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Extracellular Matrix Conditions T Cells for Adhesion to Tissue Interstitium¹

Kimberly A. Krivacic* and Alan D. Levine^{2*†‡}

The activation and differentiation of peripheral blood T cells (PBT) are known to correlate with increased surface expression and adhesive capacity of β_1 integrins, which mediate adhesion to the extracellular matrix (ECM). However, little is known about the regulation of integrin expression, affinity, and avidity on tissue T cells after they are embedded in the interstitial ECM. In this study we show that tissue T cells, freshly isolated from their residence in the interstitial ECM of the intestinal lamina propria, express a distinct subset of functionally active integrins that contribute to enhanced adhesion to purified collagen, fibronectin, and cell-derived ECM when compared with freshly isolated, short term activated, and long term cultured PBT. Furthermore, integrin usage is distinct between circulating and tissue-derived T cells, in that lamina propria T cells prefer to bind to collagen, while PBT lymphoblasts choose fibronectin when presented with a complex, three-dimensional, cell-derived matrix. To identify the extrinsic factors that regulate the conversion from a nonadhesive PBT to highly adhesive tissue T cell, we demonstrate that activation of PBT in the presence of fibronectin or collagen rapidly generates a surface integrin expression profile, an integrin usage pattern, and adhesive capacity mirroring that of a tissue T cell. These results indicate that the tissue ECM microenvironment instructs newly arrived T cells for further interactions with the underlying matrix and thereby imprints them with a signature tissue adhesive phenotype. *The Journal of Immunology*, 2003, 170: 5034–5044.

Naive and memory/effector T lymphocytes continually circulate in search of foreign Ag and rapidly transform from a nonadherent cell while in the blood and lymph to an adherent polarized cell when crawling through tertiary lymphoid organs or inflamed tissues. The recruitment and retention of T lymphocytes within inflamed tissues are dependent on adhesion to an acellular network of proteins, glycoproteins, and proteoglycans known as the extracellular matrix (ECM)³ (1, 2). Adhesion is a critical component in a T cell-mediated immune response and is therefore tightly regulated (3–6).

Activated effector T lymphocytes mediate immune protection in solid tissue and are thus distinguished from their circulating naive and memory counterparts as a distinct subclass of T cells, the tissue T cell. While T lymphocytes rarely reside in solid tissue of most healthy nonlymphoid organs, the intestinal lamina propria is richly populated with effector CD45RO⁺ T cells (LPT) and is therefore an excellent source of tissue T cells, which are maintained in close physical and functional contact with the surrounding interstitial ECM. This interstitial matrix fills the extracellular space within tissue and serves as a working platform for nonanchored cells. Local fibroblasts synthesize interstitial ECM, which is composed of primarily the glycoproteins fibrillar collagen, fibronectin, tenascin, vitronectin, and proteoglycans (7). Since LPT

possess phenotypic and functional properties distinct from those of naive and memory peripheral blood T cells (PBT) (8, 9), we propose that the close association of LPT with the ECM might be a mechanism by which the acellular components of tissue modulate the retention and function of tissue T cells.

Adhesive interactions with the ECM microenvironment are mediated by integrins, a large family of heterodimeric glycoproteins composed of noncovalently associated α and β transmembrane subunits. While each $\alpha\beta$ heterodimer has its own ligand binding specificity, individual integrins often bind to more than one ligand, and individual ligands are recognized by more than one integrin (10). T lymphocytes express members of both the β_1 and β_2 integrin families of cell surface adhesion molecules, which mediate cell-ECM and cell-cell interactions, respectively. T cell expression of various β_1 integrin subfamily members mediates binding to components of the interstitial ECM: collagen ($\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$) and fibronectin ($\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$) (11).

T cells rely on integrins to sense and respond to different matrices, known as outside-in signaling (10). These interactions initiate intracellular signaling cascades involved in T cell growth, survival, and migration (3, 12–15). Conversely, T cells are able to modulate their adhesive phenotype via inside-out signaling by controlling the expression of surface integrins and their relative functional adhesiveness (12). Integrin-mediated adhesion is regulated at three levels: 1) by cell surface expression (11), or post-translationally, 2) by changes in integrin affinity for their ligand, attributed to conformational changes in the ligand binding site, and 3) by modulating their avidity via integrin clustering in the plane of the plasma membrane (13, 14). Thus, integrins exist in several different conformational states. In the closed conformation or inactive state, integrins are unable to bind ligand. A partially active integrin in a low affinity conformation can bind certain ligands, while a fully active integrin, in a high affinity conformation, is readily occupied by ligand (15, 16).

Circulating blood T cells express moderate amounts of certain β_1 integrins on their cell surface, yet these resting cells adhere poorly to ECM, suggesting that their integrins are expressed in the

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³ Abbreviations used in this paper: ECM, extracellular matrix; DPBS, Dulbecco's PBS; HIF, human intestinal fibroblast; LPMC, lamina propria mononuclear cell; LPT, lamina propria T cell; MFI, mean fluorescence intensity; PBT, peripheral blood T cell; VLA, very late Ag.

inactive state (17). Activation of blood T cells stimulates a rapid augmentation in β_1 -mediated adhesion associated with enhanced integrin affinity and avidity (17), followed by a slow increase in cell surface expression (11). Agents that modulate integrin-mediated adhesion in circulating T cells include engagement of the Ag-specific TCR/CD3 complex, CD2, CD7, CD28 costimulation, and chemokines (17–20). Increased adhesion can be mimicked *in vitro* with activating Abs to integrins, divalent cations, and soluble ligand binding, all of which induce rapid dynamic changes in integrin affinity for their ligands (12, 20, 21), and treatment with phorbol esters, which stimulates integrin clustering and increased avidity (22). As predicted by these changes after T cell activation, integrin expression and functional status on effector/memory CD45RO⁺ PBT are greater than those in naive CD45RA⁺ PBT (17, 23). Furthermore, ligation of one subset of integrins regulates the affinity/avidity of another subset of integrins, termed integrin cross-talk (24–26).

These previous reports have only evaluated adhesion of PBT to purified protein components of the ECM. In addition, these earlier studies do not address the possibility that PBT may not have been previously exposed to a full complement of matrix proteins either in the blood or in secondary lymph nodes, where some components of the matrix are shielded from lymphocytes by appendages of reticular cells (27, 28). No studies have addressed whether residence within tissue ECM may modulate T cell adhesion or function. Therefore, we hypothesize that the interstitial ECM microenvironment imprints upon newly arriving blood T cells a signature tissue adhesive phenotype that regulates tissue T cell responses. In this report we use native human intestinal fibroblast-derived ECM as well as two of its purified components to investigate the competence and activation requirements of tissue-specific LPT for matrix adhesion. Our studies reveal that freshly isolated LPT adhere strongly to the ECM with or without activation via the $\alpha_1\beta_1$ and $\alpha_4\beta_1$ integrins with a response that is quantitatively and qualitatively distinct from their circulating peripheral blood T cell counterparts. We also demonstrate that although activation of PBT induces the global surface expression of many integrins, neither the distinguishing integrin profile nor the high adhesion of tissue T cells can be mimicked in long term activated PBT. Instead, the distinct adhesiveness of the β_1 integrin family on tissue T cells can be induced in matrix-sheltered PBT by ECM preconditioning, suggesting that a combination of activation and matrix exposure is required for generating a tissue adhesive phenotype. These results demonstrate that naive and memory circulating T cells, once enmeshed within the tissue ECM microenvironment, are imprinted with high affinity and avidity for future interactions with that tissue.

Materials and Methods

Antibodies

For functional studies anti-CD3 mAb OKT3 was purchased from Ortho Diagnostic Systems (Raritan, NJ), and the pair of anti-CD2 Abs T11₂ and T11₃ were a gift from Dr. E. Reinherz (Dana-Farber Cancer Institute, Boston, MA). For immunofluorescence, anti-human fibronectin mAb clone FN-15 (Sigma-Aldrich, St. Louis, MO), rabbit anti-human collagen type I (Accurate Chemicals, Westbury, NY), fluorescein-labeled polyclonal anti-rabbit (Santa Cruz Biotechnology, Santa Cruz, CA), and Texas Red-labeled polyclonal anti-mouse (Vector Laboratories, Burlingame, CA) were used. For flow cytometry and blocking experiments, anti-human α_1 integrin mAb clone FB12 (Chemicon, Temecula, CA), anti-human integrin α_2 mAb clone P1E6, anti-human integrin α_3 mAb clone P1B5, anti-human integrin α_4 clone P4C2, anti-human integrin α_5 clone P1D6, and anti-human integrin β_1 clone P4C10 were used (Life Technologies, Grand Island, NY). Anti-human CD3 clone UCHT1 and FITC-labeled polyclonal anti-mouse IgG were obtained from DAKO (Carpinteria, CA) and BioSource (Camarillo, CA), respectively.

Isolation and culture of HIF

Human intestinal fibroblasts (HIF) were isolated as described previously (29). Surgical specimens from patients undergoing bowel resection for colon cancer were washed in calcium- and magnesium-free HBSS (BioWhittaker, Walkersville, MD), and strips of normal mucosa, at least 10 cm from the margin, were dissected, cut into small fragments, and laid on the bottom of scored tissue culture dishes. Medium consisting of DMEM supplemented with 10% heat-inactivated FCS; 2.5% L-glutamine; 1% mixture of penicillin, streptomycin, and fungizone; and 25 mM HEPES buffer (all from BioWhittaker) was added to the dishes, which were incubated at 37°C in humidified 5% CO₂. Fibroblast growth emanating from the fragments was observed within 3–5 days. At 3–4 wk, subconfluent fibroblasts were detached with a trypsin-versene mixture (BioWhittaker) and established in long term cultures. Cells were fed twice weekly and were split at confluence every 4–7 days.

Preparation of cell-derived, ECM-coated wells

HIF established from normal donors were grown in flat-bottom, 12-well tissue culture plates (Costar, Cambridge, MA). At confluence, monolayers were maintained in culture for an additional 12 days in the presence of 100 U/ml of human recombinant TNF- α (R&D Systems, Minneapolis, MN). Fibroblast monolayers were washed with HBSS and then lysed using 0.5% Triton X-100 (Sigma-Aldrich) in Dulbecco's PBS with calcium and magnesium (DPBS; BioWhittaker) for 10 min, followed by 0.025 N ammonium hydroxide (Fisher Scientific, Hanover Park, IL) for 2 min (30). The resultant native ECM was washed six times with 2 ml of DPBS.

Isolation of lamina propria, PBMC, and T cells

Lamina propria mononuclear cells (LPMC) were isolated as described previously (31). Briefly, surgical specimens from patients undergoing bowel resection for colon cancer were washed with HBSS. The mucosa was carefully dissected from the underlying submucosa in 5-mm strips and stirred with 10 mM DTT (Sigma-Aldrich) in HBSS for 30 min at room temperature. The tissue then underwent three washes at room temperature in 1 mM EDTA (Sigma-Aldrich), then a series of washes in HBSS to remove the epithelium, followed by an overnight digestion at 37°C, with stirring, in HBSS supplemented with 0.1 mg/ml DNase and 0.1 mg/ml collagenase type 3 (both from Worthington Biochemical Corp., Freehold, NJ) and 2.5% penicillin, streptomycin, and fungizone. Digested tissue was filtered through a 100- μ m pore size Nitex mesh (Tetko, Lancaster, NY). LPMC were separated by Ficoll-Hypaque density gradient centrifugation (Histopaque; Sigma-Aldrich) at 2000 \times g for 5 min at room temperature. For the isolation of PBMC, blood was obtained from healthy volunteers, and PBMC were isolated by Ficoll-Hypaque density gradient centrifugation at 1000 \times g for 20 min at room temperature.

To obtain purified LPT and PBT, adherent cells were removed from LPMC or PBMC by plating 30 \times 10⁶ cells in RPMI 1640 (BioWhittaker) with 5% FCS on 100-mm tissue culture dishes (Corning, Corning, NY) at 37°C in 5% CO₂ for 1 h. Nonadherent cells were collected and incubated for 30 min at 4°C with magnetically labeled anti-CD19, -CD14, and -CD16 Abs (Miltenyi Biotec, Sunnyvale, CA) against B cells, monocytes, and neutrophils, respectively. T cells were negatively selected by MACS. The T cell populations were routinely >97% CD3⁺, as assessed by flow cytometry. The surface phenotypes of freshly isolated LPT and PBT were previously described (32).

LPT and PBT activation

Freshly isolated LPT and PBT were suspended at 1 \times 10⁶ cells/ml in RPMI 1640 with 10% FCS alone or with 20 U/ml of human rIL-2 (Chiron, Emeryville, CA), 5 ng/ml PMA, and 500 ng/ml ionomycin (both from Sigma-Aldrich) or with a 1/1000 dilution of each of the stimulatory anti-CD2 Abs (T11₂ and T11₃) and incubated in T25 flasks (Costar) at 37°C in 5% CO₂ for 12 h. Sixty-millimeter tissue culture dishes (Corning) were coated with 2 ml of 10 μ g/ml anti-CD3 Ab (OKT3) in borate buffer at 37°C for 2 h and washed three times with HBSS. Freshly isolated LPT and PBT were suspended at 1 \times 10⁶ cells/ml in RPMI 1640 with 10% FCS and stimulated by plate immobilized anti-CD3 Ab for 12 h.

Establishment of T cell lymphoblast lines

Freshly isolated LPMC and PBMC were suspended at 10⁶/ml in RPMI 1640 with 10% FCS containing 1 ng/ml PMA and 0.02% PHA (Sigma-Aldrich). After 48 h, cells were washed twice and kept at 37°C in 5% CO₂ in RPMI 1640 supplemented with 10% FCS and 20 U/ml of human rIL-2 (Chiron). Lymphoblast cultures were fed twice weekly and used 2 wk later

in an adhesion assay with HIF derived native ECM and its purified components. The surface expression of T cell activation markers is similar, as described in an earlier report (33).

Adhesion assay

Twelve well flat-bottom plates were precoated overnight at 4°C with 12 $\mu\text{g}/\text{well}$ fibronectin (Chemicon) in DPBS, 40 $\mu\text{g}/\text{well}$ collagen type I (Sigma-Aldrich) in 0.1 M acetic acid, or 3% BSA (Sigma-Aldrich) in DPBS as a control and then washed three times with DPBS. Freshly isolated T cells or IL-2-derived T cell lines 2 days after passage were fluorescently labeled with 4 μM calcein-AM (Molecular Probes, Eugene, OR) for 20 min at 37°C in 5% CO_2 at 2×10^6 cells/ml in DPBS containing 5% FCS. The cells were then washed three times with DPBS containing 5% FCS. Calcein-labeled T cells were resuspended in RPMI 1640 without phenol red supplemented with 10% FCS and 5×10^5 T cells/well were added to 12-well plates containing HIF-derived native ECM or purified ECM components or to plastic controls for the indicated times. Saturating concentrations of integrin-blocking mAbs (predetermined by flow cytometry) were preincubated with T cells for 30 min at 37°C before adding T cells to the wells for the adhesion assay. Nonadherent cells were removed by a standardized washing technique that was developed to minimize background binding, which includes both an orbital and a rocking motion repeated three times with DPBS. Adhesion was quantified with a multiwell fluorescent spectrophotometer (CytoFluor; PerSeptive Biosystems, Foster City, CA) set for four reads per well at eight sites within the well, which were then averaged for a single reading. For each experimental group the results were expressed as the mean percentage \pm SD of bound T cells from triplicate wells.

Flow cytometry

Single-color flow cytometric analysis was performed on freshly isolated T cells and T cell lines. Cells were washed twice in ice-cold DPBS, and 1×10^6 cells were resuspended in flow buffer (HBSS containing 1% BSA and 0.1% sodium azide). Cells were incubated with a specific mAb at predetermined saturating concentrations or with isotype-matched nonspecific mouse mAb (DAKO) for 30 min at 4°C, washed twice with flow buffer, and incubated with FITC-conjugated goat anti-mouse IgG for 30 min at 4°C. The cells were again washed twice with flow buffer, fixed in 1% paraformaldehyde, and analyzed by a single laser flow cytometer (EPICS XL-MCL; Beckman Coulter, Fullerton, CA), using WinList software (Topsham, ME). Cells were gated for lymphocytes identified by their specific location in the forward/side scatter diagram, and T cells were identified with anti-CD3. Cells staining with a negative control Ab were gated to contain <4% positive cells.

PBT preconditioning with collagen type I or fibronectin

Tissue culture dishes (100 mm; Corning) were precoated overnight at 4°C with 30 $\mu\text{g}/\text{dish}$ fibronectin in DPBS, 100 $\mu\text{g}/\text{dish}$ collagen type I in 0.1 M acetic acid, or DPBS or 0.1 M acetic acid as a control and were then

washed three times with DPBS. Freshly isolated PBT were cultured at 1×10^6 cells/ml in RPMI 1640 with 10% FCS alone, with 20 U/ml IL-2- or anti-CD3-coated beads (25 $\mu\text{g}/\text{ml}$ OKT3) on either control dishes, collagen type I, or fibronectin-coated dishes for 3 days at 37°C in 5% CO_2 . PBT were then removed from the plates with HBSS and used in an adhesion assay or were stained for flow cytometry.

Immunofluorescence microscopy of HIF-derived native ECM

For indirect immunofluorescence analysis 12-well plates containing ECM derived from HIF that had been treated, or not, with TNF- α were washed once with DPBS and were blocked with either 3% BSA (Sigma-Aldrich) in DPBS (for collagen type I staining) or 5% horse serum (Vector Laboratories) in DPBS (for fibronectin staining). ECM was then incubated overnight at 4°C with rabbit anti-human collagen type I Ab or mouse anti-human fibronectin in the appropriate blocking buffer. ECM coated with the primary Ab was washed extensively with DPBS and incubated for 60 min at room temperature with a fluorescein-conjugated goat anti-rabbit secondary Ab for collagen type I wells or with a Texas Red-conjugated horse anti-mouse secondary Ab for fibronectin wells (both from Vector Laboratories). Fluorescence microscopy was performed using an IMT-2 inverted microscope (Olympus, Lake Success, NY). Images were acquired with an Olympus camera on 35-mm color slides.

Analysis of data

Statistical analysis was performed using the two-tailed Student's *t* test and ANOVA (Excel; Microsoft, Redmond, WA). Results are expressed as the mean \pm SD, and significance was inferred at $p < 0.05$.

Results

Intrinsic adherence of tissue-derived T cells for ECM

To study tissue T cell-matrix interactions we developed a physiologically relevant strategy using cell-derived ECM (native ECM) to investigate the regulation of integrin-mediated adhesion of tissue T cells. The intestinal LPT was chosen as a representative population of tissue lymphocytes that are embedded in the mucosal interstitial ECM produced by HIF. Adhesion of T cells to the native ECM was characterized and quantitated by labeling the cells with calcein, after which 5×10^5 cells/well were adhered for 2 h to BSA-coated plastic or native ECM. A representative fluorescent microscopic field after adhesion of freshly isolated LPT is shown in Fig. 1. In contrast to previous reports showing that PBT are nonadherent to purified components of the ECM (17), there was robust adhesion of freshly isolated tissue T cells to the native matrix and only minimal adhesion to the control. In addition, higher power examination of the adherent cells in Fig. 1A revealed a

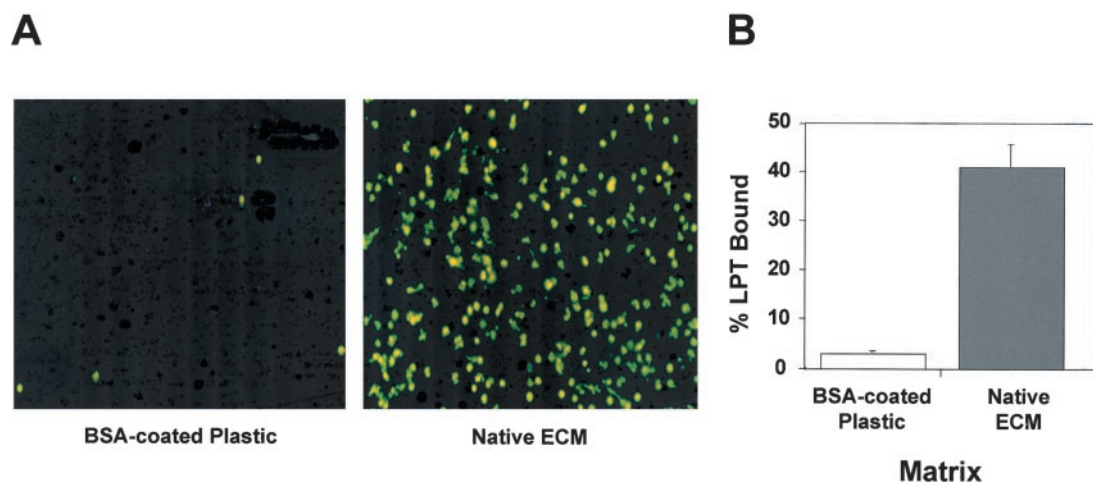


FIGURE 1. Adhesion of tissue T cells for cell-derived ECM. Calcein-labeled LPT (5×10^5 cells/well) were adhered for 2 h to BSA-coated plastic, as the control, or ECM derived from HIF. Nonadherent cells were removed by gentle washing. One representative experiment is shown of 10 donors. A, The fluorescent photomicroscopy of a representative field of LPT adherent to BSA control (left) and native ECM (right) is shown at $\times 400$ magnification. The fluorescence of adherent LPT (B) was quantified in triplicate using a fluorescence spectrophotometer. Results are the mean \pm SD percentage of input cells bound.

striking and distinctive change (i.e., spreading) in T cell morphology, directly associated with their interactions with the matrix (data not shown). To quantify the adhesion of tissue T cells for native matrix, the level of adherent T cell fluorescence was measured with a fluorescence spectrophotometer. As shown in Fig. 1B, 41% of calcein-labeled LPT cells adhered to native matrix, whereas <3% adhered to BSA-coated plastic. Maximal T cell binding to native ECM was observed within 30 min, and this adhesion was stable for >6 h (data not shown). To demonstrate that the adhesion of tissue T cells to native ECM is predominantly mediated by β_1 integrins, LPT were incubated with a blocking Ab to β_1 before their adherence to native ECM. Inhibition of β_1 interaction with native ECM decreases adhesion by >90% (Fig. 7).

Native ECM is composed of a fibrillar network of fibronectin and collagen

The dependence of tissue T cell adhesion on the β_1 family of integrins strongly suggests that both fibronectin and collagen type I are present in the native ECM-derived from mucosal fibroblasts (Fig. 2). HIF established from normal donors were grown to confluence, and resultant native ECM was prepared. HIF-derived native ECM showed intense staining with Abs against fibronectin (Fig. 2, *bottom left panel*) and collagen type I (Fig. 2, *bottom right panel*). The staining pattern indicates the presence of a fibrillar network forming a three-dimensional reticular-like structure. As a control, no fluorescence was detected in wells that were stained with the appropriate secondary Ab alone (Fig. 2, *top panels*). These findings together with those of the blocking studies demonstrate that the binding of tissue T cells to native ECM is mediated by the β_1 family of integrins and the production of fibronectin and collagen type I by mucosal fibroblasts.

Tissue T cells express integrins with a higher intrinsic affinity/avidity for ECM ligands than circulating T cells

As tissue T cells are embedded in the interstitial ECM, we hypothesized that this matrix microenvironment educates the tissue T

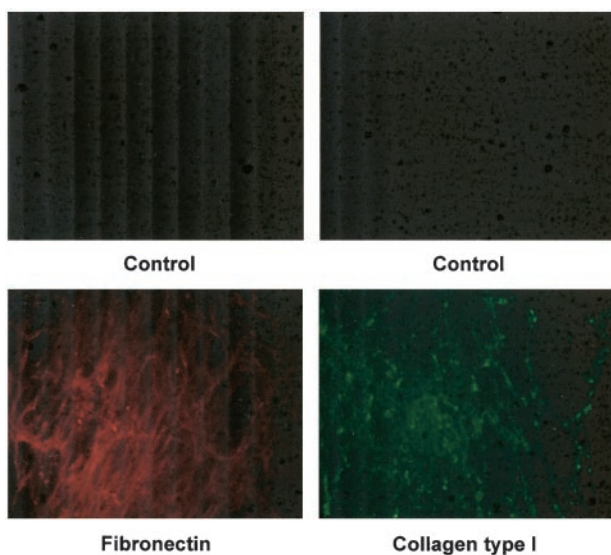


FIGURE 2. Cell-derived (native) ECM contains both fibronectin and collagen type I. Confluent HIF monolayers established from normal donors were maintained in culture for 12 days. Fibroblasts were lysed, and the resultant ECM was washed extensively. Native ECM was stained with an anti-fibronectin Ab and a Texas Red-conjugated secondary Ab (*bottom left*) or with an anti-collagen type I Ab and a fluorescein-conjugated secondary Ab (*bottom right*). Identical wells of native ECM were stained with the appropriate secondary Ab alone as a negative control (*top*).

cell to acquire a specialized adhesiveness for the ECM by shaping a distinctive β_1 integrin expression, affinity, and avidity. To test this hypothesis, freshly isolated unstimulated LPT were adhered for 2 h to native ECM and the two predominant components of this cell-derived ECM, purified collagen type I and fibronectin. As expected, LPT freshly harvested from residence in their ECM-rich microenvironment adhered strongly to all matrixes (Fig. 3). In contrast, and in agreement with other reports showing that resting CD4⁺ blood T cell adhesion to fibronectin and laminin was minimal (17), freshly isolated PBT adhered poorly (<6%) to fibronectin, collagen type I, and native ECM. Compared with PBT, the binding of LPT was 4- to 5-fold higher for fibronectin and native ECM and a striking 25-fold higher for collagen type I. Each of these differences between LPT and PBT achieved high significance ($p < 0.01$), demonstrating that interstitial tissue is populated with T lymphocytes that express the ability to adhere. Since collagenase and DNase I were used in the purification of LPT, we treated PBT under identical conditions with these enzymes. The difference in the method of purification does not change the adhesiveness of PBT (data not shown). The observation that LPT binding is significantly greater than PBT adhesion was true over a wide range of fibronectin and collagen type I concentrations (data not shown). These results suggest a distinct pattern of β_1 -mediated adhesion available to tissue T cells due to 1) increased surface expression of appropriate integrins on LPT, 2) enhanced affinity/avidity (i.e., open conformation) of LPT integrins, 3) a different profile of surface integrins, or 4) a combination of all three.

Tissue-derived T cells express a distinct β_1 integrin profile compared with circulating T cells

The increased adhesion of tissue T cells to the ECM suggests that they may express a unique pattern of β_1 integrins. Since T cells use α_3 , α_4 , and α_5 to engage fibronectin and α_1 , α_2 , and α_3 to bind collagen type I (11), we contrasted the surface expression of integrin subunits α_1 to α_5 on LPT vs PBT using single-color flow cytometry (Fig. 4). Statistical analysis of the mean for four donors revealed that a higher percentage of freshly isolated LPT express the α_1 chain (69%) compared with 16% for PBT ($p < 0.001$). There was also a modest, but significant, increase in the percentage of LPT that express α_3 (56 vs 33%; $p < 0.048$), consistent with increased adhesion of LPT for collagen type I. However, high adhesion to fibronectin by LPT was not easily explainable by surface expression of β_1 integrins, since LPT exhibited a dramatic decrease in the percentage of cells expressing α_5 (8 vs 47% for PBT; $p < 0.004$), and an identical high percentage expressed the

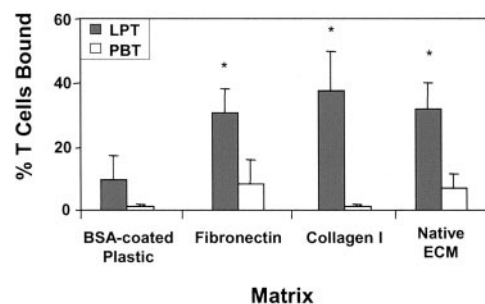
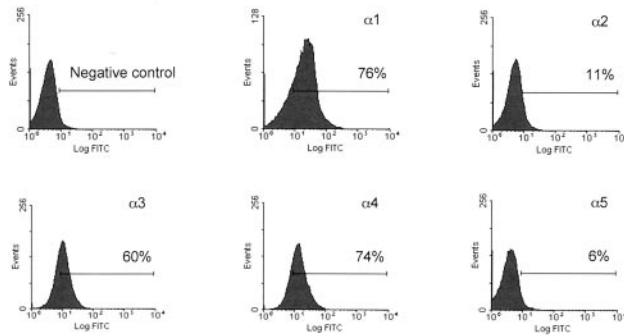
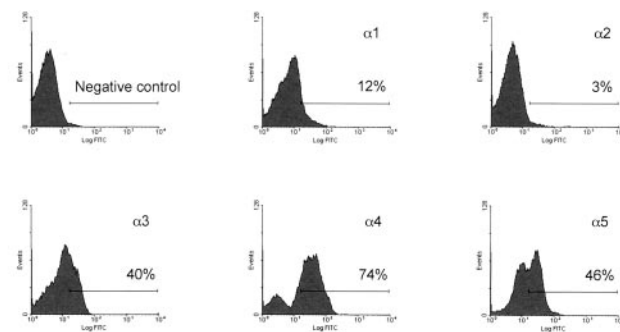


FIGURE 3. Freshly isolated tissue T cells are more adhesive for ECM than blood T cells. Freshly isolated LPT (■) and PBT (□) were labeled with calcein, and 5×10^5 cells/well were allowed to adhere for 2 h to BSA-coated plastic (as a control), fibronectin, collagen type 1, or native ECM. Nonadherent cells were removed by washing. The fluorescence of adherent T cells was quantified in triplicate using a fluorescence spectrophotometer. Results are the mean \pm SD percentage of input cells bound ($n = 3$). *, $p < 0.01$.

A. Freshly isolated LPT



B. Freshly isolated PBT



C. Statistical comparison between LPT and PBT (n=4)

	LPT	PBT	p
Percent positive			
α_1	69 \pm 7	16 \pm 3	0.001
α_2	14 \pm 3	7 \pm 3	0.01
α_3	56 \pm 6	33 \pm 7	0.048
α_4	73 \pm 8	75 \pm 4	n.s.
α_5	8 \pm 5	47 \pm 6	0.004
Mean Fluorescence Intensity			
α_1	25 \pm 3	25 \pm 6	n.s.
α_2	17 \pm 3	49 \pm 26	0.1
α_3	16 \pm 3	25 \pm 5	0.06
α_4	17 \pm 2	39 \pm 7	0.019
α_5	16 \pm 2	30 \pm 1	0.001

FIGURE 4. Unique integrin surface expression on tissue-derived T cells. Flow cytometric analysis of freshly isolated LPT (A) and PBT (B) was performed. Negative control cells were gated to contain <3% positive cells. Both PBT and LPT populations were >97% CD3 and β_1 integrin positive. Representative histograms of four experiments are shown. Statistical analysis of all four donors (C) revealed that that percentages of cells that express α_1 , α_2 , α_3 , and α_5 integrins are significantly different between LPT and PBT, and the MFIs for α_4 and α_5 are significantly lower on LPT compared with PBT.

α_4 (73%; Fig. 4C) and β_1 (94%; data not shown) chains. Furthermore, greater LPT adhesiveness to ECM was not easily explainable by differences in surface integrin subunit expression on a per cell basis. There was no significant difference in the per cell expression of α_1 , α_2 , and α_3 between PBT and LPT (Fig. 4C). In fact, the mean fluorescence intensity (MFI) for α_4 and α_5 on PBT was greater than that observed for LPT ($p = 0.019$ and 0.001 , respectively). Similarly, differences in integrin expression between PBT and LPT were not accountable for by the differences in naive and memory T cell populations. The profiles of α_1 and α_3 expression on CD45 RO⁺ and CD45 RA⁺ PBT were similar and did not reflect the CD45RO⁺ LPT pattern (data not shown). Similarly, α_5 expression was low in LPT and was actually greater in CD45RO⁺ vs CD45RA⁺ PBT (data not shown). Therefore, the enhanced adhesiveness of LPT to native ECM and its components is not simply accountable for by differences in β_1 integrin surface expression, but suggests that integrins on LPT and PBT differ in both their state of activation as well as their unique expression profile.

Short term activation of freshly isolated PBT does not recapitulate LPT adhesiveness

To define the basis for the greater integrin-mediated adhesiveness of LPT, we investigated whether short or long term activation of PBT in the absence of matrix can induce a tissue-like adhesive phenotype and whether these immunological stimuli would alter the adhesiveness of LPT. As reported previously, short term activation of PBT induced an active conformation among β_1 integrins (17, 23, 34, 35). Freshly isolated PBT were incubated in short term cultures with no stimulus, IL-2, anti-CD2, and plate-immobilized anti-CD3 for 12 h, and then calcein labeled and allowed to adhere

to collagen type I, fibronectin, or native ECM (Fig. 5). Consistent with previous studies, unstimulated PBT were nonadherent for all matrixes, but could acquire the ability to bind to both fibronectin and native ECM weakly with IL-2 and modestly with anti-CD2 or anti-CD3 (Fig. 5B). In contrast, unstimulated LPT adhered to all matrixes, and stimulation resulted in only a minimal increase in their adhesiveness (Fig. 5A). PBT did not bind as well as LPT to collagen type I, fibronectin, or native ECM after stimulation with any activator studied, including the strong stimulant PMA/ionomycin (data not shown). The reduced adhesion of freshly isolated (Fig. 4C) and short term activated (not shown) PBT compared with LPT was not due to less integrin expression per cell. Poor binding of resting and activated PBT to collagen was probably due to the low percentage of cells expressing α_1 and α_2 on their cell surface. While PBT expressed a subset of integrins with the capacity to bind fibronectin and, thus, native matrix, these integrins must be activated by immunological stimuli to engender PBT adherent. Despite a change to an active integrin conformation without a change in surface expression of β_1 integrins (data not shown), the degree of adhesiveness of activated PBT did not approach that of tissue T cells. Furthermore, LPT were strongly adherent both before and after stimulation. These results indicate that tissue T cells, having been previously exposed to interstitial ECM, express a higher intrinsic adhesion for ECM.

The distinct expression of very late Ag (VLA) integrins and the high adhesiveness of tissue T cells for ECM are not attained by long term culture of PBT

Surface expression of β_1 integrins on T cells, i.e., VLA, slowly increases with time during a long period of activation. Therefore,

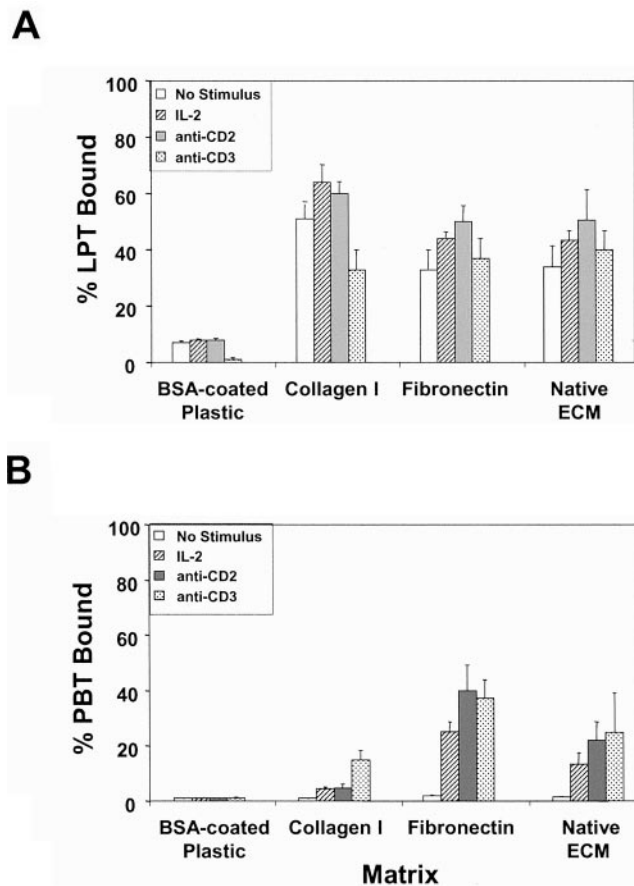


FIGURE 5. Freshly isolated tissue T cells express surface integrins in their fully activated conformation, whereas integrin-mediated adhesion must be induced on blood T cells with short term activation. Freshly isolated LPT (A) or PBT (B) were incubated in medium alone, IL-2, activating anti-CD2 (T11₁ and T11₂) mAbs, or plate immobilized anti-CD3 mAb (OKT3). T cells were labeled with calcein, and 5×10^5 cells/well were allowed to adhere for 2 h to BSA-coated plastic (control), fibronectin, collagen type I, or native ECM. Nonadherent cells were removed by washing. The fluorescence of adherent T cells was quantified in triplicate using a fluorescence spectrophotometer. Results are the mean \pm SD percentage of input cells bound from one representative experiment of four donors.

we investigated whether the adhesiveness and integrin profile of PBT in the absence of matrix would approach that of tissue T cells after expansion in culture with IL-2 (Fig. 6). Even after PBT were maintained in culture for 2 wk, the profile of VLA integrin expression on PBT lymphoblasts did not replicate the pattern observed with freshly isolated LPT (compare Fig. 6C to Fig. 4A). In addition, PBT lymphoblast adhesiveness was less than that of similarly cultured LPT (Fig. 6A). Notably the binding of LPT lymphoblasts was $42 \pm 5.4\%$ higher for collagen type I ($p < 0.04$). To investigate whether differences in surface expression of integrin receptors in PBT vs LPT lymphoblasts explain the reduced adhesion of PBT lymphoblasts, we quantified α_1 through α_5 expression by flow cytometry (Fig. 6, B vs C). Statistical analysis of the mean for four donors revealed that the percentage of T cells expressing α_2 was higher in LPT vs PBT lymphoblasts (86 vs 58% ; $p < 0.004$), while the percentages of α_1 -, α_3 -, α_4 -, and α_5 -positive T cells were identical. However, on a per cell basis, α_1 surface expression was significantly greater on LPT vs PBT (Fig. 6D; $p = 0.044$), possibly contributing to the greater adhesion of LPT for collagen. Furthermore, the levels of LPT lymphoblast adhesion to both fibronectin and native ECM were 23 ± 11 and $14.1 \pm 2.2\%$

higher than that for PBT ($p < 0.04$), respectively. Consistent with this increased binding, on a per cell basis the surface expression of the α_4 fibronectin receptor on the LPT lymphoblast was significantly greater ($p < 0.007$) than that expressed by PBT lymphoblasts (Fig. 6D). In summary, LPT lymphoblasts express greater levels of VLA-1 and VLA-4 than do PBT lymphoblasts, corresponding to the heightened LPT adhesion to all matrixes (Fig. 6A). The greatest difference in adhesion between LPT and PBT lymphoblasts, previously observed with freshly isolated T cells, is accentuated on collagen type I matrix. Taken together, these results indicate that the increased adhesiveness of tissue T cells is not due to differences in their state of activation, and that long term culture of PBT in IL-2 does not replicate the integrin expression profile characteristic of the tissue T cell. Thus, tissue T cells may have been instructed by the surrounding interstitial ECM present in their native environment to express a unique adhesiveness, as defined by their integrin profile and conformation.

Distinct integrin usage between tissue and circulating T cells

In light of the persistent differences between tissue and circulating T cells, it is likely that the integrins responsible for mediating their adhesion are distinct. ECM adhesion is mediated by multiple integrins, and conversely, integrins interact with multiple ECM ligands. T cells use α_3 , α_4 , and α_5 to engage fibronectin, and α_1 , α_2 , and α_3 to bind to collagen. We therefore used a panel of functional blocking mAbs to identify the integrins involved in both PBT and LPT adhesion (Fig. 7). Integrin usage by both LPT and PBT for adhesion to purified collagen type I was similar, predominately mediated by α_1 . In contrast, adhesion to fibronectin was mediated by different integrins. LPT adhesion to fibronectin was entirely mediated by α_4 , while PBT adhesion was markedly predominated by α_5 . Not surprisingly, the integrins responsible for adhesion to native ECM were more complex. When presented with a complex composite matrix, PBT adhesion to the native ECM was mediated by a subset of integrins that recognize fibronectin (i.e., α_4 and α_5). On the other hand, tissue T cell adhesion to native matrix was predominated mediated by α_1 and α_2 , integrins that recognize collagen, with a modest contribution of the fibronectin-binding α_4 integrin. These results extend our earlier observations that integrin expression, conformation, and now usage are distinct between tissue and circulating T cells.

Conditioning of fresh PBT with fibronectin or collagen type I dramatically increases their subsequent adhesion to ECM and modulates their integrin usage

The high adhesion of tissue T cells was not emulated by short and long term in vitro activation of PBT in the absence of matrix. Since the ligation of one subset of integrins influences the expression and affinity of another subset of integrins (25, 26, 36, 37), we predicted that activating PBT in the presence of fibronectin or collagen type I for a short time period (3 days) would increase PBT cell adhesion for ECM. Freshly isolated PBT were cultured with IL-2 in the presence or the absence of fibronectin or collagen type I for 3 days. These PBT were harvested, calcein labeled, and allowed to adhere for 2 h to BSA-coated plastic (control), fibronectin, collagen type I, and native matrix (Fig. 8). IL-2 activation of PBT in the presence of fibronectin for 3 days nearly doubled the percentage of T cells bound to both fibronectin and native matrix and modestly increased T cell adhesion to collagen type I. Culturing PBT for 3 days in the presence of collagen type I markedly increased the percentage of cells that were able to adhere to fibronectin and native ECM. These findings were similarly replicated with anti-CD3-coated bead stimulation in the presence of matrix (Table I). An additional feature that distinguishes fresh tissue T cells from

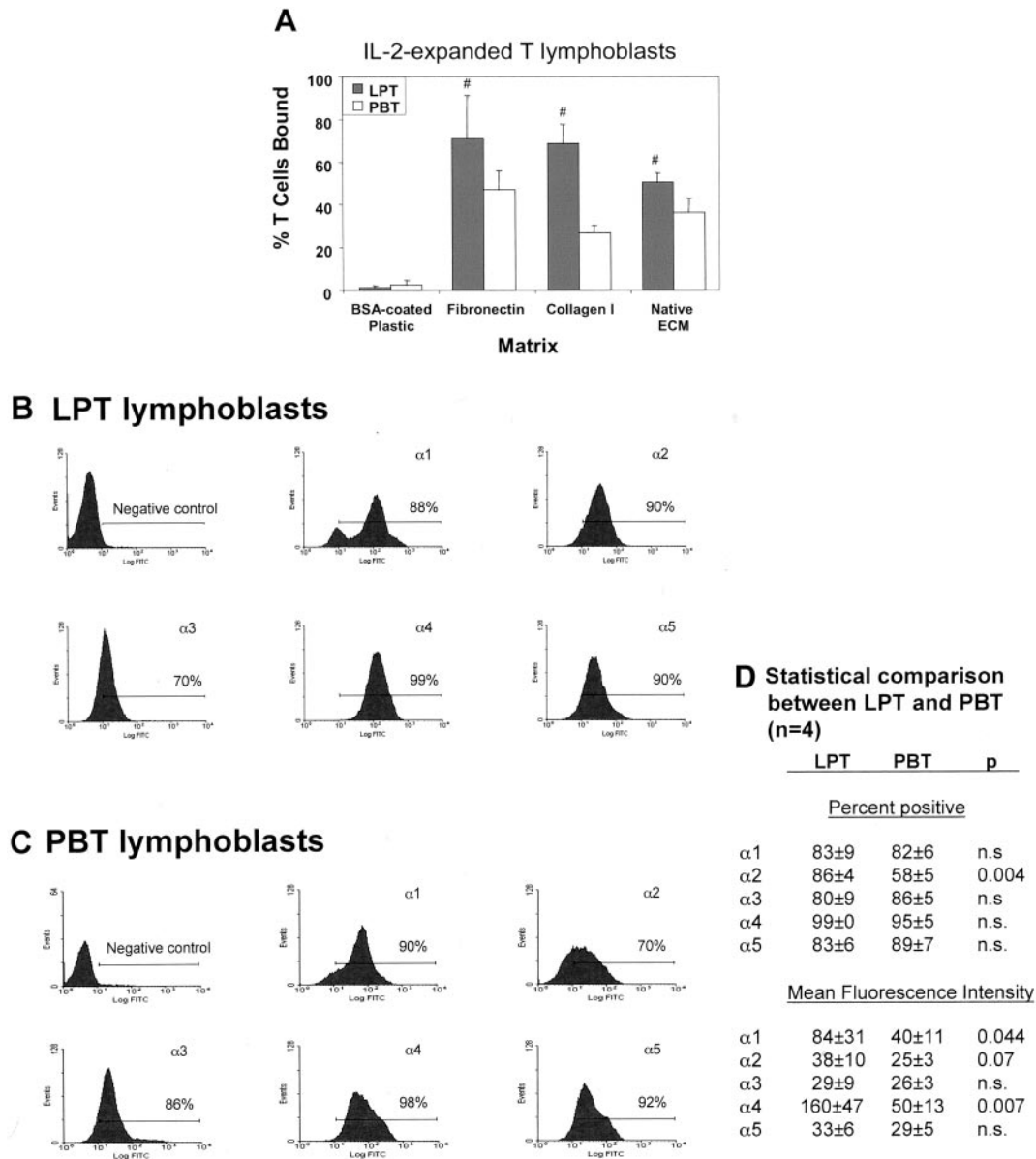


FIGURE 6. Tissue T cells retain their more adhesive phenotype after long term activation. T cells were stimulated with PHA and PMA and were expanded in culture with IL-2 for 2 wk. A, LPT (■) and PBT (□) were labeled with calcein, and 5×10^5 cells/well were allowed to adhere for 2 h to BSA-coated plastic (as a control), fibronectin, collagen type 1, or native ECM. Nonadherent cells were removed by washing. The fluorescence of adherent T cells was quantified in triplicate using a fluorescence spectrophotometer. Results for adhesion are the mean \pm SD percentage of input cells bound ($n = 3$). #, $p < 0.04$. Flow cytometric analysis was performed on LPT (B) and PBT (C) lymphoblasts. Negative control cells were gated to contain $<3\%$ positive cells. Representative histograms of four experiments are shown. Statistical analysis of all four donors (D) revealed that the percentage of cells that express the α_2 integrin and the average expression of α_1 and α_4 on a per cell basis are significantly less for PBT vs LPT.

fresh circulating PBT is the use of α_5 by LPT and α_4 for PBT adhesion to fibronectin. We therefore investigated whether increased adhesion of fresh PBT to fibronectin following preconditioning coordinated predominantly with α_4 usage (Table I). Fresh PBT were preconditioned in the presence of either IL-2 and fibronectin or anti-CD3-coated beads and fibronectin for 3 days, labeled with calcein, and adhered to fibronectin and collagen in the absence and the presence of blocking Abs to the α_1 to α_5 integrins, as described in Fig. 7. The addition of anti- α_4 functional blocking Ab inhibited preconditioned PBT binding to fibronectin by 83%. On the other hand, there was only a minimal α_5 contribution to fibronectin adhesion following preconditioning, as shown by a 15% inhibition in the presence of anti- α_5 functional blocking Ab (Table I). The pattern of preconditioned PBT binding to collagen

by α_1 through α_3 was unchanged from that of fresh PBT and fresh LPT (data not shown). The increase in adhesion to collagen after collagen preconditioning was particularly striking, with the T cells changing from no detectable adherence to $>60\%$ adherence. Adhesion to fibronectin, collagen type I, and native ECM by collagen-preconditioned PBT equaled or exceeded the strong adhesion noted for freshly isolated and blasted LPT, a level of adhesion we could not achieve for short term (12 h or 3 days) or long term (2 wk) activation in the absence of matrix. These results demonstrate that tissue T cells, which are in constant contact with the interstitial ECM, acquire a distinct adhesive preference and integrin usage pattern from and for their environment. On the other hand, circulating T cells rarely encounter collagen in blood and secondary lymph nodes, thus never acquiring the ability to strongly adhere.

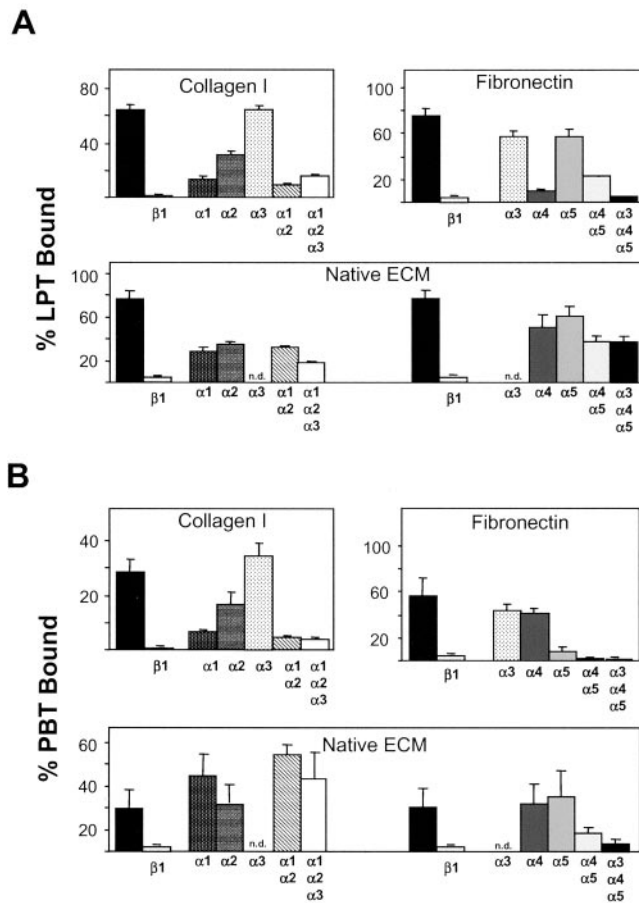


FIGURE 7. Tissue and blood T cells rely on distinct integrin subsets to mediate their interactions with native and purified matrix. Calcein-labeled LPT (A) and PBT (B) lymphoblasts were adhered to collagen type I, fibronectin, and native ECM in the absence or the presence of blocking mAbs specific for α_1 - through α_5 -chains of integrins, alone and in combination. The fluorescence of adherent cells was quantified in triplicate using a fluorescence spectrophotometer. Results are the mean \pm SD percentage of adherent cells for one representative experiment of three performed.

However, when these circulating cells do ultimately contact ECM ligands in the tissue, they are imprinted with a distinct adherent tissue phenotype.

Short term activation of PBT in the presence of collagen type I or fibronectin modulates the integrin expression profile, recapitulating the tissue-like T cell phenotype

Adhesion of PBT activated in the presence of components of the ECM, modeling a physiological process, recapitulates the binding of tissue T cells and suggests that the signature integrin profile of tissue T cells may be generated as well. Freshly isolated PBT were activated in the absence or the presence of IL-2, anti-CD3-coated beads, fibronectin, or collagen type I alone and in combination for 3 days. Expression levels of α_1 through α_5 were measured by flow cytometry (Fig. 9). Marked increases in the expression of α_1 were induced by fibronectin or collagen alone and were further enhanced by coactivation of PBT with IL-2 or anti-CD3 and matrix. α_3 expression was stimulated by collagen alone and more so in combination with IL-2 or anti-CD3. In particular, collagen preconditioning in the presence of IL-2 or CD3 induced α_1 and α_3 expression to levels approaching those of freshly isolated LPT, which paralleled the increase in adhesiveness of the same T cell popula-

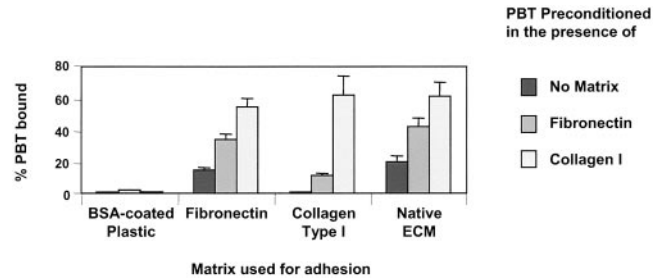


FIGURE 8. Activation of freshly isolated PBT in the presence of ECM preconditions T cells for adhesion. Freshly isolated PBT were cultured with IL-2 in the presence or the absence of purified fibronectin or collagen type I for 3 days. These PBT were then harvested, calcein labeled, and allowed to adhere for 2 h to BSA-coated plastic (as a control), fibronectin, collagen type I, and native ECM. The fluorescence of adherent T cells was quantified in triplicate. Results are the mean \pm SD percentage of input cells bound ($n = 3$) from one representative experiment of three donors.

tion (as shown in Fig. 8). In support of the role of matrix in educating PBT, we also found that incubation of fresh PBT with fibronectin alone and in combination with anti-CD3 activation, in particular, decreased α_5 expression from 49 to 18%, approaching the low α_5 expression observed in fresh LPT. Reduced expression of α_5 in preconditioned fresh LPT is fully consistent with the functional findings in Table I that demonstrate a shift in integrin usage from α_5 to α_4 . Surface expression of α_2 and α_4 was relatively low and high, respectively (<10% and ~75%, respectively), in a resting PBT. Neither surface expression nor MFI was changed after T cell activation in the presence of matrix protein (data not shown). Therefore, activation of PBT in the presence of newly encountered matrix rapidly transforms these circulating T cells into an adherent lymphocyte, and exposure to fibronectin stimulates a decrease in the expression of the α_5 integrin, resembling that of a tissue T cell freshly isolated from its interstitial microenvironment.

Discussion

The effectiveness of host defense is ultimately gauged by the ability of the host to eliminate infectious agents. Therefore, the final accountability for host survival is dependent on the successful migration and retention of Ag-educated T cells in compromised tissue, which is composed of a fine network of interstitial extracellular matrix. While it is known that T cells acquire receptors that

Table I. *The primary integrin mediating adhesion to fibronectin switches from α_5 dependence to the distinct character of a tissue T cell (α_4 predominance) in freshly isolated activated PBT preconditioned with fibronectin*

Fresh PBT: Preconditioned in the Presence of	% Inhibition of Adhesion in the Presence of Neutralizing Abs to	
	α_4	α_5
IL-2 ^a	0 ^b	84
IL-2 + fibronectin	83	15
Anti-CD3 coated beads	0	90
Anti-CD3 + fibronectin	69	0

^a Freshly isolated PBT were cultured with IL-2 or anti-CD3-coated beads in the presence or the absence of purified fibronectin for 3 days. These PBT were then harvested, calcein labeled, and allowed to adhere for 2 h to BSA-coated plastic as a control or fibronectin.

^b Inhibition of adherence in the absence or the presence of blocking mAb specific for α_4 - and α_5 -chains of integrins. Fluorescence of adherent cells was quantified in triplicate using a fluorescence spectrophotometer. Results are the mean percentage in the inhibition of adherence for one representative experiment of three performed.

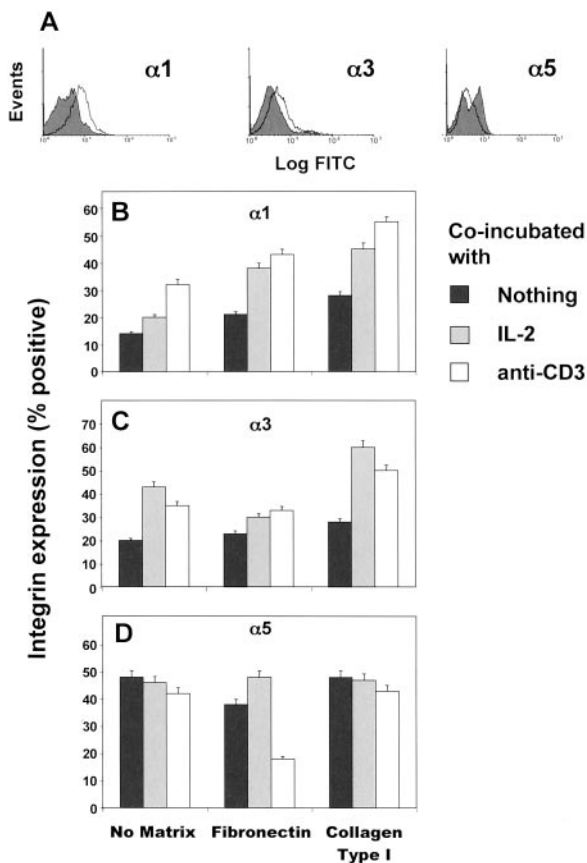


FIGURE 9. Preconditioning PBT in the presence of ECM rapidly imprints a distinct integrin surface expression, mirroring a tissue phenotype. Freshly isolated PBT were cultured in medium alone, IL-2, or suboptimal concentrations of anti-CD3 in the absence of matrix or the presence of purified fibronectin or collagen type I for 3 days. Flow cytometric analysis was performed on these preconditioned lymphoblasts for α_1 through α_5 surface expression. A, Representative histograms of four experiments for α_1 (■, medium alone; □, anti-CD3 plus collagen), α_3 (■, medium alone; □, anti-CD3 plus collagen), and α_5 (■, medium alone; □, anti-CD3 plus fibronectin) surface expression are shown. Negative control cells were gated to contain <3% positive cells, allowing for the determination of a quantitative modulation of α_1 (B), α_3 (C), and α_5 (D) surface expression.

facilitate their tissue-specific extravasation (38–41), the signals that orchestrate the selective retention of T cells in infected tissue have not been fully elucidated. Therefore, we define the mechanism by which the nonadherent circulating T cell rapidly differentiates *in vitro* into the highly adherent tissue T cell. Within 3 days of their initial exposure to collagen and fibronectin, fibrillar components of the ECM, and their re-exposure to Ag, circulating T cells not only express a similar pattern of cell surface integrins as that of tissue-derived T cells, but also exhibit adherence properties that closely resemble those of the tissue T cell. We propose that the interstitial ECM is capable of imprinting upon a newly recruited T cell a signature integrin phenotype that not only assures the long term residence of that T cell in the tissue, but also modulates the longevity and hence the efficacy of an immune response.

The regulation of integrin expression and function is frequently studied on PBL and is commonly accepted to be representative of all T cell subsets (11, 17, 23). However, the T cell area of lymph nodes is a cellular environment with minimal accessible collagen due to stromal cell processes that sheath the abundant collagen fibers forming the reticular fiber network (27, 28, 42). In contrast, once an activated T cell enters tissue, it is exposed to Ag again and

to interstitial ECM (43, 44). We demonstrate that tissue T cells, due to residency in an ECM microenvironment, possess differences in their ability to adhere to various ECMs and in their integrin expression profile compared with their circulating counterparts. Our finding that short term activation (12 h) of LPT does not increase their intrinsic high adhesiveness implies that tissue T cells express integrins in an activated or high ligand binding state. This property of tissue T cells is in stark contrast to what we and others show for PBT, whose integrins are expressed in a closed or inactive conformation and upon short term stimulation rapidly acquire modest adherence to both fibronectin and native ECM, but not collagen (17). In addition, although surface expression of a subset of integrins (α_1 , α_2 , α_3) is slowly increased in long term activated peripheral blood T cells, leading to their label as VLA (11, 34), VLA integrin expression is universally increased in long term cultured PBT and bears little resemblance to the profile of β_1 integrins on tissue T cells extracted from their residence in the ECM. Furthermore, the heightened affinity of integrins expressed by tissue T cells to components of the ECM is unattainable by these long term activated circulating blood T cell. In addition, the extended length of *in vitro* culture required for the appearance of these VLA integrins (i.e., 14 days) is inconsistent with the 3–5 days observed for the redistribution and accumulation of activated T cells into tissues in mouse models (41).

While tissue T cells derived from the intestinal mucosa differentially express the β_7 family of integrins, adhesion to the ECM is mediated predominantly by β_1 integrins, as evidenced by the blocking of T cell adhesion by neutralizing Abs directed against β_1 (Fig. 7). It was previously reported that the state of T cell differentiation, memory vs naive, modulates the expression and functional status of integrins (23). Memory T cells express 3- to 4-fold more α_4 and α_5 than do naive cells and bind more efficiently to fibronectin (17). However, CD45RO⁺ tissue LPT express 7- to 8-fold more α_1 , 20-fold more α_3 , and 7-fold less α_5 than do CD45RO⁺ PBT (data not shown). In addition, a larger percentage of CD45RO⁺ LPT adhere to both fibronectin and collagen than what was reported for CD45RO⁺ PBT (17). These results demonstrate that the tissue origin of T cells is the over-riding determinant of the adhesive phenotype, more so than the state of T cell activation and differentiation.

Tissue LPT express a significantly different profile of β_1 integrins compared with that on short term activated and long term cultured PBT. An increased percentage of tissue T cells bear integrins mediating adhesion to collagen (α_1 and α_3), explaining their greater adhesion to collagen. Thus, these findings support our premise that exposure to ECM contributes to the distinct tissue T cell integrin adhesive phenotype. Our hypothesis is further supported *in vivo*, in that the number of resident lymphocytes in the intestine is reduced by 50% in the VLA1-deficient mouse (45), suggesting that the $\alpha_1\beta_1$ integrin is required to support the retention of T cells in the interstitial matrix in the gut. In addition, freshly isolated tissue LPT express α_4 in an active conformation, mediating their adherence to fibronectin despite their lack of surface expression of α_5 . It was reported that migration of activated PBT on fibronectin is mediated by $\alpha_5\beta_1$, and that suppressing $\alpha_4\beta_1$ activity enhanced the level of migration (26). We propose that the high expression of α_4 and loss of α_5 on resident tissue T cells decrease their mobility and thus increase their long-term retention.

When PBT are activated for only 3 days in the presence of collagen or fibronectin, their ability to adhere to different matrixes, their surface integrin expression, and their integrin usage pattern recapitulate those of the tissue T cell, respectively. Activation of PBT in the presence of fibronectin preconditions these cells for enhanced binding to both fibronectin and native ECM, but not

collagen, and induces the distinctive decrease in expression of α_5 , with a concomitant increase in α_4 -mediated adhesion, a hallmark of the tissue-derived T cell. Therefore, we conclude that a fibronectin-rich microenvironment for PBT modifies integrin surface expression and affinity of those integrins that mediate adhesion to fibronectin and promotes a tissue T cell phenotype. In adherent cells, integrin binding can result in the generation of outside-in signals that modulate the function and expression of integrins (26, 36, 37). Preconditioning PBT in the presence of collagen stimulates the expression of α_1 and α_3 to levels equivalent to those observed on tissue T cells and induces optimal adhesion to fibronectin, collagen, and native ECM compared with cells activated in the presence of fibronectin. We propose that as the newly extravasated PBT enters the tissue, its expression of α_5 permits its migration deep into the matrix. Subsequently, exposure to interstitial collagen stimulates increased expression of α_1 and α_3 coupled with an increase in cell adhesion. Once completely embedded, fibronectin stimulates the down-regulation of α_5 , transforming the migratory, newly recruited T cell to a static, defensive tissue T cell, and collagen crafts a highly adherent cell. Therefore, tissue cells are imprinted with the capacity for adherence to matrix, which may be one mechanism by which these cells establish and maintain residency within their tissue microenvironment.

Our results also underscore the need to fully assess the impact of the native matrix on T cells, since both collagen and fibronectin are needed to imprint a tissue adhesive and an integrin phenotype, respectively, on an activated PBT. Furthermore, when PBT lymphoblasts are presented with native matrix containing both collagen and fibronectin, they exclusively adhere to fibronectin despite expressing all VLA molecules. Similarly, when LPT encounter native matrix, they preferentially bind to collagen despite exhibiting complete binding to purified fibronectin. These results, which truly reflect the biologically relevant T cell adhesive response, highlight the critical differences between adhesion complexes formed with two-dimensional substrates and the narrowed integrin usage we observe with a cell-derived three-dimensional matrix (46). While a full chemical, structural, and functional evaluation of the native ECM is beyond the scope of this report, our results highlight the importance of considering both the topology and the composition of the ECM as an immunoregulatory mediator.

T cells isolated from their residency in tissue are routinely identified as effector/memory cells. Yet, one important characteristic of the tissue T cell has often been overlooked. Tissue T cells are intimately associated with the ECM during Ag exposure, cytokine stimulation, and cell migration. This study extends the function for the ECM beyond its classic structural role in tissue architecture to imprint upon the extravasating blood T cell its adaptation to its new home within the tissue interstitium. First exposure to ECM rapidly alters the integrin phenotype of the newly arrived cell, optimizing its ability to assume residence within its new microenvironment. It therefore seems likely that the ECM may also regulate other T cell responses within tissue, thereby assuming an active role in modulating T cell morphology, polarization, proliferation, and effector function.

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