L-plastin (LPL) is a leukocyte actin binding protein previously implicated in the activation of the integrin αvβ3 on polymorphonuclear neutrophils. To determine the role for LPL in integrin activation, K562 cell adhesion to vitronectin via αvβ3, a well-studied model for activable integrins, was examined. Cell permeant versions of peptides based on the N-terminal sequence of LPL and the LPL headpiece domain both activated αvβ3-mediated adhesion. In contrast to adhesion induced by treatment with phorbol 12-myristate 13-acetate (PMA), LPL peptide-activated adhesion was independent of integrin β3 cytoplasmic domain tyrosines and was not inhibited by cytochalasin D. Also in contrast to PMA, LPL peptides synergized with RGD ligand or Mn2+ for generation of a conformational change in αvβ3 associated with the high affinity state of the integrin, as determined by binding of a ligand-induced binding site antibody. Although LPL and ligand showed synergy for ligand-induced binding site expression when actin depolymerization was inhibited by jasplakinolide, LPL peptide-induced adhesion was inhibited. Thus, both actin depolymerization and ligand-induced integrin conformational change are required for LPL peptide-induced adhesion. We hypothesize that the critical steps of increased integrin diffusion and affinity enhancement may be linked via modulation of the function of the actin binding protein L-plastin.

A fundamental property of leukocyte integrins is the ability to modulate their adhesive functions. As cells circulate through blood and lymph, adhesion is minimal. In response to inflammatory signals, integrin-mediated adhesion is markedly augmented. This property of the integrins is physiologically critical, because it is required for appropriate migration out of the vasculature into sites of inflammation and at the same time limits the potentially host-damaging inflammatory response to those sites alone. Because this activity of integrins is so important to their role on leukocytes, much attention has been devoted to the molecular mechanisms by which integrin adhesion is regulated. Two distinct alterations in integrins are likely to be involved in enhancement of adhesion. A rapid response to cell activation is an increase in integrin diffusion, due to loss of cytoskeletal constraint of integrin mobility. Increased diffusion may lead in turn to integrin clustering at sites of cell interaction with ligand. A second response to activation is a conformational change in the integrins, which often reflects increased affinity for ligand. This conformational change may require initial interaction with ligand and can be reflected in the generation of new epitopes recognized by monoclonal antibodies, the so-called ligand-induced binding sites (LIBS)1. Although a number of signaling molecules, such as PKC and phosphatidylinositol 3-kinase, have been implicated in this process (1–3), these enzymes may be many steps upstream from the actual change in integrin behavior. GTPases of the Ras and Rho families also have been implicated in regulation of integrin function (4–6), but their downstream targets for this function have not been identified. Therefore, much remains to be learned about the mechanisms involved in regulation of integrin avidity.

Our own studies have implicated the actin-binding protein L-plastin (LPL) in regulation of integrin function (7, 35). Recently, we have shown that cell-permeant peptides from the N terminus of LPL can rapidly activate αvβ3 (Mac-1)-mediated adhesion in polymorphonuclear neutrophils (PMN). When the peptides introduced into PMN cytosol contained phosphoserine at position 5, which is the major if not exclusive site of phosphorylation in LPL (7, 35), integrin activation was not inhibited by blockade of PKC or phosphatidylinositol 3-kinase, suggesting that LPL phosphorylation might be a mechanism by which these enzymes signal changes in integrin function. Thus, LPL is likely a downstream effector of multiple signaling pathways leading to integrin activation, and LPL peptide induction of adhesion is a useful experimental system in which to begin to understand regulation of integrin avidity.

A synthetic peptide based on amino acids 2–19 of Tat fused to 12 amino acids of the HIV tat sequence; tatHead, a recombinant protein containing amino acids 1–105 of T-plastin fused at the C terminus to 12 amino acids of the HIV tat sequence; HSA, human serum albumin; Kαvβ3, K562 cells transfected with αvβ3 integrin; LPLorf, a synthetic peptide based on amino acids 2–19 of T-plastin followed by 12 amino acids of the HIV tat sequence; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear neutrophils; RAD, Arg-Ala-Asp peptide; RGD, Arg-Gly-Asp peptide; SCRTat, a synthetic peptide in which amino acids 2–19 of L-plastin have been scrambled, followed by 12 amino acids of the HIV tat sequence; Vn, vitronectin; FACS, fluorescence flow cytometry; PBS, phosphate-buffered saline; mAb, monoclonal antibody; HBSS, Hank’s balanced salt solution; RT, room temperature; HIV, human immunodeficiency virus.

*This work was supported by Grant AI35811 from the National Institutes of Health (to E. J. B.). 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.

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1 The abbreviations used are: LIBS, ligand-induced binding site; LPL, L-plastin; Head/tat, a recombinant protein containing amino acids 1–105 of L-plastin fused at the C terminus to 12 amino acids of the HIV tat sequence; HSA, human serum albumin; Kαvβ3, K562 cells transfected with αvβ3 integrin; LPLorf, a synthetic peptide based on amino acids 2–19 of T-plastin followed by 12 amino acids of the HIV tat sequence; tatHead, a recombinant protein containing amino acids 1–105 of L-plastin fused at the N terminus to 12 amino acids of the HIV tat sequence; tatLPL, a synthetic peptide based on amino acids 2–19 of T-plastin fused at the N terminus to 12 amino acids of the HIV tat sequence; TPLorf, a synthetic peptide based on amino acids 2–19 of T-plastin followed by 12 amino acids of the HIV tat sequence; Vn, vitronectin; FACS, fluorescence flow cytometry; PBS, phosphate-buffered saline; mAb, monoclonal antibody; HBSS, Hank’s balanced salt solution; RT, room temperature; HIV, human immunodeficiency virus.
L-plastin in $\alpha_\beta_3$ Integrin Activation

characterized model for studying integrin activation. $\alpha_\beta_3$ is not normally expressed in undifferentiated K562 cells, but when expressed through transfection of $\alpha_\beta_3$ DNAs, $\alpha_\beta_3$-mediated adhesion in K562 cells is dependent on cell activation (8). Moreover, adhesion in response to PMA or thrombin is dependent on phosphorylation of Tyr-747 in the $\beta_3$ cytoplasmic tail, an event that is itself dependent on the presence of the $\alpha_\beta$ cytoplasmic tail (8, 9). Using these cells, we have examined the requirements for LPL peptide-induced adhesion. We have found that a cell permeant version of the entire headpiece domain of LPL, as well as synthetic LPL peptides, can induce adhesion. In contrast to PMA, LPL peptide-induced adhesion does not require tyrosine phosphorylation of the $\beta_3$ cytoplasmic tail, suggesting that LPL is downstream of the tyrosine signal in the activation cascade. Nonetheless, LPL peptide-induced adhesion does require actin depolymerization, presumably to induce integrin release from cytoskeletal constraint (10–12), and LPL peptide does cooperate with Arg-Gly-Asp (RGD) ligand to induce LIBS epitope expression. These data suggest that the steps of increased integrin diffusion and conformational change may be linked via modulation of the function of the actin binding protein L-plastin.

EXPERIMENTAL PROCEDURES

Cells and Reagents—The human erythroleukemic cell line K562 transfected with cDNA encoding $\alpha_\beta_3$ (K562(ts)) and $\alpha_\beta_3$ in which the tyrosine residues at position 747, 759, or both were mutated to phenyl-
LPL-related peptides used in this study were LPL (RGSVDEEM-(HSA) was from Therapeutic Corp. (Los Angeles, CA). Purified human Life Technologies, Inc. (Gaithersburg, MD). Human serum albumin Hybridolab, Institut Pasteur, Paris, France. Calcein was purchased mouse antibody against the tat-(49–58) epitope (19) was a gift from human LPL) have been previously described (8, 13–15). Monoclonal antibodies L230 (anti-human isochoyctane-conjugated Fab-specific anti-mouse IgG (Sigma Chemical Co., St. Louis, MO) for 45 min on ice. Cells were washed with FACs buffer and analyzed by flow cytometry. For MnCl2-treated samples, 250 $\mu$M MnCl2 was always present in the subsequent incubation and washing buffers.

F-actin Content—F-actin content of K562$\beta_3$ was determined as previously described (41) with minor modifications. Briefly, cells were incubated with buffer, 50 $\mu$M LPLtat, 1 $\mu$M jasplakinolide, or both for 15 min at 37°C and then solubilized by incubation with an equal volume of Tris-buffered saline, pH 7.4, containing 4% Triton X-100, 4 mM EDTA, and 5 $\mu$g/ml leupeptin. After centrifugation at 38,000 rpm for 30 min in a TL100.2 rotor (Beckman Optima TL Ultracentrifuge, Beckman Instruments, Fullerton, CA), the supernatant was carefully removed, and the gelatinous pellet was dissolved in SDS-polyacrylamide gel electrophoresis sample buffer. After separation by SDS-polyacrylamide gel electrophoresis, actin content of the Triton-insoluble pellet was analyzed by Western blot with rabbit anti-actin (Sigma) and quantitated by densitometry.

RESULTS

LPL N-terminal Peptide Induces $\alpha_\beta_3$-mediated Adhesion—We previously demonstrated that a cell permeant peptide containing LPL amino acids 2–19 fused at the C terminus with the HIV tat sequence (LPLtat) induces $\alpha_\beta_3$-mediated adhesion in PMN (7). LPLtat also causes $\alpha_\beta_3$-dependent adhesion to C3bii, an $\alpha_\beta_3$-specific ligand, in Jurkat cells transfected with $\alpha_\beta_3$ integrin. These data suggest that LPL has a role in $\alpha_\beta_3$ activation in leukocytes. We next asked whether LPL was specifically involved in $\alpha_\beta_3$ activation or if it was involved in the regulation of other integrins. The K562 cell, transfected with exogenous integrins, is a well-established model system for studying integrin functions in a myeloid background (8, 9, 14, 17), and our laboratory has shown previously that cell activation is required for adhesion mediated by $\alpha_\beta_3$ expressed in these cells (8). Therefore, we tested the effects of LPLtat on $\alpha_\beta_3$-mediated adhesion to Vn in stable K562 transfectants (K562$\beta_3$). LPLtat induced K562$\beta_3$ adhesion to Vn not but to HSA (Fig. 1A). Although LPLtat did not induce cell adhesion in the absence of ligand, its effect was apparent even at a Vn coating concentration of 0.1 $\mu$g/ml (Fig. 1B). This effect was specific to LPLtat, because control peptides, including tatLPL, in which the tat sequence mediating membrane

2 J. Wang and E. J. Brown, unpublished data.
permeability of the peptide was N-terminal to the LPL sequence, SCR tat, in which the LPL sequence was scrambled, and TPL tat, which contained amino acids 2–19 of the LPL homologue T-plastin, did not increase adhesion (Fig. 1C). LPL tat did not induce adhesion of the parental K562 cells, which do not express αvβ3, to Vn (K562 +TPL tat in Fig. 1C). LPL tat-induced adhesion of Kαβ3 cells was inhibited by the anti-αv antibody L230 and the anti-β3 antibody IA2.1 (Fig. 1D), further demonstrating the requirement for the αvβ3 integrin. LPL tat, but not tatTPL, SCR tat, or TPL tat, also induced Kαβ3 adhesion to osteopontin (data not shown). Like adhesion to Vn, Kαβ3 adhesion to osteopontin was inhibited by an anti-β3 mAb but not by antibodies recognizing αv, β5, or HLA. Thus, LPL tat activates αvβ3, as well as αMβ2-mediated adhesion. In contrast, αvβ3-mediated adhesion to fibronectin does not require activation in K562 cells (14), and LPL tat had no significant effect on adhesion of untransfected K562 to fibronectin (data not shown). Thus, LPL tat affects integrin activation for adhesion, rather than adhesion by already active integrins.

To determine whether LPL tat could activate αvβ3 in a cell constitutively expressing this integrin, we examined adhesion of the JY B lymphoma line, which is known to have activable αvβ3 (23, 37). These cells also adhered to Vn when treated with LPL tat (Fig. 2A), in a manner dependent on Vn-coating concentration (Fig. 2B) and β3 integrin (Fig. 2C).

**The LPL Headpiece Inhibits αvβ3-mediated Adhesion in Kαβ3 Cells—**In addition to the site for phosphorylation, the N-terminal domain, or headpiece, of LPL contains two EF-hand-type Ca2+ binding motifs (18), and Ca2+ markedly affects the conformation of this domain. To determine whether LPL tat induction of adhesion could be recapitulated with the entire LPL N-terminal domain, we expressed an LPL truncation mutant that contained the entire headpiece domain fused to the
tat peptide sequence either at the C terminus (Head tat) or the N terminus (tat Head) (Fig. 3A). The purified proteins were recognized by specific monoclonal antibodies directed toward the tat-(49–58) epitope (19) or toward the LPL N terminus (Fig. 3B). As in the case of LPL peptides, Head tat, but not tat Head induced Kαβ3 adhesion to Vn. Head tat-induced adhesion was completely inhibited by both anti-αv and anti-β3 antibodies (Fig. 3C). These data demonstrate that, in addition to short peptides, the intact headpiece domain of LPL can activate αvβ3-dependent adhesion in Kαβ3 cells.

LPL tat-induced Adhesion Does Not Require the β3 Cytoplasmic Tail Tyr-747 or Tyr-759—PMA- and thrombin-activated Kαβ3 adhesion to Vn requires Tyr-747 of the β3 cytoplasmic tail, because Y747F mutants fail to adhere in response to these
stimuli (8). To determine whether LPLtat-induced adhesion was also dependent on Tyr-747, we examined the effect of LPLtat on adhesion to Vn in K562 cells expressing αβ3 with single amino acid mutations Y747F, Y759F, and the double mutant Y747F/Y759F. As previously observed, the Y747F but not the Y759F mutation abolished PMA-stimulated adhesion to Vn (Fig. 4). However, Kα3β3Y747F, Kα3β3Y759F, and Kα3β3Y747F/Y759F adhered equally well as cells expressing wild-type αβ3 when treated with LPLtat (Fig. 4). Thus, unlike PMA, LPLtat-activated adhesion does not require β3 Tyr-747 or its phosphorylation. This demonstrates that the LPLtat effect on αβ3 is either downstream or independent of the Tyr-747-dependent signaling cascade (20, 21) involved in PMA-induced adhesion.

**Fig. 4.** LPLtat-induced adhesion does not require β3 cytoplasmic tail Tyr-747. K562 cells transfected with αβ3 and normal β3 (WT), β3 encoding a Y to F mutation at Tyr-747 (Y747F), β3 encoding a Y to F mutation at Tyr-759 (Y759F), or β3 expressing the Y747F/Y759F double mutation (Y747F/Y759F) have been described (8). Cells of each type with equal surface expression of αβ3 were treated with buffer control (open bar), 50 ng/ml PMA (hatched bar), or 50 μM LPLtat (filled bar). Adhesion assays were performed as described under “Experimental Procedures.” Data shown are representative of at least three independent experiments.

**LPLtat and RGD Act Synergistically to Increase Ligand-induced Binding Site (LIBS) Expression—Leukocyte integrin activation can be achieved by several mechanisms. For many integrins, binding of ligand or exposure to the divalent cation Mn2+ induces a conformational change that results in increased adhesion because of enhanced affinity for ligand (22). In the case of αβ3, RGD peptide or Mn2+ binding can induce this conformational change, which can be identified by the binding of monoclonal antibodies (mAb) that recognize LIBS epitope (8, 23, 24). This conformational change is associated with increased affinity for Vn, apparently due to a decreased off-rate of the receptor-ligand interaction (25). As shown in Fig. 5A, the anti-β3 mAb 7G2 (13) recognizes an LIBS epitope (8) and did not cause 7G2 reactivity to increase; 7G2 reactivity was further increased when cells were treated with RGD together with LPL tat (Fig. 5A), demonstrating marked synergy between the cytoplasmic peptide and the ligand (Fig. 5A). Treatment of Kα3β3 with LPLtat alone only minimally increased 7G2 binding (Fig. 5A and B). However, even at concentrations of RGD peptide that caused maximum 7G2 binding, 7G2 reactivity was further increased when cells were treated with RGD in combination with LPLtat, demonstrating marked synergy between the cytoplasmic peptide and the ligand (Fig. 5A). LPLtat synergized with TPLtat, tatLPL, and SCRtat, did not cause 7G2 reactivity to increase over the level induced by RGD alone (Fig. 5B). Total αβ3 expression on the surface of the transfected K562 cells was not altered by LPLtat treatment as assessed by the (non-LIBS) anti-β3 antibody AP3 (data not shown). LPLtat synergized with

**Fig. 3.** LPL headpiece domain induces αβ3-mediated adhesion in αβ3 cells. A, schematic representation of the recombinant tat-expressing domain. TatHead had an myc epitope at its N terminus, whereas Headtat had the tat peptide in that position. B, 15 μl of purified Headtat and tatHead proteins were Western-blotted with anti-tat mAb (upper panel) or the anti-LPL mAb LPL 4A.1 (lower panel). C, Kα3β3 cells were treated with W6/32 (control antibody), L230 (anti-alphaV), or 1A2.1 (anti-beta3) mAb as in Fig. 1 prior to measuring adhesion to Vn in the presence of buffer (open bar), 2 μM tatHead (hatched bar), or 2 μM purified Headtat (filled bar). The bar labeled K562+Headtat depicts adhesion to the Vn-coated surface of parental untransfected K562 cells activated by Headtat. Adhesion assays were performed as described under “Experimental Procedures.” Shown is a representative of three independent experiments with similar results.
RGD to induce LIBS expression in $\alpha_\beta_3$ with the Y747F and Y759F mutations (Fig. 5C), consistent with the finding that LPLtat-induced adhesion does not require these $\beta_3$ cytoplasmic tail tyrosines. LPLtat also synergized with Mn$^{2+}$ to increase LIBS expression in wild type and mutant receptors (Fig. 5D and data not shown). In contrast, PMA treatment did not induce 7G2 binding either with or without LPLtat (Fig. 5D). These data indicate that LPLtat induces $\alpha_\beta_3$-mediated adhesion by cooperating with ligand to induce expression of the high affinity conformation of the integrin, independent of Tyr-747 or Tyr-759.

Inhibition of LPLtat-induced Adhesion Requires a Higher Concentration of RGD Peptide Than PMA- or Mn$^{2+}$-induced Adhesion—As has been shown repeatedly, RGD peptide competes with Vn for the ligand binding site in $\alpha_\beta_3$ and inhibits adhesion to this protein. Because LPLtat cooperated with RGD to induce an LIBS epitope and presumably the high affinity state of the integrin, we asked whether adhesion induced by PMA, Mn$^{2+}$, and LPLtat had similar sensitivity to RGD peptide inhibition. If LPLtat increased the number of high affinity receptors, it should shift the ID$_{50}$ for RGD to a higher concentration. As this hypothesis predicted, LPLtat-activated adhesion was much more resistant to RGD peptide inhibition than either PMA or Mn$^{2+}$ stimulation (Fig. 6A). Mn$^{2+}$- or PMA-induced adhesion was completely inhibited by 50 $\mu$M RGD peptides, with an ID$_{50}$ at about 20 $\mu$M (Fig. 6, B and C). LPLtat-induced adhesion, however, required 100-fold more RGD peptide for equivalent inhibition, with an ID$_{50}$ of 2.5 mM RGD (Fig. 6A). This result is consistent with the higher LIBS expression induced by RGD and LPLtat than by RGD and Mn$^{2+}$ or RGD and PMA (Fig. 5D).

LPLtat-induced Adhesion Is Inhibited by Jasplakinolide, but Not Cytochalasin D—It is generally thought that integrins require attachment to actin filaments for firm adhesion. However, adhesion mediated by high affinity integrin receptors can be unaffected by cytochalasin D (26), implying that new actin microfilament formation is not required for this mechanism of attachment. Therefore, the sensitivity of LPLtat-induced adhesion to cytochalasin D was tested. As expected, PMA-induced adhesion, which requires the actin cytoskeleton in post-receptor events, was completely inhibited by cytochalasin D at 1 $\mu$g/ml. In contrast, cytochalasin D had no inhibitory effect on either LPLtat or Mn$^{2+}$-induced $\alpha_\beta_3$-mediated adhesion even at 10 $\mu$g/ml (Fig. 7A). These results demonstrate that LPLtat induces adhesion mediated by high affinity receptors, independent of actin microfilaments. It is recognized that actin microfilaments can also negatively regulate integrin activation by restricting integrin lateral mobility in resting cells (10). As a result, low doses of cytochalasin D actually induces LFA-1- and Mac-1-mediated adhesion by releasing integrins from cytoskeletal constraints leading to integrin activation (10, 27–29). In this study, low concentration of cytochalasin D (0.1 $\mu$g/ml) activated $\alpha_\beta_3$-mediated adhesion as well (Fig. 7B). Apparently, as in the case of LFA-1 and Mac-1, release of unactivated integrins from cytoskeletal constraint can lead to $\alpha_\beta_3$-mediated adhesion.

Because actin polymerization can induce $\alpha_\beta_3$-mediated adhesion, the requirement for actin polymerization in $\alpha_\beta_3$-mediated adhesion was examined. Jasplakinolide stabilizes pre-existing F-actin, prevents actin depolymerization, and induces a net increase in actin polymerization (30). It was previously shown to inhibit LFA-1-dependent adhesion (12). Similarly, jasplakinolide inhibited $\alpha_\beta_3$-mediated adhesion induced by LPLtat, Mn$^{2+}$, or PMA in K$\alpha_\beta_3$ (Fig. 8A) and in JY cells (data not shown). Taken together, the cytochalasin D and jasplakinolide data suggest that, although actin polymerization is not required for LPLtat-initiated $\alpha_\beta_3$-mediated adhesion, actin depolymerization is. F-actin content of cells treated with LPLtat and jasplakinolide was determined (Fig. 8B). Although LPLtat caused about a 50% decrease in total F-actin, jasplakinolide treatment more than doubled cellular F-actin. Jasp-
plakinolide also prevented the LPLtat-induced decrease in F-actin. Because both actin depolymerization and increase in receptor affinity are involved in LPLtat-induced adhesion, we asked whether actin depolymerization was required for the generation of high affinity $\alpha v\beta_3$. Surprisingly, jasplakinolide had little effect on synergistic expression of the 7G2 epitope by RGD and LPLtat (Fig. 8C). In addition, cytochalasin D had no effect on the ability of RGD, LPLtat, or their combination to induce the LIBS epitope recognized by 7G2 (data not shown). Thus, although actin depolymerization is required for $\alpha v\beta_3$-mediated adhesion, it is neither necessary nor sufficient for achieving a high affinity conformation of the receptor.

**DISCUSSION**

The term “integrin activation” is sometimes used to mean the modulation of affinity that can be induced in many integrins by Mn$^{2+}$, activating antibodies, ligand, and sometimes other physiologic or pharmacologic stimuli. If used in this way, integrin activation is thought be related to a conformational change in the extracytoplasmic domain of the integrins and can occur in a wide variety of cell types. However, $\beta_2$ and $\beta_3$ integrins on bone marrow-derived cells, including leukocytes and platelets,
are different from integrins on other cell types, because without physiologic or pharmacologic activation, they do not mediate adhesion. Thus, for these integrins, cell activation is an absolute requirement for function. Activation of adhesion likely results from integrin clustering within the membrane as well as changes in affinity of individual integrins (10–12). Moreover, leukocyte integrins can be distinguished from platelet integrin α_{IIb}β_{3} because, although α_{IIb}β_{3} activation for ligand binding is irreversible, activation of leukocyte integrins is a reversible process. Indeed, reversible activation of integrin-mediated adhesion is thought to be important in a variety of critical leukocyte functions, including migration, phagocytosis, and cell-mediated cytotoxicity.

Despite the importance of this reversible activation of leukocyte integrin-mediated adhesion, the mechanisms involved in regulation of leukocyte integrin function remain obscure. Although a number of signaling molecules have been identified that can be involved in the signal transduction pathways of inside-out signaling, the effector mechanisms leading to changes in integrin function have not been elucidated. In general, two not mutually exclusive models have been invoked, involving receptor rearrangement on the plasma membrane and another involving conformational change in the integrin itself (31, 32). Our previous work implicates LPL phosphorylation in a final common pathway influencing integrin function after cell activation with G-protein coupled, tyrosine kinase-mediated, and PKC-dependent stimuli (7, 33–35). The fact that peptides that mimic the phosphorylation site in the N terminus of LPL rapidly stimulate integrin activation when introduced into PMN or monocyte cytoplasm (7) presents an excellent opportunity to gain insight into the effector mechanisms of inside-out signaling. Therefore, in this work we have investigated the mechanism by which the LPL N terminal activates integrin-mediated adhesion, using the well-characterized model of α_{IIb}β_{3}-mediated adhesion in K562 cells.

A cell-permeant version of the LPL N-terminal domain (headpiece) activated α_{IIb}β_{3}-mediated adhesion as well as the 18-amino acid LPL peptides previously shown to activate α_{IIb}β_{3}-mediated adhesion. As previously discussed (7), integrin activation by the LPL N-terminal peptides requires their entry into the cytoplasm, because peptides of identical sequence without the tat addition have no effect on integrin function. Placing the tat sequence at the N-terminal end of either the peptide or the headpiece domain abrogates function even though this placement does not decrease entry into the cytoplasm. This suggests the possibility that a free LPL N terminus is essential for its integrin regulatory function and that addition of the 12-amino acid tat peptide blocks some essential function of this region of the protein. However, this domain binds neither actin nor vimentin (36), and there are no known cytoplasmic proteins that interact with the LPL headpiece. Although the headpiece does bind Ca^{2+} through its tandem EF-hand domains, the active peptides do not, and modulation of intracytoplasmic Ca^{2+} by itself is not known to affect integrin function. Thus, the molecular interactions by which the LPL N terminus activates integrin avidity are unknown. Nonetheless, both the headpiece domain and the N-terminal peptides induce α_{IIb}β_{3}-mediated adhesion, and the peptides clearly synergize with RGD ligand to activate a conformational change in α_{IIb}β_{3} associated with increased ligand affinity. We hypothesize that this occurs as the result of an interaction between the N-terminal domain of LPL and an as yet unknown target, which could be the integrin itself.

The requirements in the β_{3} cytoplasmic domain for LPLtat induction of adhesion are distinct from PMA or thrombin, because the β_{3} Y747F mutant, which fails to support adhesion stimulated by PMA, supports normal adhesion induced by the LPL peptides. Because the Y747F mutant also supports adhe-
sion in cells with constitutively active integrins (8), it is clear that phosphorylation of this tyrosine is not required for αβ₃-mediated adhesion. Rather, Tyr-747 phosphorylation is likely required in a signal transduction pathway involving the integrin, perhaps by recruiting an SH2- or PTB-containing protein, that leads to a final common pathway of integrin adhesion that does not itself require Tyr-747. If this is the case, then LPL functions downstream of Tyr-747 phosphorylation.

LPLat acts synergistically with RGD ligand to induce the expression of an LIBS epitope on β₃ recognized by mAb 7G2. The RGD peptide can induce 7G2 binding in a dose-dependent manner, as is expected for a LIBS mAb; in contrast, at optimal concentration, LPLat induces little 7G2 binding. Thus, LPLat does not act like an activating antibody or certain α₅β₁ integrin ligands, for which LIBS expression becomes independent of ligand. Instead, LPLat appears to augment the conformational change induced by RGD. In K562, as in other cells, only a minority of integrins expresses the LIBS epitope in the presence of ligand. Virtually nothing is known about what distinguishes receptors undergoing conformational change from those when exposed to ligand. In studying purified αβ₃, Orlando and Cheresh (25) noted that prior exposure to RGD markedly decreased the off-rate of its binding to Vn and suggested that this was the mechanism by which the ligand-induced conformational change in the integrin induced stable cell adhesion. It is likely that, in the context of an intact cell, LPLat exaggerates this normal response, leading to marked strengthening of adhesion. It is noteworthy that LPLat-mediated adhesion is insensitive to cytoskeleton disruption with cytochalasin D, suggesting that the LPL effect on integrin interaction with ligand dominates any potential indirect effect of adhesion strengthening through modulation of actin-integrin interactions.

Although the change in integrin conformation is essential for LPLat-induced adhesion, it is not sufficient, because jasplakinolide blocks adhesion without significant inhibition of the conformational change. It is now clear that integrin release from cytoskeletal constraint is an early and essential aspect of adhesion. It is noteworthy that LPLat-mediated adhesion is insensitive to cytoskeleton disruption with cytochalasin D, suggesting that the LPL effect on integrin interaction with ligand dominates any potential indirect effect of adhesion strengthening through modulation of actin-integrin interactions.

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