The Nonintegrin Laminin Binding Protein (p67 LBP) Is Expressed on a Subset of Activated Human T Lymphocytes and, Together with the Integrin Very Late Activation Antigen-6, Mediates Avid Cellular Adherence to Laminin

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The Nonintegrin Laminin Binding Protein (p67 LBP) Is Expressed on a Subset of Activated Human T Lymphocytes and, Together with the Integrin Very Late Activation Antigen-6, Mediates Avid Cellular Adherence to Laminin

Stephen M. Canfield and Aarif Y. Khakoo

A search for genes expressed in activated T cells revealed that the nonintegrin, 67-kDa laminin binding protein (p67 LBP) is expressed on the surface of a subset (10–15%) of activated peripheral blood T cells. Surface p67 LBP expression is detectable by FACS using the anti-p67 LBP mAb, MLuC5, within 6 h of T cell activation with phorbol dibutyrate and ionomycin, peaks 18–36 h postactivation, and persists for 7–10 days. The subset of T cells expressing p67 LBP is composed of mature, single-positive cells (85% CD4+8+, 15% CD4+8−) of memory cell phenotype (100% CD45RO+/CD45RA−). The p67 LBP+ T cells also express the integrin α6 chain (CD49f), which is known to associate with p67 LBP on tumor cells. In addition, the p67 LBP+ T cells express the integrin β1, which associates with α6 in the laminin-specific integrin receptor very late activation Ag (VLA)-6 (α6β1). Expression of an exogenous cDNA encoding the 37-kDa LBP precursor (p37 LBPP) confers p67 LBP surface expression on a p67 LBP-negative Jurkat T cell line (B2.7). Expression of p67 LBP induces B2.7 transfectants to adhere to laminin, but avid laminin binding depends on coexpression of VLA-6. Taken together, these data indicate that p67 LBP is an activation-induced surface structure on memory T cells that, together with VLA-6, mediates cellular adherence to laminin. The Journal of Immunology, 1999, 163: 3430–3440.

Activation of T cells induces binding to laminin, an extracellular matrix (ECM) glycoprotein that characterizes endothelial and epithelial cell basement membranes (1, 2). The characterized lymphocyte receptors for laminin are members of the integrin family of αβ heterodimers (3, 4). The β1, or very late activation Ags (VLA) consist of at least six members (VLA 1–6), whose ligands include laminin, collagen, and fibronectin (5, 6). Within this group, VLA-6 (α6β1) mediates laminin binding exclusively, while VLA-1, -2, and -3 bind other ECM ligands in addition to laminin (7). T cell activation results in augmentation of VLA-6-dependent laminin binding by mechanisms that remain incompletely characterized, but include increased VLA-6 surface expression (8) and possibly conformational alterations within the VLA-6 dimer (9–11). Whether T cell activation recruits other nonintegrin factors to augment VLA-6-dependent laminin binding is unclear.

The 67-kDa nonintegrin laminin binding protein (p67 LBP) was first identified on tumor cells (12–14). The p67 LBP appears to be a homodimer of a 37-kDa precursor polypeptide (p37 LBP) (15) encoded by a single gene (16), for which >17 nonproductive pseudogenes exist (17). The p37 LBPP cDNA encodes a polypeptide precursor of 295 amino acids, which is posttranslationally modified to the mature 67-kDa form (18). The posttranslational processing is not completely understood and may involve other cytoplasmic factors (15, 19–21). For example, a variety of recent evidence suggests that p67 LBP interacts with the integrin α6 chain and that this interaction is required for p67 LBP surface expression (22). On tumor cells, p67 LBP is coexpressed with α6 (23), colocalizes with α6 in the same cytoplasmic structures (24), and coimmunoprecipitates with α6 (22). Treatment of tumor cells with TNF-α and IFN-γ, which down-modulates surface expression of α6, induces a coordinate down-regulation of p67 LBP (22). Moreover, treatment with α6 anti-sense oligonucleotides that inhibit α6 expression results in a coordinate reduction of surface p67 LBP (without reducing total cellular p67 LBP) (22). Together, these studies suggest that α6 plays a role in the surface expression of p67 LBP.

In addition to its role in p67 LBP surface expression, α6 may be required for high-avidity laminin binding by p67 LBP. Though p67 LBP, isolated by affinity chromatography from cell membrane extracts, was initially reported to bind laminin with an affinity (Kd) of 5 × 10^4 M−1 (13), recent work has shown that highly purified p67 LBP loses much of its avidity for laminin (15). High-avidity laminin binding by the purified p67 LBP fraction can be reconstituted by adding back two other (nonlaminin-binding) column fractions, suggesting that other polypeptides combine with p67 LBP to form a complex that binds laminin avidly, and it has been suggested that the integrin α6 chain participates in this complex (15).

The biology of p67 LBP has been defined largely in tumor cells, and the expression level of p67 LBP correlates with local invasive and metastatic potential on a variety of malignancies (18, 25–31). These data suggest a role for p67 LBP in cell metastasis; however, the biological function of p67 LBP in the migration or trafficking...
of normal cells remains undefined. The p67 LBP has been described on a number of normal cells, including pulmonary macrophages and neutrophils (32), though its role with respect to the inflammatory response remains uncharacterized. Moreover, p67 LBP has not been described on T lymphocytes.

In the course of investigations aimed at identifying genes associated with T cell activation, p37 LBPP mRNA was found to be selectively expressed by a T cell tumor subclone with altered phenotype. In addition, p67 LBP protein was found to be expressed by a subset of activated, human memory T lymphocytes. Expression of p67 LBP on Jurkat T cell transfectants induces cellular laminin adherence, and avid laminin binding depends on expression of high level α6.

Materials and Methods

Cell lines

The Jurkat T leukemia lines B2.7 and D1.1 have been described previously (33). Briefly, Jurkat B2.7 is a CD4+ human T leukemia line. D1.1 is a Jurkat subclone that lacks surface CD4 expression, constitutively expresses surface CD8, and is capable of providing contact-dependent T cell help to B cells (34). Cells were maintained in IMDM (Life Technologies, Grand Island, NY) supplemented with 10% FBS (Summit Biotechnologies, Fort Collins, CO), 5 U/ml penicillin, and 5 μg/ml streptomycin (Sigma, St. Louis, MO) in a 5% CO2 incubator at 37°C.

Flow cytometry

Cells analyzed by flow cytometry were pelleted at 300 × g for 5 min at 4°C, and resuspended at 2 × 10⁶ cells/ml in DMEM (Life Technologies) supplemented with 1% FBS and 20 μg/ml heat-aggregated human IgG (International Enzymes, Fallbrook, CA). Cells were aliquoted at 50 μl/well into a polystyrene 96-well plate (Becton Dickinson, San Jose, CA), and the appropriate mAb was added to a final concentration of 2.5 μg/ml. Cells were incubated at 4°C for 30 min, pelleted at 800 × g for 5 min at 4°C, and washed with 200 μl 1% DMEM/1% FBS. Cells stained with fluorochrome-conjugated primary mAbs were resuspended in 100 μl PBS containing 1% formaldehyde. Cells stained with nonconjugated primary mAbs were resuspended in 50 μl fluorochrome-conjugated goat anti-mouse (GAM) IgG/IgM (Jackson ImmunoResearch, West Grove, PA) at 1.5 μg/ml in DMEM/1% FBS and incubated at 4°C for 15 min, washed, and resuspended in 100 μl PBS containing 1% formaldehyde. In some experiments, fluorochrome-conjugated GAM IgG (γ-chain-specific) and anti-mouse IgG or IgM (whole IgG) used were in dual color staining to distinguish mAb MLαC5 (mouse IgM) from any of several un conjugated anti-integrin mAbs (all mouse IgGs).

The following mAbs directed against human cell surface Ags were used in flow cytometry: anti-p67 LBP mAb MLαC5, nonconjugated (NeoMarkers/Lab Vision, Fremont, CA); anti-CD3 mAb HIT3A, R-PE-conjugated (PharMingen); anti-CD8 mAb RPA-T4, R-PE-conjugated (PharMingen); anti-CD6 mAb RPA-T8, R-PE-conjugated (PharMingen); anti-CD45RO mAb UCHL1, R-PE-conjugated (PharMingen); anti-integrin αL α4 (CD94) mAb HSB26, unconjugated (Beckman Coulter, Fullerton, CA); anti-integrin αL α5 (CD49b) mAb 12F1-H6, R-PE-conjugated (PharMingen); anti-integrin αL (CD49c) mAb C3-3, R-PE-conjugated (PharMingen); anti-integrin αL (CD49f) mAb GoH3, R-PE-conjugated (PharMingen); anti-integrin β1 (CD29) mAb 4B4, PE-conjugated (Beckman Coulter); anti-integrin α5 (CD11a) mAb B-15, PE-conjugated (Biosource International, Camarillo, CA); anti-CD69 mAb L78, R-PE-conjugated (Becton Dickinson); anti-IL-2 receptor α-chain (CD25) mAb 143-13, R-PE-conjugated (Biosource International); anti-transferrin receptor (CD71) mAb YDJ.1.2.2, R-PE-conjugated (Beckman Coulter); anti-CD26 mAb M-A261, R-PE-conjugated (PharMingen); and anti-CD44 mAb A3D8, R-PE-conjugated (Sigma). The unconjugated mAbs TS2/7 (anti-integrin αL) and P1H5 (anti-integrin αL) were the gift of Dr. Eugene Marcantonio (Columbia University, New York, NY).

Flow cytometry was performed on a FACScan (Becton Dickinson) and analyzed using CellQuest software (Becton Dickinson), except in experiments involving cell sorting by cytometry. All cell sorting was performed on a FACStar sorter (Becton Dickinson). Cells were prepared for sorting as described above, except that all solutions were prestained by filtration through 0.2-μm pore cellulose acetate filters (Nalgene Nunc International, Rochester, NY), and cells were resuspended in ice-cold IMDM/10% FBS after the final wash step, instead of PBS/1% formaldehyde.

Differential display and cloning of p67 LBP

Oligo(dT)-primed cDNA sets derived from the Jurkat cell lines B2.7 and D1.1 were screened for messages differentially expressed in the D1.1 subclone, using the method of differential display RT-PCR (DDRT-PCR) (35, 36). Briefly, mRNA was isolated from each line using the FastTrack System (Invitrogen, Carlsbad, CA) and reverse transcribed using oligo(dT) and Superscript II reverse transcriptase (Life Technologies). A set of 23 pairs of arbitrary decanucleotides (Operon Technologies, Alameda, CA) was used to screen the cDNA sets by PCR at low annealing temperature (42°C) designed to amplify DNA fragments of 150–500 bp in length. This amplification was performed in the presence of α32PdCTP (NEN Life Sciences, Boston, MA), and products derived from B2.7 and D1.1 were compared by PAGE followed by autoradiography, on 10% polyacrylamide gels. (Bio-Rad, Hercules, CA). All reactions were performed in duplicate with independently isolated mRNA preparations, and PCR products reproducibly amplified from D1.1 cDNA, but not B2.7 cDNA, were excised from the gel, eluted in TE buffer (10 mM Tris (pH 8.0), 1 mM EDTA), and reamplified using the same primer pair. These putatively differentially expressed cDNA fragments were cloned using the TA Cloning System (Invitrogen) and partially sequenced using an automated ABI 373 Sequence (Perkin-Elmer, Norwalk, CT). Fragment-specific cDNA primers were designed based on cloned fragment sequence, and PCR used to confirm the differential expression of the represented messages by RT-PCR. Finally, the cDNAs amplified using the fragment-specific primers were radiolabeled with α32PdCTP (NEN Life Sciences) by random hexanucleotide priming using the NEBlot system (New England Biolabs, Beverly, MA), and used to probe the cDNA library. Several independent cDNA inserts cloned directionally into the expression vector pCDNA1 (Invitrogen). A 180-bp PCR product from DDRT-PCR was used to screen 180,000 colonies from the D1.1 cDNA library, yielding two independent clones (no. 74 and no. 79) that were further characterized. Both showed >98% identity to the published sequence of the p67 laminin receptor (NCBI BLAST Search: www.ncbi.nlm.nih.gov). Clone no. 74 was incomplete, lacking 10 base pairs of 5′ coding sequence; clone no. 79 represented the complete coding sequence, as well as 27 bp of 3′ untranslated sequence. Clone no. 79 was excised from the cDNA vector by restriction endonuclease digestion, and ligated into the vector pCE4 (Invitrogen), yielding pCPE-p37 LBPP. This vector confers stable episomal replication in eukaryotic cells selectable on the basis of hygromycin resistance. Transcription of the insert is initiated within the vector-encoded CMV immediate-early promoter.

Transfection of Jurkat tumor cells

The plasmid pCE-p37 LBPP was transfected into Escherichia coli strain XL1 Blue MRF′ (Strategene, La Jolla, CA) by electroporation using a Bio-Rad Gene Pulser under the following conditions: 1.8 kV, 200 Ω, 25 μF, 0.1 cm gap (Bio-Rad). A single colony was amplified by growth overnight at 37°C in 1 L Luria Broth supplemented with 50 μg/ml ampicillin. Plasmid DNA was purified from this culture by a standard alkaline lysis protocol followed by two cycles of CsCl density centrifugation (37).

Before electroporation, Jurkat B2.7 cells were grown in IMDM/10% FBS to a density of 1 × 10⁶ cells/ml, collected by centrifugation at 300 × g for 5 min, resuspended in IMDM/10% FBS at a density of 8 × 10⁶ cells/ml, and divided into 0.5-ml aliquots in sterile polypropylene tubes. Purified plasmid DNA was precipitated in ethanol, washed once with 70% ethanol, and dissolved in sterile water at a concentration of 1 μg/ml. A total of 10 μg of plasmid DNA in sterile water was added to each aliquot of cells, the cells and DNA were gently mixed by stirring with a pipet tip, and the mixture was transferred to a sterile electroporation cuvette (Bio-Rad; 0.4 cm gap). The DNA was transfected into the B2.7 cells by electroporation using a Bio-Rad Gene Pulser (280 V, 975 μF), after which transfec tants were cultured in IMDM/10% FBS for 48 h before drug selection in IMDM/10% FBS containing 800 μg/ml hygromycin B (Boehringer Mannheim, Indianapolis, IN). Finally, the hygromycin-resistant transfec tants were cloned by flow cytometric sorting into 96-well plates on the basis of staining with mAb MLαC5 on a FACSStar cell sorter (Becton Dickinson), as described above.

PCR analysis for vector-encoded p37 LBPP expression

Jurkat B2.7 transfected clones expressing the pCE-p37 LBPP cDNA were grown to a density of 1 × 10⁶ cells/ml in IMDM/10% FBS/800 μg/ml hygromycin, and 1 × 10⁵ cells were collected by centrifugation at 800 × g, washed once with PBS, and pelleted. Total RNA was isolated from each cell pellet using the RNeasy RNA Isolation System (Qiagen, Valencia, CA). A total of 5 μg of total RNA was reverse transcribed into cDNA using a dT15 primer and SuperScript II reverse transcriptase (Life Technologies).
in a 20-μl reaction. A total of 1 μl of the resulting single-stranded cDNA product was subjected to PCR amplification using Taq DNA polymerase (Fisher, Pittsburgh, PA) in a forty cycle reaction using a forward primer specific for the 5′ untranslated region of the expression vector pCEP4 (5′-AACCGTCAGATCTCTAGAAGCTGGG-3′), and a reverse primer specific for the p37 LBPP cDNA (5′-AATTCCTCCTTGGTCACTGCTTC-3′). Cycle parameters included denaturation at 94°C (0.5 min), annealing at 56°C (1 min), and chain extension at 72°C (0.5 min). PCR amplification products were separated by gel electrophoresis on a 1% agarose gel, and visualized by staining in ethidium bromide (0.3 mg/ml). The primers resulted in the generation of an 800-bp cDNA fragment in clones expressing the p37 LBPP cDNA transcribed from the CMV immediate/early promoter in pCEP4.

Substrate-specific cell adhesion assay
Cell adhesion to laminin was assayed by a method modified from St. John (38). Briefly, 96-well polystyrene tissue culture plates (Becton Dickinson) were precoated with human laminin (Life Technologies) at 5 μg/ml in PBS, 50 μl/well, in a humidified environment at room temperature overnight (control wells received PBS alone). At the end of this period, all wells were washed three times with PBS (400 μl/well) and blocked with 3% BSA in PBS, 200 μl/well, at 37°C for 3 h. Finally, wells were washed with PBS, and the plates were stored inverted and sealed at 4°C for up to 48 h before use. In other assays, plates were precoated with human fibronectin (Life Technologies), VCAM-1 (R&D Systems, Minneapolis, MN), or collagen IV (Life Technologies) by an identical procedure.

Test cells were removed from culture, washed once with IMDM/10% FBS, and resuspended in the same media supplemented with 90 μCi/ml 51Cr sodium chromate (NEN Life Sciences) at a cell density of 20 × 10⁶ cells/ml. The cells were incubated in 51Cr-loading media at 37°C with agitation for 90 min, then washed three times with 30-fold excess IMDM/10% FBS. 51Cr-loaded cells were plated at a density of 100,000 cells/well in 400 μl of the same media and allowed to adhere for 2 h in a humidified incubator at 5% CO₂ and 37°C. Following adhesion, the test plate was submerged in 1.5 L PBS at room temperature in a vessel large enough to accommodate the entire plate. The plate, entirely beneath the PBS surface, was then inverted and fixed in position using a burette stand and clamp. The PBS bath was oscillated relative to the plate for 20 min on a rotary shaker at 40 cycles/min, applying a mild shear force to all wells simultaneously. Following this shear wash, the plate, still beneath the liquid surface, was reinverted (to the upright position) and removed from the PBS bath. Half of each well volume (200 μl) was removed by pipet, and cells were lysed by the addition of 200 μl 20% SDS (final concentration 10% SDS). To determine cpm/cell, 100,000 51Cr-labeled cells from each test sample were incubated in parallel with the test plate, washed, pelleted, and lysed in 400 μl 10% SDS. All cell lysates (400 μl) were transferred to counting vials and analyzed using a Clinigamma 1272 gamma counter (EG&G Wallac, Gaithersburg, MD). All conditions were assayed in quadruplicate. The laminin-specific adhesion was calculated as the difference in average cpm per well between the laminin-coated and noncoated wells, and expressed as number of cells, based on the measured cpm/cell for each
clone tested. The results presented in Fig. 7 derive from a representative experiment. Similar results were found in at least four independent experiments for each cell line tested.

Isolation and activation of normal human PBL

Peripheral blood specimens from normal healthy human volunteers were collected in heparinized tubes by venipuncture. PBL were isolated by ficoll density centrifugation (Sigma) and subsequently enriched to >90% CD3⁺ T cells by rosetting with SRBC (Colorado Serum, Denver, CO). After isolation of erythrocyte rosetting (E⁺) lymphocytes on a second ficoll density gradient, SRBC were lysed by agitation at room temperature for 3 min in a solution of 0.5 M NH₄Cl, 33 mM KHCO₃, 0.4 mM Na₂EDTA. E⁺ lymphocytes were then washed three times in DMEM (Life Technologies) supplemented with 1% FBS (Summit Biotechnologies) before transfer to IMDM/10% FBS for culture. T cell stimulation was performed by incubating the cells for 6 h at 37°C in IMDM/10% FBS in the presence of 10 ng/ml PDB and 600 ng/ml ionomycin, as described in Materials and Methods.

FIGURE 2. Expression of p67 LBP on activated human peripheral blood T cells. Shown are dual-color FACS contour plots of resting (left column) and activated (right column) peripheral blood human T cells depicting p67 LBP expression (mAb MLuC5) on the x-axis and CD3 (mAb HIT3A), CD4 (mAb RPA-T4), CD8 (mAb RPA-T8), and CD45 RO (mAb UCHL1), as indicated on the y-axis. The percentage of total T cells represented in selected quadrants is indicated within each quadrant. T cells were activated by PDB (10 ng/ml) and ionomycin (600 ng/ml), as described in Materials and Methods.
**Results**

**Differential expression of p67 LBP in Jurkat T leukemia subclones**

To isolate genes expressed in activated T cells, DDRT-PCR (35, 36) was used to compare two subclones of the Jurkat T leukemia line. The Jurkat subclone D1.1 is a model for activated T lymphocytes, whereas the Jurkat B2.7 subclone is a model for resting T cells (33). Using DDRT-PCR, a cDNA fragment of 180 bp was reproducibly amplified from D1.1 mRNA, but not from B2.7 mRNA. This 180-bp PCR product was radiolabeled and used to screen a D1.1 cDNA library. Two independent cDNA clones were identified, both with >98% identity with the cDNA encoding the 37-kDa polypeptide precursor (p37 LBPP) to p67 LBP (16). Together, these data suggested that p37 LBPP is differentially expressed by Jurkat D1.1; however, due to the presence of p37 LBPP-hybridizing transcripts in B2.7 (presumably from pseudogenes (17)), the differential expression of productive LBPP transcripts was difficult to substantiate by Northern or RT-PCR analysis.

To determine whether the expression of p37 LBPP mRNA by Jurkat D1.1 is associated with differential surface expression of p67 LBP, the Jurkat clones were examined by flow cytometry using the anti-p67 LBP mAb, MLuC5 (39). Consistent with the DDRT-PCR results, the D1.1 subclone expresses high level surface p67 LBP, whereas B2.7 does not express detectable p67 LBP (Fig. 1). Together, these data corroborate the DDRT-PCR result and confirm that p67 LBP is differentially expressed by Jurkat D1.1, a model for activated T cells.

**Expression of p67 LBP on activated, normal T cells**

To determine the significance of the selective expression of p67 LBP in the Jurkat model of activated T cells, the next series of experiments examined the expression of p67 LBP on normal human peripheral blood T cells either resting (freshly isolated) or 36 h after activation with PDB and ionomycin. T cells were analyzed by dual channel flow cytometry for p67 LBP expression relative to the expression of CD3, CD4, CD8, and CD45RO. High level surface expression of p67 LBP was detected on a subset (10–15%) of activated T cells, but only on very few resting cells (Fig. 2). In fact, the rare freshly isolated T cells (1–2%) that stain with MLuC5 appear to represent a small population of circulating, recently activated T cells because these cells are CD69+ and CD71- (data not shown). The subset of PDB/ionomycin-activated T lymphocytes that express p67 LBP are predominantly CD4+, CD8-, and CD45RO+ (Fig. 2). Therefore, these data suggest that a subset of activated, but not resting, memory T cells expresses p67 LBP.

The next experiments studied the time course of p67 LBP expression by T cells after activation. Expression of p67 LBP is detectable within 6 h after activation and peaks between 18 and 36 h after activation (Fig. 3). Thereafter, p67 LBP expression declines gradually over 5 days (Fig. 3) and returns to baseline by 10 days (data not shown). To further define the T cell population expressing p67 LBP, 36 h-activated T cells were FACs sorted for MLuC5+ phenotype and analyzed by dual channel flow cytometry for a variety of other T cell surface molecules (Fig. 4). The p67 LBP+ subset is uniformly CD45 RO+ (Fig. 4), confirming the results of the studies on unsorted cells (Fig. 2). The p67 LBP+ subset is composed of mature, single-positive T cells, 85% CD4+ and 15% CD4-8+ (Fig. 4). The p67 LBP+ T cells express the IL-2 receptor p55 polypeptide (CD25) and CD69, consistent with their activated state. The p67 LBP+ subset also expresses CD11a, CD26, and CD44 at high levels (Fig. 4), a phenotype that has been associated with T cell migratory potential, in vitro (8). Taken together, these data indicate that a subset of activated memory T cells, including both CD4+ and CD8+ cells, expresses p67 LBP for 7–10 days after activation.

The FACs studies also revealed that p67 LBP+ T cells express higher levels of both αa (CD49d) and β2 (CD29), relative to the p67 LBP- T cell subset (Fig. 5). The αa and β2 integrin chains pair in VLA-6, the integrin laminin-specific receptor. High VLA-6 expression is consistent with the memory phenotype of these cells (9), and is of interest since, in tumor cells, p67 LBP and αa associate, are coordinately expressed, and αa is thought to play a role in p67 LBP expression (15, 22). In addition, these studies revealed that the p67 LBP+ T cell subset also expresses higher levels of surface α6, αv, and α9 than the p67 LBP- T cell subset (Fig. 5), suggesting that these cells may interact with laminin through a number of different VLA receptors.

**Expression of p37 LBPP cDNA in Jurkat B2.7**

To determine whether the differential expression of p67 LBP on D1.1 is due solely to differential expression of p37 LBPP mRNA, the next series of experiments examined whether transfection of p37 LBPP cDNA confers p67 LBP expression on the p67 LBP- Jurkat subclone B2.7. The B2.7 line was electroporated with p37 LBPP cDNA in an expression vector (pCEP4; Invitrogen), and stable transfectants were selected on the basis of hygromycin resistance. Drug-resistant clones were sorted by flow cytometry for MLuC5+ phenotype, and vector-encoded p37 LBPP expression was verified in the sorted clones by RT-PCR. Transfectant clones were analyzed by FACs for surface expression of p67 LBP, as
as \( \alpha_1 \), \( \alpha_2 \), \( \alpha_3 \), and \( \alpha_6 \) (Table I). All clones expressed high levels of \( \beta_1 \) (data not shown). B2.7 transfectants expressing pCEP4-p37 LBPP express high level surface \( \beta_6 \) LBP (Table I; Fig. 6, clones 42 and 48). In contrast, control B2.7 transfectants expressing a CD8 \( \alpha \)-chain cDNA in pCEP4 (B2.7/CD8) do not express \( \beta_6 \) LBP (Table I; Fig. 6). Together, these data show that expression of a single cDNA encoding p37 LBPP confers high level \( \beta_6 \) LBP surface expression on B2.7 cells.

**Laminin-specific adherence of \( \beta_6 \) LBP\(^+ \) Jurkat B2.7 transfectants**

To determine whether the presence of \( \beta_6 \) LBP on the surface of the Jurkat clones is associated with increased cellular adherence to laminin, chromium-labeled D1.1, B2.7, or B2.7 transfectant clones were allowed to interact with immobilized laminin (or BSA in control wells) for 2 h at 37°C, before nonadherent cells were removed and adherent cells were lysed in 10% SDS and quantified by gamma counting. Each Jurkat clone was studied in at least four independent assays, and a representative experiment is shown in Fig. 7A. As a positive control for laminin-specific adherence, D1.1, which expresses high levels of both \( \beta_6 \) LBP and VLA-6 (Fig. 1), adheres avidly to the laminin-coated surface (Fig. 7A). As negative controls, the parental B2.7 clone (Fig. 1) and a B2.7 transfectant expressing the CD8\( \alpha \)-polypeptide (B2.7/CD8) (Fig. 6) show no significant laminin-specific adherence. B2.7 transfectants that express high \( \beta_6 \) LBP display significant laminin binding (Fig. 7A, clones 18, 28, and 42). These data indicate that \( \beta_6 \) LBP expression confers laminin-specific adherence on B2.7 cells.

Since B2.7 transfectants expressing \( \beta_6 \) LBP display varying levels of laminin-specific adherence (Fig. 6), these lines were studied for their expression of \( \alpha_6 \) (CD49f) by flow cytometry (Fig. 6; Table I). Clones 18, 28, and 42 are representative of transfectants with low \( \alpha_6 \) and high \( \beta_6 \) LBP (\( \alpha_6^{\text{low}} \beta_6^{\text{high}} \)) (Table I; Fig. 7A). Clones 29, 34, and 48 are representative of transfectants with high \( \alpha_6 \) and high \( \beta_6 \) LBP (\( \alpha_6^{\text{high}} \beta_6^{\text{high}} \)) (Table I; Fig. 7A). The \( \alpha_6^{\text{low}} \beta_6^{\text{high}} \) and \( \alpha_6^{\text{high}} \beta_6^{\text{high}} \) groups of clones had similar levels of \( \alpha_1 \), \( \alpha_2 \), and \( \alpha_3 \), although \( \alpha_6 \) expression was slightly increased in the latter group (Fig. 6; Table I). Clones with
the phenotype $\alpha_6^{\text{low}}p67 \text{LBP}^{\text{high}}$ show moderate laminin-specific adherence (Fig. 7A). In contrast, B2.7 transfectants with the phenotype $\alpha_6^{\text{high}}p67 \text{LBP}^{\text{high}}$ show avid laminin-specific adherence (Fig. 7A). Interestingly, B2.7 and B2.7/CD8, which express surface $\alpha_6$ in the absence of p67 LBP, do not exhibit laminin-specific adherence. Taken together, these data indicate that p67 LBP expression on B2.7 transfectants confers increased cellular laminin adherence; however, avid laminin binding requires high level expression of both p67 LBP and $\alpha_6$. Since Jurkat B2.7 is $\beta_1^{\text{negative}}$ (data not shown) and $\alpha_6$ is a component of VLA-6 ($\alpha_6\beta_1$), these data suggest that p67 LBP and VLA-6 function together to mediate high-avidity laminin adherence.

We next sought to determine whether the adherence of the Jurkat transfectants was specific to laminin relative to other $\beta_1$-integrin substrates, such as VCAM-1 and fibronectin, or to the basement membrane matrix protein collagen IV. Similar to the case for laminin, the parental B2.7 clone and the B2.7/CD8 transfectant do not adhere to collagen IV (Fig. 7B). In contrast, all Jurkat clones show significant baseline adherence to VCAM-1 and to fibronectin (Fig. 7B). Although p67 LBP expression in the B2.7/p37 LBPP

### Table I. Surface phenotype of Jurkat B2.7, D1.1, and transfectants of B2.7 with respect to p67 LBP and the integrins $\alpha_1$, $\alpha_2$, $\alpha_3$, and $\alpha_6$

<table>
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<th>$\alpha_2$</th>
<th>$\alpha_3$</th>
<th>$\alpha_6$</th>
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* Shown are the mean fluorescence intensities for each indicated surface protein assayed by FACS and normalized in each cell line for fluorescence of the appropriate isotype control mAb. Clones are arranged in order of increasing laminin-specific adherence, as depicted in Fig. 7, and as indicated qualitatively in the second column of the table.
transfectants is associated with increased laminin adherence (Fig. 7), p67 LBP expression does not induce collagen IV adherence. In addition, expression of p67 LBP is not associated with increased adherence to either fibronectin or to VCAM-1. Therefore, p67 LBP expression is associated with increased cellular adherence that is restricted to laminin, consistent with the previously reported specificity of p67 LBP for this β1-integrin substrate.

Discussion

The studies reported herein show that a subset of human peripheral blood T cells expresses surface 67 kDa LBP within 6 h of activation. The expression of p67 LBP persists for 7–8 days before returning to baseline (undetectable) levels. The activated T lymphocytes that express p67 LBP are memory cells and include CD4 and CD8 cells. The p67 LBP-expressing T lymphocytes also express the integrin α6 and β1 chains, which form the laminin receptor VLA-6. Finally, p67 LBP acts together with VLA-6 to mediate laminin binding on Jurkat T cell transfectants.

Although the role of p67 LBP on normal T cells has not been studied, certain inferences concerning its functional significance can be drawn from studies in tumor cells. For example, evidence associating p67 LBP with cell metastasis has been accumulating. The p37 LBPP cDNA was initially isolated by a differential cloning strategy designed to identify genes specifically associated with aggressive, metastatic behavior in colon carcinomas (40). Surface expression of p67 LBP has since been recognized to correlate with risk of tumor invasion and metastasis in a variety of solid tumors (including breast, colon, and lung) (18, 25–30, 41). Given the role of laminin as the predominant glycoprotein of the basement membrane, this correlation has suggested a role for p67 LBP in cellular trafficking across blood vessel and tissue basement membrane barriers. The fact that activated T lymphocytes express p67 LBP suggests that it plays a role in lymphocyte migration. In support of this interpretation is the finding that the p67 LBP high T cells also express high level CD11a, CD26, and CD44. The CD11a high/CD26 high CD44 high phenotype on memory helper T cells has been associated with transendothelial migratory capacity in in vitro assays (8, 42). These considerations suggest that p67 LBP may function to facilitate the extravasation of memory T cells into tissue spaces, or to retain activated T cells in local inflamed tissues once extravasation has been completed.

The first evidence for the expression of p67 LBP on activated T cells came from the isolation of p37 LBPP CDNA using a differential display strategy designed to identify genes specifically expressed in a Jurkat clone with activated T cell phenotype (Jurkat D1.1), relative to a Jurkat clone with resting phenotype (Jurkat
B2.7). The differential expression of p37 LBPP was difficult to substantiate by Northern or RT-PCR analysis because at least 17 human p37 LBPP pseudogenes exist (17). These share high homology (88–98%) with the functional gene (16) and are consistent with the production of sterile transcripts. The functional p37 LBPP cDNA encodes a nascent polypeptide of 34–37 kDa (19) that is posttranslationally modified to the 67-kDa mature form without evidence of additional biosynthetic intermediates in pulse-chase studies (19). Mass spectrometric analysis indicates that p67 LBP represents a homodimer of p37 LBPP polypeptides (15). In the Jurkat system, transfection of the single cDNA encoding the p37 LBPP confers p67 LBP expression on the Jurkat B2.7 subclone. These data indicate that the differential expression of p37 LBPP cDNA accounts for the differential surface expression of p67 LBP between Jurkat D1.1 and B2.7.

The fact that expression of p37 LBPP cDNA confers p67 LBP surface expression in Jurkat B2.7 may be due in part to the fact that all B2.7 clones express some surface α6. In certain previously reported cases, attempts to confer p67 LBP surface expression by transfection of the p37 LBPP cDNA (for example, in CHO cells) resulted in the accumulation of cytoplasmic polypeptide without significant surface p67 LBP expression, leading to speculation that another unidentified factor is required for mature p67 LBP to reach the cell surface (19). Recent studies of α6 and p67 LBP coexpression in tumor cells may bear on this problem. For example, the coordinate regulation of surface p67 LBP and α6 expression in a human epidermoid carcinoma line suggests that α6 is required for p67 LBP transport to the surface (22).

Expression of both p67 LBP and α6 is required to yield maximal laminin adherence in Jurkat cells. In this regard, α6 may be required to complement purified p67 LBP in a complex with high avidity for laminin (15). The fact that p67 LBP-associated laminin binding was greatly augmented by the coexpression of α6 on B2.7 transfectants strongly supports the notion that p67 LBP and α6 may function as components of a high-avidity laminin binding complex on cells. Therefore, α6 association with p67 LBP, in addition to facilitating surface p67 LBP expression (15), may also result in high-avidity cellular adherence to laminin.

The mechanism by which p67 LBP expression augments VLA-6-mediated laminin binding remains to be defined. Previous work by others has shown that p67 LBP and VLA-6 bind to distinct sites allowed to adhere for 2 h to polystyrene wells coated either with laminin or BSA (control). Following adherence, nonadherent cells were removed, and the cpm remaining in each well obtained. The cpm/cell was determined separately by counting 100,000 labeled cells. Laminin-specific adherence refers to the difference in average number of cells remaining between the laminin-coated and control wells. In all experiments, a given clone was assayed in quadruplicate wells, and each clone was assayed in at least four independent experiments. The results shown derive from one experiment, and are typical of performance over multiple trials. Horizontal bars depict laminin-specific adherence (in number of cells) for parental B2.7 and D1.1 (light grey bars), the B2.7 transfectant expressing pCEP-CD8 (B2.7/CD8, light stippled bar), and B2.7 transfectants expressing pCEP-p37 LBPP (B2.7/p37 LBPP, dark stippled bars). The phenotypes of all clones with respect to p67 LBP and α6 are shown in the table to the left of the bar plot.

**FIGURE 7.** Laminin-specific adherence of Jurkat B2.7, D1.1, and transfectants of Jurkat B2.7. A, The laminin-specific binding of Jurkat B2.7, D1.1, and selected transfectants of B2.7. Cells were labeled with 51Cr and...
on the laminin macromolecule, that are contained in nonoverlapping proteolytic laminin fragments, P1 and E8, respectively (43, 44). In cells expressing both p67 LBP and VLA-6, the anti-α6 blocking mAb GoH3 inhibits cellular adherence to E8, but not to P1 (45). The nonapeptide CDPGYIGSR, derived from the laminin P1 fragment, specifically elutes p67 LBP from a laminin affinity column (44, 46). In addition, this nonapeptide specifically inhibits cellular adherence to whole laminin (46), but has no effect on adherence to E8 (45). Therefore, though p67 LBP and α6 may be physically associated at the cell surface and appear to function together to mediate high-affinity laminin binding, they most likely interact independently with distinct sites on the laminin molecule. Avid adherence may result from the coordinate interactions of p67 LBP and VLA-6 with distinct laminin binding sites, rather than from interaction of a p67 LBP-VLA-6 heterodimer with a single laminin binding site. These data suggest that the expression of both p67 LBP and high level α6 after T cell activation may contribute to the enhanced laminin binding, which is known to be a feature of activated T cells (47). Our data do not address whether p67 LBP associates with α6 (independently of β1) or whether p67 LBP associates with the α6β1 heterodimer, VLA-6. If p67 LBP interacts with VLA-6 on the surfaces of activated T cells, it will be of interest to determine whether this interaction results in conformational changes in VLA-6, similar to those that have been characterized by mAbs to "neoeptopes" in other integrins (9–11, 48). Alternatively, since purified p67 LBP itself has significant affinity for laminin (15), the possibility exists that p67 LBP forms a laminin receptor complex independently of VLA-6. Although the molecular details of how p67 LBP, α6, and VLA-6 collaborate in mediating avid cellular adherence to laminin remain to be elucidated, these studies strongly suggest that p67 LBP plays a normal physiologic role in T cell biology in addition to its previously defined role in tumor metastasis.

Acknowledgments

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References