766   | 244    |
| K781A | 316   | 1244  | 393   | 103   | 413   | 1540  | 782   | 271    |
| V782A | 287   | 1451  | 158   | 120   | 396   | 1658  | 629   | 134    |
| D783A | 429   | 1688  | 128   | 88    | 427   | 2137  | 1086  | 134    |
| L784A | 578   | 1138  | 127   | 75    | 323   | 1658  | 913   | 164    |
| S785A | 667   | 806   | 383   | 230   | 294   | 2160  | 582   | 170    |
| T786A | 81    | 1193  | 170   | 169   | 343   | 1516  | 897   | 57     |

FIG. 4. Adhesion of mock-, ß6-, and ß6 alanine substitution mutant-transfected SW480 cells to fibronectin. 5 × 10^4 transfec-

tants were allowed to adhere to fibronectin-coated wells in the presence of ß1-blocking antibody P5D2. Nonadherent cells were removed by 
centrification, and the attached cells were stained with crystal violet. Cell adhesion was quantitated by measuring the absorbance at 595 nm 
in a Microplate Reader. The bars represent mean values from nine data points obtained from triplicate wells, in three separate experiments. 
The lines above each bar represent standard errors.

The results of the present study confirm our previous finding that distinct regions of the ß6 cytoplasmic domain are required for 
localization to focal adhesions and mediating stable cell adhesion and for enhancing cell proliferation in three-di-

...that form at the ends of actin stress fibers in spread cells 

...probability of ß6-mediated proliferation in this culture system. Although 
alanine substitution for serine at position 785 also abolished 
proliferation, this amino acid does not appear to be essential, 
because the mutant (T-4) from which it was deleted was able to 
support substantially enhanced proliferation (albeit 50% of 
that induced by full-length ß6). The carboxyl-terminal 11 

...in the present study inhibited ß6-mediated proliferation in this culture system. Although 
alanine substitution for serine at position 785 also abolished 
proliferation, this amino acid does not appear to be essential, 
because the mutant (T-4) from which it was deleted was able to 
support substantially enhanced proliferation (albeit 50% of 
that induced by full-length ß6). The carboxyl-terminal 11 
amino acids of ß6 and the critical amino acid sequence we have 
identified within this region are not homologous with any 
known signaling motifs. Although the carboxyl-terminal 11 
amino acids includes two potential phosphorylation sites (ser-
ine 785 and threonine 786), neither of these residues appears to 
be critical.

The cytoplasmic domain of the integrin ß1 subunit has been 
 extensively studied and has regions within it that have been 
 shown to be important for association with the linker proteins 
a-actinin and talin that connect integrins to the actin cytoskel-

ton and lead to localization of integrins to the focal adhesions 
that form at the ends of actin stress fibers in spread cells 
(14–17). Each of these regions is highly homologous to regions 
within the membrane-proximal 48 amino acids of the ß6 
subunit cytoplasmic domain (6, 13). It is therefore not surpris-
ing that none of the mutations analyzed in the present study, 
all of which involved amino acids outside this conserved region, 
affected localization to focal adhesions. Similarly, because we 
have previously reported that deletion of the carboxyl-terminal 
11 amino acids of the ß6 cytoplasmic domain does not interfere 
with ß6-mediated adhesion to fibronectin (6), it is not surpris-
ing that none of the smaller deletions or point mutations 
examined in the present study inhibited ß6-mediated cell adhesion.

**DISCUSSION**

The results of the present study confirm our previous finding that distinct regions of the ß6 cytoplasmic domain are required for 
localization to focal adhesions and mediating stable cell adhesion and for enhancing cell proliferation in three-dimen-
sional culture (6). For focal adhesion localization and cell ad-
hesion, regions within the highly conserved, membrane-proxi-
mal 48 amino acids are sufficient, as confirmed by the lack of 
effects of any of the deletion or substitution mutants examined 
in the present study on either of these end points. In contrast, 
proliferation in three-dimensional culture is absolutely de-
pendent on the presence of a region within the unique 11-amino 
acid carboxyl-terminal extension of ß6, which has the minimal 
essential sequence EKXKVDL. Either deletion or alanine sub-
stitution for any of these amino acids markedly attenuated 

...mediated proliferation in this culture system. Although 
alanine substitution for serine at position 785 also abolished 
proliferation, this amino acid does not appear to be essential, 
because the mutant (T-4) from which it was deleted was able to 
support substantially enhanced proliferation (albeit 50% of 
that induced by full-length ß6). The carboxyl-terminal 11 
amino acids of ß6 and the critical amino acid sequence we have 
identified within this region are not homologous with any 
known signaling motifs. Although the carboxyl-terminal 11 
amino acids includes two potential phosphorylation sites (ser-
ine 785 and threonine 786), neither of these residues appears to 
be critical.

The cytoplasmic domain of the integrin ß1 subunit has been 
 extensively studied and has regions within it that have been 
 shown to be important for association with the linker proteins 
a-actinin and talin that connect integrins to the actin cytoskel-
ton and lead to localization of integrins to the focal adhesions 
that form at the ends of actin stress fibers in spread cells 
(14–17). Each of these regions is highly homologous to regions 
within the membrane-proximal 48 amino acids of the ß6 
subunit cytoplasmic domain (6, 13). It is therefore not surpris-
ing that none of the mutations analyzed in the present study, 
all of which involved amino acids outside this conserved region, 
affected localization to focal adhesions. Similarly, because we 
have previously reported that deletion of the carboxyl-terminal 
11 amino acids of the ß6 cytoplasmic domain does not interfere 
with ß6-mediated adhesion to fibronectin (6), it is not surpris-
ing that none of the smaller deletions or point mutations 
examined in the present study inhibited ß6-mediated cell adhesion.

**FIG. 5.** Proliferation of mock-, wild type ß6-, and ß6 alanine substitution mutant-transfected SW480 cells in three-di-

...of 10 data points from duplicate wells in five separate experiments, and the lines above each bar represent standard errors.
The results of mutagenesis experiments should always be interpreted with caution, because deletions or substitutions in one portion of a polypeptide could produce effects by changing the conformation of other regions. For example, in the present study, as noted above, substitution of alanine for serine 785 abolished αvβ6-mediated enhancement of proliferation, but deletion of this same amino acid did not, a finding that suggests an effect of the alanine at this position on the conformation of another region of the cytoplasmic domain. Nonetheless, several lines of evidence suggest that at least amino acids with the linear sequence KVDL are directly involved in the proliferative effects of αvβ6. Deletion or alanine substitution of each of these amino acids consistently eliminated αvβ6-mediated enhancement of proliferation. Furthermore, none of the deletion or substitution mutations within this region affected proliferation. Over the past few years, studies from several different laboratories have described a number of intracellular signaling pathways that are specific for specific family members are likely to involve distinct pathways. The stable transfectants we have described in the present study should be useful tools for identifying the signaling pathways involved in the unique cellular responses to αvβ6.

REFERENCES
1. Busk, M., Pytela, R., and Sheppard, D. (1992) J. Biol. Chem. 267, 5790–5796
2. Prieto, A. L., Edelman, G. M., and Crossin, K. L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10154–10158
3. Weinacker, A., Chen, A., Agrez, M., Cone, R. I., Nishimura, S., Wayner, E., Pytela, R., and Sheppard, D. (1994) J. Biol. Chem. 269, 6940–6948
4. Breuss, J. M., Gallo, J., Delisser, H. M., Klimanskaya, I. V., Folkesson, H. G., Pittet, J. F., Nishimura, S. L., Aldape, K., Landers, D. V., Carpenter, W., Gillett, N., Sheppard, D., Matthay, M., Albehd, S. M., Kramer, R. H., and Pytela, R. (1995) J. Cell Sci. 108, 2241–2251
5. Breuss, J. M., Gillett, N., Lu, L., Sheppard, D., and Pytela, R. (1993) J. Histochim. Cytochem. 41, 1521–1527
6. Agrez, M., Chen, A., Cone, R. I., Pytela, R., and Sheppard, D. (1994) J. Cell Biol. 127, 547–556
7. Turner, C. E. (1994) Bioessays 16, 47–52
8. Kornberg, L., Earp, H. S., Parsons, J. T., Schaller, M., and Juliano, R. L. (1992) J. Biol. Chem. 267, 23439–23442
9. Guan, J. L., Trevithick, J. E., and Hynes, R. O. (1991) Cell Regul. 2, 951–964
10. Burridge, K., Turner, C. E., and Romer, L. H. (1992) J. Cell Biol. 116, 893–903
11. Engvall, E., and Ruoslahti, E. (1977) Int. J. Cancer 20, 1–5
12. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Transfection of Coprecipitates of Calcium Phosphate and DNA, pp. 16.32–16.39, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
13. Cone, R. I., Weinacker, A., Chen, A., and Sheppard, D. (1994) Cell Adhes. Commun. 2, 101–113
14. Horwitz, A., Duggan, K., Buck, C., and Burridge, K. (1986) Nature 320, 531–533
15. Hynes, R. O. (1992) Cell 69, 11–25
16. Otey, C. A., Vasquez, G. B., Burridge, K., and Erickson, B. W. (1993) J. Biol. Chem. 268, 21193–21197
17. Reszka, A. A., Hayashi, Y., and Horwitz, A. F. (1992) J. Cell. Biol. 117, 1321–1339
18. Clark, E. A., and Brugge, J. S. (1995) Science 268, 233–239
19. Schlaepfer, D. D., Hanks, S. K., Hunter, T., and van der Geer, P. (1994) Nature 372, 786–791
20. Schaller, M. D., Otey, C. A., Hildebrand, J. D., and Parsons, J. T. (1995) J. Cell Biol. 130, 1181–1187
21. Sheppard, D., Razzo, C., Starr, L., Quaranta, V., Erle, D. J., and Pytela, R. (1996) J. Biol. Chem. 271, 11562–11567

2 R. B. Dixit, A. Chen, J. Chen, and D. Sheppard, unpublished observations.
Identification of a Sequence within the Integrin β6 Subunit Cytoplasmic Domain That Is Required to Support the Specific Effect of αvβ6 on Proliferation in Three-dimensional Culture

Rashmi B. Dixit, Aileen Chen, John Chen and Dean Sheppard

J. Biol. Chem. 1996, 271:25976-25980.
doi: 10.1074/jbc.271.42.25976

Access the most updated version of this article at http://www.jbc.org/content/271/42/25976

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 20 references, 12 of which can be accessed free at http://www.jbc.org/content/271/42/25976.full.html#ref-list-1