Interchangeable α Chain Cytoplasmic Domains Play a Positive Role in Control of Cell Adhesion Mediated by VLA-4, a β₁ Integrin

By Paul D. Kassner and Martin E. Hemler

From the Dana-Farber Cancer Institute, Boston, Massachusetts 02115

Summary

Integrins can exist in a range of functional states, depending on the cell type and its state of activation. Although the mechanism that controls activity is unknown, it has been suggested that for some integrins, α chain cytoplasmic domains may exert either a negative effect or no effect on adhesion function. To address this issue for VLA-4 (an α4β1 heterodimer), we constructed an α4 cytoplasmic deletion mutant and chimeric α chains composed of the extracellular domains of α4 and the cytoplasmic domains of α2, α4, or α5. Upon stable transfection of wild-type α4, VLA-4 heterodimer was obtained that mediated (a) poor adhesion to CS1 peptide, fibronectin, or vascular cell adhesion molecule 1 (VCAM-1) (in K562 cells); (b) poor adhesion to CS1 peptide but moderate adhesion to VCAM-1 (in MIP101 cells); and (c) moderate adhesion to both CS1 peptide and VCAM-1 (in PMWK cells). Chimeric α4 constructs and wild-type α4 yielded similar results in these cell lines. In contrast, truncation of the α4 cytoplasmic domain (after the conserved GFFKR motif) caused an almost complete loss of adhesive activity in all three cell lines. Thus, several interchangeable α chain cytoplasmic domains play a fundamentally positive role in determining the state of constitutive activity for VLA-4. The α chain cytoplasmic domain is also required for agonist-stimulated adhesion, since phorbol ester stimulated the cell adhesion mediated by wild-type and chimeric α chains, but not by the cytoplasmic deletion mutant. The inactivity of both wild-type VLA-4 (in K562 cells), and truncated VLA-4 (in all three cell lines) was overcome by the addition of a stimulatory anti-β1 monoclonal antibody. Thus, the cytoplasmic domain-dependent cellular mechanism controlling both constitutive and agonist-stimulated VLA-4 activity could be bypassed by external manipulation of the integrin.

The integrin family is comprised of at least 20 heterodimers, each with different ligand-binding specificities formed by the pairing of one of 14 α chains with one of eight β chains. The α4β1 integrin VLA-4 has been implicated in a wide variety of biological processes. It is involved in cell adhesion to extracellular matrix via the alternatively spliced CS1 domain of fibronectin (FN)1 (1–4), and in cell–cell adhesion through the vascular cell adhesion molecule-1 (VCAM-1) on activated endothelium (3, 5, 6). VLA-4, which is normally expressed on T and B lymphocytes, monocytes, and eosinophils, is involved in inflammation, migration, and recirculation of lymphocytes (7, 8). Eosinophil adhesion to activated endothelium as seen in asthmatic and allergic conditions appears to be mediated by VLA-4 interaction with VCAM-1 (9). VLA-4/VCAM-1 interaction is likely to be important in lymphocyte adhesion to inflamed endothelium in rheumatoid synovium (10–12). Abs against the α4β1 integrin were shown to prevent the onset of experimental autoimmune encephalomyelitis, suggesting that VLA-4 may be involved in inflammatory diseases of the central nervous system, such as multiple sclerosis (13). Differentiation of B lymphocytes in germinal centers (14), hematopoietic stem cells in bone marrow (15–17), and T lymphocytes in thymus (18), all involve interaction of VLA-4 with VCAM-1 or FN. Additionally, α4β1 is expressed on neural crest–derived cells and interaction with the CS1 domain of FN is involved in migration of those cells from the neural crest during embryogenesis (19, 20). The VLA-4/VCAM-1 interaction has also been demonstrated during the formation of myotubes during muscle development (21). Because adhesion through α4β1 is involved in so many biological processes, it follows that regulation of the adhesion mediated by α4β1 (and other integrins) is of fundamental importance (22).

Integrins are versatile, exhibiting different levels of functional activity depending on the cell type and state of activation (23–25). For example, on platelets and leukocytes, many...
integrins exist in a relatively nonfunctional condition, but show increased binding capabilities after cell activation with a variety of agonists (23, 24, 26–30). In contrast, on many other cell types, integrins demonstrate a high level of functional activity, with relatively little capability for further up-regulation (23, 24). Elucidation of the mechanism(s) by which the functional activity of integrins is controlled is an area of intense research, and of fundamental importance. Nonetheless, this mechanism is poorly understood, although it has been variously suggested that membrane lipid composition (31, 32), divalent cations (24, 33–35), integrin phosphorylation (36–38), and glycosylation (39) may alter functional activity of integrin heterodimers. Other studies have suggested a role for the cytoplasmic domains of the integrin chains in the control of functional activity. Deletion of the β2, but not the α4, cytoplasmic domain resulted in loss of the ability to upregulate function by the phorbol ester PMA (40, 41), implicating the β but not α chain cytoplasmic domain in the control of functional regulation. Additionally, it has been demonstrated that deletion of the α2β1 cytoplasmic tail or exchange with the α2 cytoplasmic domain converted an inactive α2β1 heterodimer to a highly functional state (42). This suggested that the α2β1 cytoplasmic tail plays a negative regulatory role, and thus, alteration of that domain resulted in upregulation of function. Heretofore, there has been minimal analysis of the role of β1-associated α chain cytoplasmic domains in the regulation of integrin-mediated adhesion.

To analyze the role of the α chain cytoplasmic domains in the control of both constitutive and agonist-stimulated activity of β integrins, we have constructed a series of mutant α chains. The cytoplasmic domain of α4 was either deleted or exchanged with the cytoplasmic domains of α2 or α3, and stable transfectants expressing these mutant α4 chains or wild-type α4 were assayed for adhesive function. For transfections, we chose three cell lines (K562, MIP101, and PMWK) in which wild-type α4 was expressed to yield relatively inactive, partly active, or fully active VLA-4. Thus we were able to directly compare and determine the extent to which exchange or deletion of the cytoplasmic domain affects the variable levels of constitutive VLA-4 activity in different cell types.

**Materials and Methods**

Abs, Antisera, Cell Lines, and Purified Ligands. mAbs used in this study were anti-α4, B-5G10 (43), and HP 1/2 (44); anti-β1, A-1A5 (45) and TS2/16 (46); and J-2A2 (47) was used as a negative control Ab. Peptides corresponding to the COOH-terminal 21 amino acids of α4, and the COOH-terminal 21 amino acids of α4 were coupled to KLH and injected into rabbits for generation of antisera as previously described (48). The primary melanoma cell line PMWK (49) was a gift from Dr. H.R. Byers (Massachusetts General Hospital, Boston, MA). The 40-kD fragment of FN-40, which contains the CS1 region, was purified as described (50). Recombinant soluble VCAM-1 (51), the form with seven Ig domains, (sVCAM-1) was the gift of Dr. Roy Lobb (Biogen Inc., Cambridge, MA). CS1 peptide conjugated to BSA (CS1-BSA) was the gift of Dr. T. Shimo-Oka (Iwaki Glass Co., Tokyo, Japan).

Construction of Chimeric α Chains, Transfection, and Cell Culture. Mutations introducing a HindIII site into α2 and α4 cDNAs were as described (52). The X4C2 chimeric α subunit was first constructed in pBluescript-KS+ (pBS) by excising the 3' 1.3 kb of the α2 cDNA using KpnI and XbaI polylinker and then inserting the 516-bp HindIII fragment of α2 and the 1.9-kb HindIII-XbaI polylinker fragment of α4. For construction of the X4C5 chimera, the 0.7-kb HindIII-XbaI polylinker fragment from the α4 cDNA was used instead of the 1.9-kb fragment of α4. The α4 cDNA containing the introduced HindIII site (X4C4) encodes an α4 protein with a single conservative amino acid change from alanine to leucine at amino acid number 969. The α4 cytoplasmic deletion mutant (X4C0) was created by replacing codons for Q975 and Y976 (CAA, TAC) with termination codons (TAA, TAG) after the highly conserved GFFKK motif in the cytoplasmic domain of the integrin α4 cDNA using a PCR mutagenesis procedure (53). Sequencing of the product confirmed that only the intended mutations were introduced into the cDNA. The constructs were cloned into the pSRαNeo expression vector using the SalI and XbaI sites present in the pBS polylinker. The latter vector is a composite of pSRα (56) and pSRαNeo, prepared by Dr. Hamid Band (Brigham and Women's Hospital, Boston, MA; manuscript in preparation). KS56 (erythroleukemia), MIP101 (colon carcinoma), and PMWK (primary melanoma) were chosen as α4 recipients because they do not normally express VLA-4. K562 cells were transfected via electroporation at 960 μF and 270 mV using a gene pulser (Bio-Rad Laboratories, Cambridge, MA). Constructs were transfected into MIP101 and PMWK cells using the Lipofectin reagent (Bethesda Research Laboratories, Bethesda, MD). After selection with 2.0 mg/ml G418 (Gibco, Grand Island, NY), resistant cells were enriched for α4 expression using the B-SG10 mAb and immunomagnetic beads (Dynal, Inc., Great Neck, NY). Stably transfected cells were cultured in RPMI 1640 supplemented with 10% FCS, l-glutamine, antibiotics, and 1.0 mg/ml G418 (complete media).

Flow Cytometry and Immunoprecipitations. Indirect immunofluorescence was performed as described (3). For immunoprecipitation, lactoperoxidase and 125I were used for cell surface labeling followed by lysis with 1% Triton X-100 in Tris-buffered saline. Immunoprecipitation and SDS-PAGE were carried out as described (57).

Adhesion Assays. Adhesion assays were essentially as described (58). Briefly, cells were labeled by incubation with the fluorescent dye BCECF-AM (Molecular Probes, Inc., Eugene, OR), and then 5 x 10⁴ cells in RPMI with 0.1% BSA (assay media) were added to each well of 96-well microtiter plates (Flow Laboratories, Inc., McLean, VA) that had been coated with protein ligands and blocked with 0.1% BSA. After 20-min incubation at 37°C, unbound cells were removed (two to three washes with assay media). Cells remaining attached to the plate were analyzed using a fluorescence concentration analyzer machine (IDEXX Co., Portland, ME), or Cytofluor 2300 (Millipore Corp., Bedford, MA). Background binding (assessed using BSA-coated wells) was typically <5% of the total, and results are reported as the mean of triplicate determinations ± SD. TS2/16 (1 μg/ml) or PMA (10 ng/ml) was added to indicated samples at the start of the assay. For assays in which blocking Abs were used, cells were incubated with blocking or control Abs for 30 min before the time of plating.

**Results**

Chimeric α Chains Associate with β1 and Are Expressed on the Cell Surface. To examine the role of the α chain cytoplasmic domain in control of integrin function, a series of
truncated and chimeric α4 molecules were created (Fig. 1) and stably expressed at the cell surface of K562, MIP101, and PMWK cells (Fig. 2). Notably, the chimeric molecules (X4C2 and X4C5) were expressed at roughly similar levels as unaltered α4 (X4C4), whereas α4 lacking a cytoplasmic domain (X4C0) was expressed at variably lower levels. Expression of β1 in K562 cells was increased slightly upon transfection of the α4 chain constructs, although no significant differences in β1 expression were seen in the MIP101 or PMWK cell lines because of the large number of α4 chains endogenously expressed.

β1 can form a stable heterodimer with each of the chimeric α chains, as shown by coprecipitation of the α chimeras with β1-specific mAbs (Fig. 3, lanes e, i, m, and q) as well as coprecipitation of β1 with the α4-specific mAb B-5G10 (Fig. 3, lanes f, j, n, and r). Also coprecipitating with β1 was α5, seen as a faint band at ~150 kD (Fig. 3, lanes a, e, i, m, and q). The presence of this other integrin partly accounts for the level of total β1 being greater than the level of β1 associated with α4. Identity of the chimeric α4 chains was confirmed using antisera recognizing specific cytoplasmic tails. Antisera raised against a peptide from the cytoplasmic domain of α4 precipitated chimeric proteins from X4C2 transfected cells (Fig. 3, lane h) but not from pFNEO, X4C4, X4C5, or X4C0 transfected cells (Fig. 3, lanes d, l, p, and t). Similarly, antisera raised against a peptide from the α4 cytoplasmic domain precipitated proteins from cells transfected with X4C4 (Fig. 3, lane k) but not from pFNEO, X4C2, X4C5, or X4C0 constructs (Fig. 3, lanes c, g, o, and s). These results demonstrated that substitution of the cytoplasmic domain of α4 did not alter heterodimer assembly or expression. Although the X4C0 mutant was expressed in association with β1, deletion of the α4 cytoplasmic domain consistently resulted in lower expression (Fig. 2), perhaps because of slightly altered heterodimer assembly or stability. Exchange or deletion of the cytoplasmic domain did not alter the ability of α4 to be processed into its various structural forms (59). In K562 cells, wild-type and modified α4 chains were all equally cleaved into α4/β1 and α5/β1 fragments (Fig. 3). The α5/β1 fragment (derived from the COOH-terminal portion of α4) of X4C0 migrates at a lower apparent molecular weight (Fig. 3, lanes q and r) than the same fragment of X4C4 (Fig. 3, lane k).
lanes i–k) consistent with deletion of the COOH-terminal 25 amino acids. In MIP101 and PMWK transfectants, the appearance of the α4^135 and α4^130 structural forms (60), was not altered by cytoplasmic domain modification (data not shown). The αβ7 complex, examined by flow cytometry using the Act-1 mAb (61), was not detected on K562, MIP101, or PMWK transfectants, consistent with α1 being entirely associated with β1.

Variable Constitutive Activity of VLA-4 in Different Cell Types. K562, MIP101, and PMWK transfectants expressing X4C4 were assayed for their basal level of adhesion to sVCAM-1 and to CS1-BSA. Although surface expression of VLA-4 on each of these transfectants was similar, they exhibited widely variable adhesive properties (Fig. 4 A). Without stimulation, K562-X4C4 transfectants showed very little binding to either CS1-BSA or sVCAM-1, the MIP101-X4C4 transfectants displayed low level adhesion to CS1-BSA yet moderate adhesion to sVCAM-1, and the PMWK-X4C4 transfectants exhibited substantial adhesion to both ligands. Adhesion of each cell line was differentially amplified after stimulation with the TS2/16 mAb (previously shown to enhance β1 integrin mediated adhesion [23, 24, 62, 63]). Cell adhesion to sVCAM-1 was enhanced by 9.9, 3.0, and 1.4-fold (K562, MIP101, and PMWK, respectively), and cell adhesion to CS1-BSA was stimulated by 26.8, 6.6, and 1.4-fold (K562, MIP101, and PMWK, respectively) (Fig. 4 B). Thus we conclude that the K562 cell line had low constitutive activity but had a high potential for stimulation, the MIP101 cell line had an intermediate level of constitutive activity and moderate potential for stimulation, and the PMWK cell line had high constitutive activity with a low capacity for further stimulation. In several other experiments (data not shown), the magnitude of stimulation within each cell line was variable but the trend was consistent. Thus α4 derived from a single cDNA expressed in different cellular environments resulted in αβ1 with different levels of constitutive activity. As previously demonstrated, endogenous αβ7 can exist in a range of functional states depending on the cellular environment, and the degree of stimulation is inversely proportional to the basal level of activity (24). This is analogous with results obtained with αβ-transfected cells demonstrating differential ability to bind to collagen and laminin (23).

Because PMWK cells showed substantially more constitutive adhesion to VLA-4 ligands than did K562 cells, it was considered that these cell types could differ in their general overall adhesivity, independent of VLA-4 function. However, this was not the case because adhesion of PMWK cells was not greater than K562 cells on poly-L-lysine (Fig. 5 B), and both showed similarly low adhesion to BSA (Fig. 5 A). Fur-
Figure 5. Comparison of α4β1-transfected K562 and PMWK cell adhesion to control surfaces. Adhesion of K562 and PMWK transfectants was determined as described in Materials and Methods, except that background binding was not subtracted. K562 and PMWK cell lines transfected with X4C0 (filled) or X4C4 (diagonal stripes) were examined for their adhesion to plastic surfaces coated with 0.1% heat-denatured BSA (A) or 5 μg/ml poly-L-lysine (B).

Furthermore, in these control experiments, there was essentially no difference between X4C0 and X4C4 constructs, despite their marked differences in mediating adhesion to VLA-4 ligands (see Figs. 6–8).

Functional Activity of α4β1: Dependence on the Identity of the Cytoplasmic Domain. It has been suggested that deletion or substitution of the α chain cytoplasmic domain can release an integrin from an inactive conformation and allow it to display adhesive function (42). To determine how the identity of the α chain cytoplasmic tail affected the level of constitutive function of the α4β1 integrin heterodimer, cells transfected with αc chimeras were tested for adhesion to sVCAM-1 and CS1-BSA in a standard adhesion assay (58). Dose-dependent adhesion of all transfectants to sVCAM-1, CS1-BSA, or to a 40-kD fragment of FN containing the CS1 region (FN-40) was VLA-4 specific, as evidenced by nearly complete inhibition by the αc-specific antibody HP1/2 (data not shown). Additionally, mock transfectants displayed only minimal binding to either ligand.

Adhesion of K562 transfectants was uniformly low at the lower doses of FN-40 (0–20 μg/ml) and sVCAM-1 (0–2.5 μg/ml), regardless of the cytoplasmic domain (Fig. 6, A and B). Thus replacement or removal of the cytoplasmic tail of αc did not lead to increased constitutive activity of VLA-4. At higher doses of FN-40 (>20 μg/ml, data not shown) or sVCAM-1 (>2.5 μg/ml) a moderate level of adhesion was observed, but still there was no consistent difference between
cells transfected with the different chimeric α4 constructs. However, at the higher ligand doses (sVCAM-1 5 μg/ml) a lower level of adhesion was mediated by X4C0 than by X4C4, X4C2, or X4C5 in K562 cells. It appears unlikely that the slightly lower level of surface expression of the X4C0 construct could account for the greatly diminished adhesion at the high ligand level. When tested for adhesion to CS1-BSA (data not shown), the panel of K562 transfectants yielded results very similar to those obtained using FN-40.

For MIP101 and PMWK transfectants, the functions of the X4C4, X4C2, and X4C5 constructs were again not markedly different from one another (Fig. 6, C–F). Multiple other experiments (data not shown) confirmed that each of these constructs supported adhesion essentially to a similar extent, with minor variations perhaps due to slightly different expression levels.

In marked contrast to the chimeric constructs, the truncated α4 (X4C0) supported essentially no adhesion above that seen with mock-transfected MIP101 and PMWK cells (Fig. 6, C–F). Although cell surface expression of X4C0 was at a slightly lower level in those cells (Fig. 2), the nearly complete absence of adhesion appeared to be greater than could be explained by lower expression.

To test whether variation in expression levels caused the differences seen in levels of adhesion, cell surface expression of chimeric α4 chains and α4-mediated adhesion were examined in parallel, and then flow cytometry results were used to normalize adhesion results. Upon dividing cell adhesion (cells bound/mm²) by α4 expression (mean fluorescence intensity units [MFI]), we obtained a normalized result that clearly showed that adhesion mediated by α6β1 was not altered by exchange of the cytoplasmic tail (Fig. 7). As indicated, whether constitutive activity was low (K562), intermediate (MIP101), or high (PMWK), similar normalized results were obtained using X4C2, X4C4, or X4C5.

However, even after normalizing for expression differences, the X4C0 construct yielded substantially lower adhesive activity in both MIP101 and PMWK cells. In K562 cells, the adhesive activity of VLA-4 was already at such a low level, that truncation of the cytoplasmic domain could not result in a further decrease in activity.

To verify that the normalization of adhesion to cell surface expression was valid over a range of expression levels, we examined a series of six MIP101-X4C4 clones and lines, and compared their adhesion to either CS1-BSA or sVCAM-1, with their surface expression of α4. It was apparent that over a wide range of expression levels, adhesion to sVCAM-1 (Fig. 8 A) and to CS1-BSA (Fig. 8 B) correlated well with cell expression.
inherent defect that prevented them from binding ligand under X4C0 construct in K562 (Fig. 9 A), MIP101 (Fig. 9 B), or Where there was minimal unstimulated activity for the cells (Fig. 9 C) X4C4 was already active, and therefore only the nearly identical activities of X4C0 and X4C4 in the pres-

examined, similar results were obtained (data not shown). it was VLA-4 mediated. When adhesion to CS1-BSA was any circumstance, we tested their ability to adhere to sVCAM-1 elevated level of adhesion in each case. Also, the low (Fig. 9 A) or intermediate (Fig. 9 B) activity or the X4C4 con-
struct was greatly elevated by TS2/16 stimulation. In PMWK

Adhesion of X4C0 and X4C4 transfectants to both CS1-BSA and to sVCAM-1 than would be predicted based on α4 expression (Fig. 8, A and B), providing further evidence that the loss of cell adhesion was due to the deletion of the α4 cytoplasmic domain, and not to low surface expression of X4C0.

**Ab Stimulation of α Chain Cytoplasmic Deletion Mutants.** To determine whether α4 cytoplasmic deletion mutants had an inherent defect that prevented them from binding ligand under any circumstance, we tested their ability to adhere to sVCAM-1 after stimulation by the anti-β1 mAb TS2/16 (23, 24, 62). Whereas there was minimal unstimulated activity for the X4C0 construct in K562 (Fig. 9 A), MIP101 (Fig. 9 B), or PMWK cells (Fig. 9 C), TS2/16 stimulation caused a greatly elevated level of adhesion in each case. Also, the low (Fig. 9 A) or intermediate (Fig. 9 B) activity or the X4C4 construct was greatly elevated by TS2/16 stimulation. In PMWK cells (Fig. 9 C) X4C4 was already active, and therefore only slightly stimulated. For each cell line, adhesion (unstimulated or stimulated) was entirely blocked by HP1/2, indicating that it was VLA-4 mediated. When adhesion to CS1-BSA was examined, similar results were obtained (data not shown).

The nearly identical activities of X4C0 and X4C4 in the presence of TS2/16 emphasizes again (as also seen in Fig. 5) that there is no difference in the overall adhesive properties of the transfected cells. Also, this result emphasizes that when VLA-4 is fully stimulated, there is no difference in the specific adhesive contributions of X4C4 and X4C0.

**Phorbol Ester Stimulation of Adhesion: Affect of α Chain Cytoplasmic Domain.** As it is known that cell stimulation with a variety of agonists results in enhanced β1 integrin-mediated adhesion (22, 64, 65), we sought to ascertain the role of the α chain cytoplasmic domain in agonist-stimulated adhesion. Adhesion of X4C0 and X4C4 transfectants to both CS1-BSA and sVCAM-1 was assayed in the presence of PMA (a model cellular agonist) or TS2/16, and results are shown in Fig. 10. Predictably, each of the cell lines transfected with X4C4 exhibited increased cell adhesion to either CS1-BSA or sVCAM-1 in response to either PMA and TS2/16. Notably, there was variable enhancement of cell adhesion in response to PMA, with the K562-X4C4 transfectant showing a high degree of stimulation, MIP-X4C4 exhibiting an intermediate increase in adhesion, and PMWK-X4C4 displaying only slightly elevated adhesion. In this regard, stimulation with PMA resembles that seen with TS2/16 in Fig. 4. In contrast to the X4C4 transfectants, none of the X4C0-bearing cell lines exhibited enhanced adhesion in response to PMA, although they all demonstrated markedly increased adhesion in response to TS2/16. These data suggest that the cytoplasmic domain of the α chain is critically involved in the stimulation of adhesion by a cellular agonist such as PMA.

To further examine the role of α chain cytoplasmic domains in agonist-stimulated adhesion, cell lines expressing chimeric α chains were assayed for sensitivity to PMA and to TS2/16. To help control variability in absolute levels of adhesion, results were expressed in three ratios: (a) adhesion in the presence of PMA divided by adhesion in normal media; (b) adhesion in the presence of TS2/16 over adhesion in the presence of assay media; and (c) adhesion in the presence of PMA over adhesion in the presence of TS2/16 (maximal adhesion). These three ratios were determined for each separate experiment, and the mean and SD of ratio values from multiple experiments are presented in Table 1. In all three cell lines, the PMA/media and PMA/TS2/16 ratios were not significantly diminished for X4C0- compared to X4C4-transfected cells (values, *). Thus truncation of the α cytoplasmic domain clearly resulted in loss of response to PMA. Also, the PMA/media and PMA/TS2/16 ratios from X4C2, X4C5, and X4C4 transfectants were not significantly different in any of the three cell types. Therefore substitution of the α2 or α2 tail did not alter the affect of either PMA or TS2/16 on cell adhesion. Together, these results imply that the α cytoplasmic domain is critically involved in agonist-stimulated adhesion, but the identity of the domain is not crucial. The PMA/media ratio for adhesion to sVCAM-1 did not appear to be decreased for the X4C0 transfectant MIP101 or PMWK cell lines. However this may be due to the very low levels of initial binding seen by these cells. When the PMA/TS2/16 ratios for these cell lines are compared, it is clear that PMA stimulation did not enhance adhesion to the levels seen with the X4C4 transfected cell lines.

---

Figure 9. Adhesion mediated by X4C4 and X4C0 after stimulation with mAb TS2/16. Adhesion of X4C0+ and X4C4+ cell lines was examined in assay media alone (filled), assay media in the presence of 1 μg/ml α4-blocking mAb HP1/2 (gray crosshatch), assay media including 1 μg/ml TS2/16 (diagonal stripes), or assay media including 1 μg/ml TS2/16 and 1 μg/ml HP1/2 (tire tread). (A) K562 transfectant adhesion to sVCAM-1 (10 μg/ml). (B) MIP101 transfectant adhesion to sVCAM-1 (5.0 μg/ml). (C) PMWK transfectant adhesion to sVCAM-1 (2.5 μg/ml).
Discussion

This study clearly demonstrates a role of the α chain cytoplasmic domain in regulation of constitutive avidity and agonist-stimulated activity of a prototype β1 integrin heterodimer, VLA-4. First, we established that the same α4 cDNA expressed in three different cell types yielded three distinct levels of activity. Second, we found that exchange of the α4 cytoplasmic tail with that of α2 or α5 had no effect on the level of constitutive activity of the αβ1 integrin.

Table 1. PMA and TS2/16 Stimulation of Transfected Cell Lines

| Cell line | CS1-BSA | sVCAM-1 |
|-----------|---------|---------|
| K562      |         |         |
| X4C0      | 8       | *0.52 ± 0.40 | 7.60 ± 6.50 *0.11 ± 0.14 |
| X4C2      | 8       | 9.78 ± 11.56 | 15.58 ± 15.37 0.61 ± 0.37 |
| X4C4      | 8       | 13.25 ± 14.14 | 30.69 ± 22.27 0.45 ± 0.24 |
| X4C5      | 8       | 4.27 ± 2.85  | 22.68 ± 23.92 0.26 ± 0.09 |
| MIP101    |         |         |
| X4C0      | 3       | *0.30 ± 0.42 | 5.78 ± 5.94 *0.02 ± 0.04 |
| X4C2      | 3       | 1.70 ± 0.19  | 3.26 ± 1.16 0.59 ± 0.31 |
| X4C4      | 3       | 1.74 ± 0.23  | 4.66 ± 4.14 0.56 ± 0.31 |
| X4C5      | 3       | 1.48 ± 0.37  | 3.63 ± 2.65 0.52 ± 0.27 |
| PMWK      |         |         |
| X4C0      | 7       | *0.51 ± 0.87 | 3.42 ± 3.14 *0.16 ± 0.23 |
| X4C2      | 7       | 2.24 ± 1.72  | 2.29 ± 1.19 0.97 ± 0.37 |
| X4C4      | 7       | 1.48 ± 0.41  | 1.83 ± 0.87 0.87 ± 0.16 |
| X4C5      | 7       | 1.67 ± 0.51  | 1.72 ± 0.52 0.99 ± 0.17 |

Adhesion assays were as described in Materials and Methods. The PMA/media ratio was determined by dividing the adhesion (cells bound/mm²) in the presence of PMA by the adhesion in the presence of assay media alone. The (TS2/16)/(media) ratio was calculated by dividing adhesion in the presence of TS2/16 by adhesion in assay media, and the (PMA)/(TS2/16) ratio was computed by dividing the adhesion in the presence of TS2/16 by the adhesion in the presence of PMA. Ratios from multiple experiments were averaged and presented as the mean ± SD. Values in bold-face type demonstrate significant differences (p < 0.01, Student's t test) when compared with values for X4C4 transfectants.

* Discussed in text.
whether the activity was low (K562 cells), intermediate (MIP101 cells), or high (PMWK cells). Third, deletion of the \( \alpha^4 \) cytoplasmic domain led to greatly diminished adhesion relative to that observed using X4C4 or the chimeras (X4C2, X4C5). This diminished activity (X4C0 mutant) was especially obvious in both the MIP101 and PMWK cell lines, and was not merely a result of lower expression. Fourth, cell stimulation with a model cellular agonist (PMA) was able to upregulate integrin-mediated adhesion regardless of which cytoplasmic tail (\( \alpha^2 \), \( \alpha^4 \), or \( \alpha^5 \)) was used, whereas truncation of the cytoplasmic tail eliminated the capability of PMA to stimulate cell adhesion. A fifth major finding in this work is that the anti-\( \beta_1 \) mAb TS2/16 could stimulate adhesive function of all three cells bearing the X4C0 construct. Thus, whereas the absence of a cytoplasmic domain may disable the “natural” mechanism for generating a functionally active integrin, this mechanism can be bypassed by direct action of the TS2/16 mAb on the integrin. Notably, the natural constraints on integrin function in K562 cells expressing X4C4 were similarly bypassed through the addition of TS2/16.

Although results presented here strongly suggest that several interchangeable \( \alpha \) chain cytoplasmic domains play a positive role in regulating cell adhesion, studies involving the \( \alpha \) cytoplasmic domains of other integrins have yielded confusing and dissimilar results. The cytoplasmic domain of \( \alpha_{1b} \) appeared to play a negative regulatory role because deletion or exchange of the \( \alpha_{1b} \) tail with that of \( \alpha^2 \) caused the affinity of the \( \alpha_{1b}\beta_3 \) complex for its ligand to increase (42). In a separate study, the cytoplasmic tail of \( \alpha^4 \) was truncated with no apparent alteration of the adhesive properties of the \( \alpha^4\beta_2 \) heterodimer (40, 41), suggesting that the \( \alpha \) chain cytoplasmic domain has no effect on the level of constitutive avidity of the \( \beta_3 \) integrin. There are several possible reasons why our results are contrary to those found with the \( \alpha_{1b} \) and \( \alpha^4 \) integrin subunits. First, the cells transfected in those studies were different than those in our study, and it is becoming increasingly evident that integrins behave differently in distinct cellular environments (23, 24, 66, 67). Second, in the \( \alpha_{1b} \) study, the \( \alpha_{1b} \) cytoplasmic tail was truncated before the highly conserved GFFKR sequence, whereas we have deleted \( \alpha^4 \) after that sequence. Third, the cytoplasmic tail of \( \alpha^5 \) was placed onto the \( \alpha_{1b} \) extracellular domain, forcing the unnatural association of the \( \alpha^5 \) and \( \beta_3 \) cytoplasmic domains, which could be responsible for the increase in ligand binding. Fourth, it is possible that integrin \( \alpha \) subunit cytoplasmic domains are regulated in distinct ways depending on whether they are associated with \( \beta_1 \), \( \beta_2 \), or \( \beta_3 \). This could explain why in three studies of \( \alpha \) cytoplasmic domains, involving three different \( \beta \) chain partners, three distinct answers regarding regulation of constitutive activity were obtained.

We do not know the mechanism whereby integrins are maintained in different states of constitutive activity in different cell types. Also, it is not clear how PMA stimulation causes a rapid upregulation in integrin function. Nonetheless, we propose that the regulation of constitutive activity and agonist-stimulated activity could have similar mechanistic elements because (a) neither is influenced by exchange of \( \alpha^4 \) with \( \alpha^2 \) or \( \alpha^5 \) cytoplasmic tails; (b) both are suppressed upon \( \alpha^4 \) cytoplasmic domain deletion; and (c) in both cases, the negative effects of cytoplasmic domain deletion can be overcome by the addition of the anti-\( \beta_1 \) mAb TS2/16. Thus, we hypothesize that PMA, on a rapid scale, introduces a change into cells (such as K562) that is already constitutively present in cells such as PMWK.

It is quite clear that VLA-4-mediated cell adhesion varies in different cell types, is suppressed upon cytoplasmic tail deletion, and is upregulated upon PMA treatment. However, it is not clear whether any of these changes are due to variations in post-ligand binding events necessary for cell adhesion, or involve direct alterations in integrin-ligand binding affinity.

In one model, cytoplasmic tail deletions or PMA treatment could alter integrin interaction with the cytoskeleton or other key proteins, and thus influence the overall cellular adhesiveness without changing ligand binding affinity. Consistent with this, agents that disrupt the cytoskeleton are known to cause diminished cell adhesion (62, 68, 69). Also, PMA stimulation of LFA-1 (\( \alpha^\beta_2 \)) function correlated with coclustering of LFA-1 with the cytoskeletal protein talin (70, 71), and PMA-treated macrophages showed increased cytoskeletal anchoring (38). Furthermore, in one study, PMA stimulated increased cell adhesion to FN without altering the apparent affinity for soluble FN binding (64).

On the other hand, \( \alpha \) cytoplasmic domain–dependent changes in cell adhesion may be due to alterations of ligand-binding affinity. For example, others have demonstrated that agonist-stimulated cell adhesion correlated with integrin conformational changes (72, 73), and cytoplasmic tail mutations resulted in altered ligand-binding constants (42). Although it bypasses normal cellular regulation mechanisms, the anti-\( \beta_1 \) mAb TS2/16 and other similar Abs can stimulate cell adhesion, most likely by causing an increase in integrin affinity (23, 74, 75), thus providing another precedent for upregulated adhesion through modulation of integrin affinity.

To determine whether regulation through \( \alpha \) chain cytoplasmic domains does or does not involve alteration in integrin-ligand binding affinity, direct affinity measurement will be required. However, multiple attempts at direct ligand binding of CS1 peptide to VLA-4 have thus far proven unsuccessful because of high levels of nonspecific binding.

Regardless of whether \( \alpha \) chain cytoplasmic domains influence integrin affinity, it is clear that their role is not highly specific since three different sequences (\( \alpha^2 \), \( \alpha^4 \), and \( \alpha^5 \)) were interchangeable. Whereas the details of the roles of \( \alpha \) chain cytoplasmic domains remain to be elucidated, we suggest that \( \alpha \) chain–specific association with other proteins, or specific phosphorylation events are unlikely, given the extent of sequence divergence. Most likely, the role of these \( \alpha \) chains in regulating cell adhesion is closely connected with the role of the \( \beta_1 \) cytoplasmic domain. Previous work has indicated that the cytoplasmic domains of \( \beta_1 \) (76) and \( \beta_3 \) (40, 41) are also very important for regulating integrin contributions to
normal and agonist-stimulated cell adhesion. Notably, although the specific sequences of three different $\alpha$ chain cytoplasmic domains are interchangeable with respect to integrin-mediated cell adhesion, in other functional contexts, involving cell migration and collagen gel contraction, the $\alpha$ cytoplasmic domain appeared to be quite distinct from those of $\alpha\beta$ and $\alpha\gamma$ (52).

In conclusion, our data establishes that the $\alpha$ chain cytoplasmic domain plays a critical positive role in determining the level of adhesive function of VLA-4 and perhaps other $\beta_1$ integrins. Although its exact identity is not critical, the presence of an $\alpha$ chain cytoplasmic domain is essential for the integrin to manifest the full activity permitted by the cellular environment in which it is expressed. We postulate that the $\alpha$ chain cytoplasmic domain is involved in "inside-out" signaling, such that cellular factors acting at least partly through the $\alpha$ chain cytoplasmic domain are altering the external conformation of the $\alpha\beta_1$ heterodimer and therefore controlling the level of both constitutive and agonist stimulated integrin function.

We thank Dr. Francisco Sanchez-Madrid (Hospital de la Princesa, Madrid, Spain) for mAb HP1/2, and Tassie L. Collins for critical review of the manuscript.

This work was supported by National Institutes of Health grant GM-46526 to M. E. Hemler.

Address correspondence to Dr. Martin E. Hemler, Dana-Farber Cancer Institute, M613, 44 Binney Street, Boston, MA 02115.

Received for publication 20 January 1993 and in revised form 11 May 1993.

References

1. García-Pardo, A., and O.C. Ferreira. 1989. Adhesion of human T-lymphoid cells to fibronectin is mediated by two different fibronectin domains. ImmunoI. 69:121.
2. Guan, J.-L., and R.O. Hynes. 1990. Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor $\alpha_4\beta_1$. Cell. 60:53.
3. Elices, M.J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M.E. Hemler, and R.R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell. 60:577.
4. Wayner, E.A., A. García-Pardo, M.J. Humphries, J.A. McDonald, and W.G. Carter. 1989. Identification and characterization of the lymphocyte adhesion receptor for an alternative cell attachment domain in plasma fibronectin. J. Cell Biol. 109:1321.
5. Rice, G.E., J.W. Munro, and M.P. Bevilacqua. 1990. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes: a CD11/CD18-independent adhesion mechanism. J. Exp. Med. 171:1369.
6. Schwartz, B.R., E.A. Wayner, T.M. Carlos, H.D. Ochs, and J.M. Harlan. 1990. Identification of surface proteins mediating adherence of CD11/CD18-deficient lymphoblastoid cells to cultured human endothelium. J. Clin. Invest. 85:2019.
7. Isserl, T.B., and A. Wyrkelot. 1991. Effect of a new monoclonal antibody, TA-2, that inhibits lymphocyte adherence to cytokine-stimulated endothelium in the rat. J. Immunol. 147:109.
8. Hemler, M.E., M.J. Elices, C. Parker, and Y. Takada. 1990. Structure of the integrin VLA-4 and its cell-cell and cell-matrix adhesion functions. ImmunoI. Rev. 114:45.
9. Dobrina, A., R. Menegazzi, T.M. Carlos, E. Nardon, R. Cramer, T. Zacchi, J.-L. Harlan, and P. Patriarca. 1991. Mechanisms of eosinophil adherence to cultured vascular endothelial cells. Eosinophils bind to the cytokine-induced endothelial ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. J. Clin. Invest. 88:20.
10. Laffón, A., R. García-Vicuña, A. Humbría, A.A. Postigo, A.L. Corbi, M.O. De Landázuri, and F. Sánchez-Madrid. 1991. Upregulated expression and function of VLA-4 fibronectin receptors on human activated T cells in rheumatoid arthritis. J. Clin. Invest. 88:546.
11. van Dinten-Janssen, A.C.H.M., E. Horst, G. Koopman, W. Newmann, R.J. Sheper, C.J.L.M. Meijer, and S.T. Peals. 1991. The VLA-4/VCAM-1 pathway is involved in lymphocyte adhesion to endothelium in rheumatoid synovium. J. Immunol. 147:4207.
12. Postigo, A.A., R. García-Vicuña, F. Díaz-Gonzalez, A.G. Arroyo, M.O. De Landázuri, G. Chi-Russo, R.R. Lobb, A. Laffón, and F. Sánchez-Madrid. 1992. Increased binding of synovial T lymphocytes from rheumatoid arthritis to endothelial-leukocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1). J. Clin. Invest. 89:1445.
13. Yednock, T.A., C. Cannon, L.C. Fritz, F. Sánchez-Madrid, L. Steinman, and N. Kari. 1992. Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha_4\beta_1$ integrin. Nature (Lond.) 356:83.
14. Freedman, A.S., J.M. Munro, G.E. Rice, M.P. Bevilacqua, C. Morimoto, B.W. McIntyre, K. Rhyhah, J.S. Pober, and L.M. Nadler. 1990. Adhesion of human B cells to germinal centers in vivo involves VLA-4 and INCAM-110. Science (Wash. DC). 249:1030.
15. Williams, D.A., M. Rios, C. Stephens, and V.P. Patel. 1991. Fibronectin and VLA-4 in haematopoietic stem cell-microenvironment interactions. Nature (Lond.) 352:438.
16. Teixidó, J., M.E. Hemler, J.S. Greenberger, and P. Anklesaria. 1992. Role of $\beta_1$ and $\beta_2$ integrins in the adhesion of human CD34+ stem cells to bone marrow stroma. J. Clin. Invest. 90:358.
17. Miyake, K., I.L. Weissman, J.S. Greenberger, and P.W. Kin...
18. Utsumi, K., M. Sawada, S. Narumiya, J. Nagamine, T. Sakata, S. Iwagami, Y. Kita, H. Teraoka, H. Hirano, M. Ogata, et al. 1991. Adhesion of immature thymocytes to thymic stromal cells through fibronectin molecules and its significance for the induction of thymocyte differentiation. Proc. Natl. Acad. Sci. USA. 88:5685.

19. Bednarzyczyk, J.L., and B.W. McIntyre. 1992. Expression and ligand-binding of the integrin alpha 4 beta 2 (VLA-4) on neural crest-derived tumor cells. Clin. & Exp. Metastasis. 10:281.

20. Dufour, S., J.-L. Duband, M.J. Humphries, M. Obara, K.M. Yamada, and J.P. Thiery. 1988. Attachment, spreading, locomotion of avian neural crest cells are mediated by multiple adhesion sites on fibronectin molecules. EMBO (Eur. Mol. Biol. Organ.) J. 7:2661.

21. Rosen, G.D., J.R. Sanes, R. LaChance, J.M. Cunningham, J. Roman, and D.C. Dean. 1992. Roles for the integrin VLA-4 and its counter receptor VCAM-1 in myogenesis. Cell. 69:1107.

22. Shimizu, Y., G.A. Van Seventer, K.J. Horgan, and S. Shaw. 1990. Regulated expression and binding of three VLA (B1) integrin receptors on T cells. Nature (Lond.). 345:250.

23. Chan, B.M.C., and M.E. Hemler. 1993. Multiple functional forms of the integrin VLA-2 derived from a single e2α2 cDNA clone: interconversion of forms induced by an anti-β1 antibody. J. Cell Biol. 120:537.

24. Masumoto, A., and M.E. Hemler. 1993. Multiple activation states of VLA-4: mechanistic differences between adhesion to CSF1/fibronectin and to VCAM-1. J. Biol. Chem. 268:228.

25. Hynes, R.O. 1992. Integrins: versatility, modulation and signalling in cell adhesion. Cell. 69:11.

26. Plow, E.F., and M.H. Ginsberg. 1989. Cellular adhesion: GPIIb/IIIa as a prototypic adhesion receptor. Prog. Hemostasis Thromb. 9:117.

27. Phillips, D.R., I.F. Charo, and R.M. Scarborough. 1991. GPIIb/IIIa: the responsive integrin. Cell. 65:359.

28. Dustin, M.L., and T.A. Springer. 1989. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature (Lond.). 341:619.

29. Van Kooyk, Y., P. Van DeWiel-Van Kemenade, P. Weder, T.W. Kuipers, and C.G. Figdor. 1989. Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. Nature (Lond.). 342:811.

30. Wright, S.D., and B.C. Meyer. 1986. Phorbol esters cause sequential activation and desactivation of complement receptors on polymorphonuclear leukocytes. J. Immunol. 136:1759.

31. Conforti, G., A. Zanetti, I. Pasquali-Ronchetti, D. Quaglino, Jr., P. Neyroz, and E. Dejana. 1990. Modulation of vitronectin receptor binding by membrane lipid composition. J. Biol. Chem. 265:4011.

32. Hermanowski-Yosatsa, A., J.A.G. Van Strijp, W.J. Swiggard, and S.D. Wright. 1992. Integrin modulating factor-1: a lipid that alters the function of leukocyte integrins. Cell. 68:341.

33. Grzesiak, J.J., G.E. Davis, D. Kirchhofer, and M.D. Pierschbacher. 1992. Regulation of αβ3-mediated fibroblast migration on type I collagen by shifts in the concentrations of extracellular Mg2+ and Ca2+. J. Cell Biol. 117:1109.

34. Kirchhofer, D., J.J. Grzesiak, and M.D. Pierschbacher. 1991. Calcium as a potent physiological regulator of integrin-mediated cell adhesion. J. Biol. Chem. 266:4471.

35. Dransfield, I., and N. Hogg. 1989. Regulated expression of Mg2+ binding epitope on leukocyte integrin α subunits. EMBO (Eur. Mol. Biol. Organ.) J. 8:3759.

36. Chatila, T.A., R.S. Geha, and M.A. Arnaout. 1989. Constitutive and stimulus-induced phosphorylation of CD11/CD18 leukocyte adhesion molecules. J. Cell Biol. 109:3435.

37. Buyon, J.P., S. Slade, J. Reibman, S.B. Abramson, M.R. Phillips, G. Weissman, and R. Winchester. 1990. Constitutive and induced phosphorylation of the α- and β-chains of the CD11/CD18 leukocyte integrin family. J. Immunol. 144:191.

38. Shaw, L.M., J.M. Messier, and A.M. Mercurio. 1990. The activation dependent adhesion of macrophages to laminin involves cytoskeleton anchoring and phosphorylation of the α6-β1 integrin. J. Cell Biol. 110:2167.

39. Akiyama, S.K., S.S. Yamada, and K.M. Yamada. 1989. Analysis of the role of glycosylation of the human fibronectin receptor. J. Biol. Chem. 264:18011.

40. Hibbs, M.L., H. Xu, S.A. Stacke, and T.A. Springer. 1991. Regulation of adhesion to ICAM-1 by the cytoplasmic domain of LFA-1 integrin beta subunit. Science (Wash. DC). 251:1611.

41. Hibbs, M.L., S. Jakes, S.A. Stacke, R.W. Wallace, and T.A. Springer. 1991. The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 β subunit: sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site. J. Exp. Med. 174:1227.

42. O'Toole, T.E., D. Mandelman, J. Forsyth, S.J. Shattil, E.F. Plow, and M.H. Ginsberg. 1991. Modulation of the affinity of integrin α1β3 (GPIIb-IIIa) by the cytoplasmic domain of αIb. Science (Wash. DC). 254:845.

43. Hemler, M.E., C. Huang, Y. Takada, L. Schwarz, J.L. Strominger, and M.L. Clabby. 1987. Characterization of the cell surface heterodimer VLA-4 and related peptides. J. Biol. Chem. 262:11478.

44. Sánchez-Madrid, F., M.O. De Landazuri, G. Morago, M. Cebrian, A. Acevedo, and C. Bernabeu. 1986. VLA-3: a novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterization. Eur. J. Immunol. 16:1343.

45. Hemler, M.E., C.F. Ware, and J.L. Strominger. 1983. Characterization of a novel differentiation antigen complex recognized by a monoclonal antibody (A1A5): unique activation-specific molecular forms on stimulated T cells. J. Immunol. 131:334.

46. Hemler, M.E., F. Sánchez-Madrid, T.J. Flotte, A.M. Krensky, S.J. Burakoff, A.K. Bhan, T.A. Springer, and J.L. Strominger. 1984. Glycoproteins of 210,000 and 130,000 m.w. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. J. Immunol. 132:3011.

47. Hemler, M.E., and J.L. Strominger. 1982. Monoclonal antibody reacting with immunogenic mycophasma proteins present in human hematopoietic cell lines. J. Immunol. 129:2734.

48. Chan, B.M.C., J. Wong, A. Rao, and M.E. Hemler. 1991. T cell receptor dependent, antigen specific stimulation of a murine T cell clone induces a transient VLA protein-mediated binding to extracellular matrix. J. Immunol. 147:398.

49. Byers, H.R., T. Etoh, J.R. Doherty, A.J. Sober, and M.C. Mihm, Jr. 1991. Cell migration and actin organization in cultured human primary, recurrent cutaneous and metastatic melanoma. Am. J. Pathol. 139:1.

50. Garcia-Pardo, A., A. Rostagho, and B. Frangione. 1987. Primary structure of human plasma fibronectin: characterization of a 38 kDa domain containing C-terminal heparin-binding site (Hep III site) and a region of molecular heterogeneity. Biochem. J. 241:923.

51. Lobb, R.R., G. Chi-Rosso, D.R. Leone, M.D. Rosa, B.M. Newman, S. Luhowskyj, L. Osborne, S.G. Schiffer, C.D. Benjamin, I.G. Douglas, et al. 1991. Expression and functional char-
acterization of a soluble form of vascular cell adhesion molecule 1 (VCAM1). Biochem. Biophys. Res. Commun. 178:1498.
52. Chan, B.M.C., P.D. Kassner, J.A. Schiro, H.R. Byers, T.S. Kupper, and M.E. Hemler. 1992. Distinct cellular functions mediated by different VLA integrin α subunit cytoplasmic domains. Cell. 68:1051.
53. Helmsley, A., N. Arnheim, M.D. Toney, G. Cortopassi, and D.J. Galaz. 1989. A simple method for site directed mutagenesis using the polymerase chain reaction. Nucleic Acids. Res. 17:6545.
54. Ohashi, P., T.W. Mak, P. Van den Elsen, Y. Yanagi, Y. Yoshikai, A.F. Calman, C. Terhorst, J.D. Stobo, and A. Weiss. 1985. Reconstitution of an active surface T3/T-cell antigen receptor by DNA transfer. Nature (Lond.). 316:606.
55. Saito, T., A. Weiss, J. Miller, M.A. Norcross, and R.N. Germain. 1987. Specific antigen-1a activation of transfected human T cells expressing Ti αβ-human T3 receptor complexes. Nature (Lond.). 325:125.
56. Takebe, Y., M. Seiki, J. Fujisawa, P. Hoy, K. Yokota, K.-I. Arai, M. Yoshida, and N. Arai. 1988. SRαα promotor: an efficient and versatile mammalian cDNA expression system composed of simian virus 40 early promoter and the R-U5 segment of human T cell leukemia virus type I long terminal repeat. Mol. Cell. Biol. 8:466.
57. Hemler, M.E., C. Huang, and L. Schwarz. 1987. The VLA protein family. Characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight β subunit. J. Biol. Chem. 262:3300.
58. Chan, B.M.C., M.J. Elices, E. Murphy, and M.E. Hemler. 1992. Adhesion to VCAM-1 and fibronectin: comparison of α4β1 (VLA-4) and α4β7 on the human cell line JY. J. Biol. Chem. 267:8366.
59. Teixidó, J., C.M. Parker, P.D. Kassner, and M.E. Hemler. 1992. Functional and structural analysis of VLA-4 integrin α subunit cleavage. J. Biol. Chem. 267:1786.
60. Parker, C.M., C. Pujades, M.B. Brenner, and M.E. Hemler. 1993. α1481α180, a novel form of the integrin α4 subunit. J. Biol. Chem. 268:7028.
61. Lazarovits, A.I., R.A. Moscicki, J.T. Kurnick, D. Camerini, A.K. Bhan, L.G. Baird, M. Erikson, and R.B. Colvin. 1984. Lymphocyte activation antigens I. A monoclonal antibody, anti-Act I, defines a new late lymphocyte activation antigen. J. Immunol. 133:1857.
62. Arroyo, A.G., P. Sánchez-Mateos, M.R. Campanero, I. Martín-Padura, E. Dejana, and F. Sánchez-Madrid. 1992. Regulation of the VLA integrin-ligand interactions through the β1 subunit. J. Cell Biol. 117:659.
63. van de Wiel-van Kemenade, E., Y. Van Kooyk, A.J. de Boer, R.J.F. Huijbens, P. Weder, W. van de Kastelee, C.J.M. Melief, and C.G. Figdor. 1992. Adhesion of T and B lymphocytes to extracellular matrix and endothelial cells can be regulated through the β subunit of VLA. J. Cell Biol. 117:461.
64. Danilov, Y.N., and R.L. Juliano. 1989. Phorbol ester modulation of integrin-mediated cell adhesion: a postreceptor event. J. Cell Biol. 108:1925.
65. Wilkins, J.A., D. Stupack, S. Stewart, and S. Caixia. 1991. β1 integrin-mediated lymphocyte adherence to extracellular matrix is enhanced by phorbol ester treatment. Eur. J. Immunol. 21:517.
66. Larson, R.S., M.L. Hibbs, and T.A. Springer. 1990. The leukocyte integrin LFA-1 reconstituted by cDNA transfection in a nonhematopoietic cell line is functionally active and not transiently regulated. Cell Regul. 1:359.
67. Kieffer, N., L.A. Fitzgerald, D. Wolf, D.A. Cheresh, and D.R. Phillips. 1991. Adhesive properties of the β3 integrins: comparison of GP IIb-IIIa and the vitronectin receptor individually expressed in human melanoma cells. J. Cell Biol. 113:451.
68. Mercurio, A.M., and L.M. Shaw. 1988. Macrophage interactions with laminin: PMA selectively induces the adherence and spreading of mouse macrophages on a laminin substratum. J. Cell Biol. 107:1873.
69. Kovach, N.L., T.M. Carlos, E. Yee, and J.M. Harlan. 1992. A monoclonal antibody to β1 integrin (CD29) stimulates leukocyte adherence to human umbilical vein endothelial cells and matrix components. J. Cell Biol. 116:499.
70. Burn, P., A. Kupfer, and S.J. Singer. 1988. Dynamic membrane-cytoskeletal interactions: specific association of integrin and talin arises in vivo after phorbol ester treatment of peripheral blood lymphocytes. Proc. Natl. Acad. Sci. USA. 85:497.
71. Kupfer, A., P. Burn, and S.J. Singer. 1990. The PMA-induced specific association of LFA-1 and talin in intact cloned T helper cells. J. Mol. Cell. Immunol. 4:317.
72. Shattil, S.J., and L.F. Brass. 1987. Induction of the fibrinogen receptor on human platelets by intracellular mediators. J. Biol. Chem. 262:992.
73. O'Toole, T.E., J.C. Loftus, X. Du, A.A. Glass, Z.M. Ruggeri, S.J. Shattil, E.F. Flow, and M.H. Ginsberg. 1990. Affinity modulation of the αMβ2 integrin (platelet GPIIb-IIIa) is an intrinsic property of the receptor. Cell Regul. 1:883.
74. Faull, R.J., N.L. Kovach, J.M. Harlan, and M.H. Ginsberg. 1993. Affinity modulation of integrin α5β1: regulation of the functional response by soluble fibronectin. J. Cell Biol. 121:155.
75. Arroyo, A.G., A. García-Pardo, and F. Sánchez-Madrid. 1993. A high affinity conformational state on FLA integrin heterodimers induced by an anti-β1 chain monoclonal antibody. J. Biol. Chem. 268:9863.
76. Hayashi, Y., B. Haimovich, A. Reszka, D. Boettiger, and A. Horwitz. 1990. Expression and function of chicken integrin beta-1 subunit and its cytoplasmic domain mutants in mouse NIH 3T3 cells. J. Cell Biol. 110:175.