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*J Immunol* 1999; 162:7120-7127; ;
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Regulation of Integrin-Mediated T Cell Adhesion by the Transmembrane Protein Tyrosine Phosphatase CD45

Hemanth Shenoi,*† John Seavitt, †‡ Alexander Zheleznyak,* Matthew L. Thomas, ‡ and Eric J. Brown‡*‡

The transmembrane protein tyrosine phosphatase CD45 is required for Ag receptor signal transduction in lymphocytes. Recently, a role for CD45 in the regulation of macrophage adhesion has been demonstrated as well. To investigate further the role of CD45 in the regulation of adhesion, we examined integrin-mediated adhesion to fibronectin of two T cell lines and their CD45-deficient variants. The absence of CD45 correlated with enhanced adhesion to fibronectin via integrin αβ1 (VLA-5), but not αβ1 (VLA-4) in both cell lines. Adhesion returned to normal levels upon transfection of wild-type CD45 into the CD45-deficient lines. Transfection of chimeric or mutant molecules expressing some, but not all, CD45 domains and activities demonstrated that both the transmembrane domain and the tyrosine phosphatase activity of CD45 were required for regulation of integrin-dependent adhesion, but the highly glycosylated extracellular domain was dispensable. In contrast, only a catalytically active CD45 cytoplasmic domain was required for TCR signaling. Transfectants that restored normal levels of adhesion to fibronectin communoprecipitated with the transmembrane protein known as CD45-associated protein. These studies demonstrate a novel role for CD45 in adhesion regulation and suggest a possible function for its association with CD45-associated protein. The Journal of Immunology, 1999, 162: 7120–7127.

A

adhesion of leukocytes to the extracellular matrix or to other cells is mediated by members of the integrin receptor family. Organized around common β subunits, the integrin family of noncovalently associated heterodimers is found on most metazoan cells (1–3). Lymphocytes express two types of integrin receptors for the extracellular matrix protein fibronectin (FN), 1 VLA-4 (α4β1), and VLA-5 (α5β1), which recognize distinct domains of the matrix glycoprotein. α4β1 binds the CS1 region within the 12th type III repeat of FN, in which the peptide sequence EILDV is critical (4–6); α5β1 recognizes the cell binding domain (CBD) of FN in which the RGD tripeptide within the 10th type III repeat and additional sequences within the 9th type III repeat form the minimal binding site (7, 8). Ligation of FN receptors on lymphocytes contributes to events critical for the immune response, including adhesion and migration, enhancement of Ag receptor signal transduction, induction of tyrosine phosphorylation, and activation of gene transcription (2, 9–13).

Although resting T cells express both α5β1 and αβ1 FN receptors, these cells bind poorly to FN-coated surfaces. Upon cell activation by chemokines, Ag recognition, CD2 or CD28 ligation, or other stimuli, integrin receptors become competent to mediate cell adhesion without a change in receptor expression at the plasma membrane (14–18). The exact nature of this change in adhesiveness is not known, but may be a result of conformational change in the receptors themselves (19, 20), alterations in receptor diffusion rates (21), changes in receptor clustering (22), cytoskeletal organization, or integrin-cytoskeleton interaction (23, 24).

Recently, there has been increasing interest in the potential role that tyrosine phosphorylation plays in regulating cell adhesion (25–28). Integrin-dependent tyrosine phosphorylation regulates signal transduction and cytoskeletal assembly at focal adhesion sites, and tyrosine-phosphorylated proteins accumulate at integrin-mediated adhesions (29–33). The loss of Src family kinases affects both adhesion kinetics and integrin-mediated signaling, while the absence of the focal adhesion kinase pp125FAK, which is regulated by integrin ligation, affects cell motility (29, 34). Thus, it is clear that protein tyrosine phosphorylation can regulate cell adhesion and vise versa. This suggests that tyrosine phosphatases as well as kinases may affect adhesion.

CD45 is a leukocyte-specific transmembrane tyrosine phosphatase that has a large, highly sialylated extracellular domain. Because of its abundance in lymphocytes, the CD45 extracellular domain contributes significantly to the negatively charged glycosylycalyx (35). In lymphocytes, CD45 associates with a second transmembrane protein called leukocyte phosphatase-associated protein or CD45-associated protein (CD45AP) that has no known function (36). Analysis of CD45-deficient cell lines and mice has shown that CD45 is required for efficient Ag receptor signal transduction in lymphocytes (37, 38). In TCR-mediated signaling, CD45 dephosphorylates regulatory tyrosine residues in Src family kinases, allowing activation of the kinases by Ag receptor ligation (39, 40). This initiates downstream signaling events that culminate in gene activation and proliferation. The function of CD45 in the control of other leukocyte functions is less clear. Recently, Roach and colleagues showed that CD45-deficient murine macrophages were hyperadhesive via integrin contacts, but could not sustain adhesion as well as normal macrophages (29). Other studies, using anti-CD45

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Received for publication June 29, 1998. Accepted for publication April 5, 1999.

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‡ Abbreviations used in this paper: FN, fibronectin; CBD, cell binding domain; CD45AP, CD45-associated protein; DCS, double Cys→Ser mutant CD45
mAbs, has suggested that CD45 also has a role in regulation of homotypic aggregation of lymphocytes (41–44). The ability of transmembrane protein tyrosine phosphatases, including CD45, to regulate adhesion in some cell types prompted our investigation of the role of CD45 in the regulation of lymphocyte adhesion. Our studies reveal that CD45 can regulate integrin-mediated adhesion in human and murine T lymphocyte cell lines. The absence of CD45 correlates with increased adhesion via α4β1, but not α5β1. Through the expression of CD45 mutations and chimeric proteins, we found that both the transmembrane domain and tyrosine phosphatase activity of CD45 are required for regulation of integrin-dependent adhesion. Surprisingly, the highly negatively charged extracellular domain is dispensable for regulation of adhesion. Instead, regulation of adhesion correlates with the ability of CD45 and chimeric molecules to associate with CD45AP at the plasma membrane. This suggests that one function of CD45 AP may be to synergize with CD45 for regulation of lymphocyte adhesion.

Materials and Methods

Cells

The T human cell line Jurkat E-6-1 was a gift from Dr. Andrew Chan (Washington University School of Medicine, St. Louis, MO). The CD45-deficient variant of this clone, J45.01, and the J45.01 transfectants J45.LB3, expressing normal human CD45, and J45.CH11, expressing a chimeric molecule with the HLA-A2 extracellular and transmembrane domains and the CD45 intracytoplasmic domains, were gifts from Dr. Gary Koretzky (University of Iowa School of Medicine, Iowa City, IA). Jurkat cells, mutants, and transfectants were maintained in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 0.1 mM nonessential amino acids, 2-ME, gentamicin, l-glutamine, and 10% FCS (HyClone, Logan, UT). Transfected cell lines were supernatants and polyclonal rabbit anti-murine CD45AP was purchased from PharMingen (San Diego, CA). The HLA-reactive mAb W6/32 (50) was purchased from American Type Culture Collection (Manassas, VA). Anti-human CD45 mAb 12.3 (anti-mouse CD45 12.3/2/3) were used as supernatants, and polyclonal rabbit anti-murine CD45AP was previously described (45). The anti-human IFNy-α-chain mAb GIR 208, purified and conjugated to biotin, was a gift from Robert Schreiber (Washington University). HRP-conjugated goat anti-rabbit Ig was purchased from Organon Teknika-Cappel (Durham, NC). All other reagents were obtained from Sigma (St. Louis, MO) unless otherwise noted.

Cell adhesion

FN was prepared from fresh human blood as previously described (51). FN fragments recognized by α5β1 and α6β4 integrins were prepared and purified by chromatography on digel and heparin affinity purification as previously described (51). CBD is the 110-kDa chyotryptic FN fragment containing the binding site for α5β1 that does not bind gelatin or heparin (52). 33/66, an alternatively spliced carboxyl-terminal heparin-binding FN fragment containing the CS1 domain recognized by α5β1, was prepared as previously described (45). The purity of FN fragments was verified by SDS-PAGE, and isolated binding domains were transferred to a nitrocellulose membrane. FN fragments or whole FN were coated onto plates (Immuno I, Dynatech, Chantilly, VA) at 10 μg/ml in PBS overnight at 4°C. Plates were subsequently blocked with 2% human serum albumin or blocker casein (Pierce, Rockford, IL) for 1 h at room temperature. Plates were washed three times in PBS and twiced in adhesion buffer before use. All adhesion experiments were performed at 37°C in adhesion buffer (HBSS containing 1 mM Ca2+ and 1 mM Mg2+, and 1% human albumin). Cells (5 × 104) were added after centrifugation for 8 min at room temperature in a swinging bucket rotor. The fluorescence of each well was determined before and after centrifugation on a fluorescent plate reader (Molecular Probes, Eugene, OR) for 15 min at 37°C. Cells were washed twice with HBSS (Life Technologies) and resuspended at 5 × 105 cells/ml in adhesion buffer (HBSS containing 1 mM Ca2+, 1 mM Mg2+, and 1% human serum albumin). Cells (5 × 104/well) were added in a final volume of 200 μl and were gently spun into contact with substrate-coated plates at 100 rpm for 10 min. Plates were then transferred to a 37°C water bath and, after incubation, were gently washed to separate adherent cells from the substrate. Cell adhesion was quantified by a modification of the procedure of McClay et al. (53). Briefly, cells were labeled with 1 μM calcein/AM (Molecular Probes, Eugene, OR) for 15 min at 37°C. Cells were washed with acetylase plate sealers (Dynatech) and centrifuged as an inverted plate at the indicated centrifugal force for 8 min at room temperature in a swinging bucket rotor. The fluorescence of each well was determined before and after centrifugation on a fluorescent plate reader (Molecular Dynamics, Sunnyvale, CA) using 485- and 530-nm filters for excitation and emission, respectively. The fraction of adherent cells was calculated by dividing postwash prewash fluorescence. All experiments were performed at least in triplicate. Preliminary experiments comparing calcein fluorescence with cell number confirmed a linear correlation, and experiments directly comparing calcein fluorescence of adherent cells with visual inspection of stained cells also showed a linear correlation of calcein fluorescence to the number of adherent cells. In some experiments cytochalasin D was added to concentrations between 0.5–5 μg/ml to cells and was incubated for 10 min at 37°C before cell addition to adhere substrates. Cytochalasin D remained in the wells for the 30-min duration of the assay.

Analysis of immunoprecipitations

Cells (5 × 106) were pelleted from suspension culture, washed twice in cold PBS, and lysed in 1 ml of ice-cold modified RIPA (1% Nonidet P-40, 0.5% deoxycholate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 1 mM PMSF, 1 mM EDTA, and 250 μg/ml pervanadate). Cells were lysed for 30 min at 4°C, and cell debris was pelleted at 10,000 × g for 10 min at 4°C. Lysates were transferred to a tube containing 20 μl of washed Gammabind Plus protein G-Sepharose (Pharmacia, Uppsala, Sweden) and 4 μl of rabbit polyclonal anti-mouse CD45AP. Lysates were immunoprecipitated for 2 h, washed three times with modified RIPA, solubilized in reducing SDS-sample buffer, and subjected to electrophoresis on an 8% SDS-polyacrylamide gel. Samples were transferred to polyvinylidene difluoride membrane, and transferred proteins were detected with the CD45AP antiserum using protein G-Sepharose-HRP (Bio-Rad, Hercules, CA) and enhanced chemiluminescence (Amer sham, Arlington Heights, IL) for development using standard protocols.

Analysis of coinmunoprecipitations

Cells (10–50 × 106) were washed, lysed, and clarified as described above in 1 ml of lysis buffer (0.9% Brij with 0.1% sodium deoxycholic acid and 0.1% Triton X-100 or 0.15% Nonidet P-40, 50 mM Tris-HCl (pH 8.0), 0.2
mg/ml aprotinin, 10 μg/ml trypsin inhibitor, 2 mM leupeptin, 5 mM iodoacetamide, 1 mM PMSF, 1 mM pepstatin A, and 5 mM EDTA). Twenty-five microliters of a 50% slurry of 132/32-conjugated Sepharose or 5 μl of biotin-conjugated GIR-208 was added and rotated at 4°C for 2 h. Twenty-five microliters of a 50% slurry of streptavidin-agarose (Pierce) was added, and tubes were rotated at 4°C overnight. Beads were pelleted by centrifugation, washed three times in lysis buffer, and boiled in SDS-PAGE sample buffer, followed by resolution on SDS-PAGE and transfer to Trans-Blot (Bio-Rad). Filters were hybridized with anti-CD45 and anti-CD45AP and were developed with peroxidase-conjugated goat anti-rabbit antiserum as described above.

### Results

α₃β₁-mediated adhesion is increased in CD45-deficient cells

To examine the potential role of CD45 in the regulation of lymphocyte integrin-mediated adhesion, CD45-expressing Jurkat and the CD45-deficient derivative J45.01 were assessed for their ability to adhere to FN. To evaluate the individual contributions of α₃β₁ and α₅β₁, the two integrin FN receptors expressed by Jurkat, we tested adhesion to FN fragments containing the individual binding domains, CBD and CS1, separately. To compare the adhesion more precisely, populations of Jurkat and J45.01 expressing equivalent amounts of α₃β₁ and α₅β₁ were derived by FACS sorting (Table I). CD45-deficient J45.01 showed enhanced adhesion via α₃β₁ to the FN CBD compared with the CD45-expressing Jurkat cells over the entire range of ligand concentrations tested (Fig. 1A). The difference in adhesion to the FN CBD between the two cell lines was consistent over a large range of centrifugal forces as well (Fig. 1B). Cytochalasin D inhibited adhesion to both FN fragments, and the dose-response curve for inhibition did not differ between Jurkat and J45.01 cells (data not shown). Further, the adhesion difference was observed by 10 min and was not transient, since it persisted for at least 2 h (data not shown). In contrast, α₅β₁-mediated adhesion to the CS1 region of FN was similar between CD45-expressing and CD45-deficient Jurkat cell lines at all time points (Fig. 1C shows the 30 min point). In additional experiments 30-min incubation was used routinely because it represents a time when adhesion has achieved a steady state in this assay.

To determine whether the apparent CD45 effect on adhesion was limited to Jurkat, binding to FN of CD45-expressing (L3) and -deficient variants (L3M) of a murine T cell hybridoma (39) was examined. As was the case for the Jurkat lines tested, α₃β₁ and α₅β₁ expressions were equivalent on the CD45-expressing and -deficient cells (Table I). For this lymphocyte cell line as well CD45 deficiency enhanced α₅β₁-mediated adhesion (Fig. 1D). However, α₃β₁-mediated adhesion was not affected (Fig. 1E).

To determine whether Jurkat and J45.01 used identical receptors for adhesion to the FN fragments, the effects of various mAbs on adhesion were assessed. An anti-α₅β₁ mAb blocked binding of both cells to FN CBD, while anti-α₅β₁ had no effect on binding to this fragment (Fig. 2A), demonstrating that the enhanced adhesion in the absence of CD45 was not a result of recruitment of additional FN-binding integrins to CBD. Anti-α₃β₁ blocked binding of Jurkat and J45.01 to FN CS1 equivalently, and the addition of anti-α₅β₁ did not increase the inhibition (Fig. 2B). Abs directed against human HLA had no effect on adhesion.

### Phosphatase activity of CD45 is not sufficient to restore normal adhesion

To determine whether normal levels of adhesion could be restored by re-expressing CD45, we examined J45.01 cells, which had been transfected with the 180-kDa isoform of human CD45 (J45.LB3) (54). α₃β₁-mediated adhesion to CBD was reduced to wild-type levels in J45.LB3 (Fig. 3). However, reconstitution of J45.01 with a chimeric cDNA (55) containing the extracellular and transmembrane regions of HLA-A2 and with the CD45 cytoplasmic domain, which contains the protein tyrosine phosphatase activity of the molecule (J45.CH11), did not affect adhesion (Fig. 3). α₅β₁ expression was slightly higher in the J45.LB3 than in the more avidly adherent J45.CH11. This result contrasts with the equivalence of the two reconstituted cell lines in restoration of TCR-mediated signal transduction (54, 56) (data not shown). These data suggest that CD45 regulation of adhesion requires the CD45 extracellular and/or transmembrane domains and that this function of CD45 is distinct from its role in TCR signaling.

### CD45 extracellular and transmembrane domains in regulation of adhesion

To investigate further the potential dichotomy between CD45 control of TCR signal transduction and adhesion, a series of chimeric cDNA was transfected into both CD45-deficient J45.01 (human) and L3M (murine) T cell lines. In addition to the normal murine low m.w. isoform of CD45 (CD450), transfectants were made that expressed CD45 with two point mutations at aa 816 and 1132 (double Cys→Ser mutant CD45 (DCS); Fig. 4A), thus abolishing tyrosine phosphatase activity (29). Chimeric proteins also were expressed in which the CD45 extracellular domain was replaced with the IFN-γR α-chain extracellular domain (γ/45/45) and in which in addition to this substitution the CD45 transmembrane domain was replaced by the CD44 transmembrane domain (γ/44/45; Fig. 4A). Several stable populations with equivalent α₅β₁ expressions were derived for each transfected DNA, with similar results for all lines.

α₃β₁-mediated adhesion of all transfectants in both J45.01 and L3M was assessed (Fig. 5). For both the murine and human cell lines, wild-type murine CD45 reconstituted regulation of adhesion, as had wild-type human CD45 in J45.01. The chimera in which the CD45 extracellular domain was deleted (γ/45/45; Fig. 4A) was equal to wild-type CD45 in its ability to restore regulation in both cell lines (Fig. 5). Thus, the extracellular domain of CD45 is not required for regulation of integrin-mediated adhesion. In contrast, the chimeras in which the CD44 transmembrane domain was substituted for CD45 (γ/44/45; Fig. 4A) failed to regulate adhesion in either cell type (Fig. 5). This suggests that unlike the extracellular domain, the transmembrane domain is required for CD45’s ability to regulate adhesion. Differences in the abilities of the various transfectants to regulate adhesion were not due to different expression levels, since the two different chimeras with IFN-γR extracellular domains expressed equivalent levels of protein in both cell types (Fig. 4B and data not shown). In contrast to adhesion, the
chimeras expressing the CD44 and CD45 transmembrane domains reconstituted TCR signaling to the same degree, as assessed by an increase in cytoplasmic Ca\(^{2+}\) after TCR ligation with C305, an mAb that recognizes the clonotypic TCR of Jurkat (Fig. 6A). Thus, the structural requirements for CD45 in regulation of adhesion and Ag receptor signaling are distinct.

FIGURE 1. Adhesion of human and murine lymphocytes to FN fragments. Jurkat and the CD45-deficient derivative J45.01 were assessed for the ability to attach to FN CBD via α5β1 (A) or FN CS1 via α4β1 (C). B, Dose response of force (RCF) required to detach Jurkat and J45.01 adherent for 30 min from FN CBD. D, CD45-expressing L3 and CD45-deficient L3M were assessed for the ability to attach to Fn CBD. E, Binding of L3 and L3M to CS1. Data are expressed as the percentage of cells remaining after washing. Shown are the mean and SEM of at least three separate experiments, each performed in quadruplicate, comparing the CD45-sufficient and -deficient cell lines. In A, C, D, and E, cells were allowed to adhere for 30 min, and washing was performed at 250 \(\times\) g as described in Materials and Methods.
CD45 phosphatase activity is required for regulation of adhesion

The contrast between the requirement for the CD45 transmembrane domain in adhesion and TCR signaling raised the possibility that CD45 phosphatase activity was not necessary for its regulation of adhesion. To determine whether this was the case, CD45-deficient murine and human T cell lines stably expressing a murine cDNA encoding full-length but enzymatically inactive CD45 were derived (DCS; Fig. 4A). As expected, TCR-mediated signaling was not restored in these cell lines (Fig. 6B). While expression of normal murine CD45 in CD45-deficient cell lines fully reconstituted adhesion to wild-type levels, the DCS CD45 mutant did not restore normal regulation of adhesion in either cell line (Table II). FACS analysis showed that human and murine cell lines expressed nearly equivalent amounts of wild-type and DCS CD45 (Fig. 4C and data not shown), demonstrating that differences in adhesion did not result from differences in the level of protein expression. Thus,
CD45 phosphatase activity is required for its regulation of $\alpha_5\beta_1$-mediated adhesion.

Association of CD45AP correlates with regulation of adhesion

Previous studies have shown that lymphocytes express a transmembrane protein that coimmunoprecipitates with CD45, known as CD45AP (36). The CD45 transmembrane and/or extracellular domains have been shown to be necessary for this association (36), and the transmembrane domain has been shown to be sufficient in transient transfection systems (48). To determine whether CD45AP could be involved in CD45 regulation of adhesion, expression of CD45AP was examined in CD45-deficient cells and transfectants. As previously reported, the absence of CD45 inhibited expression of CD45AP (36). When the association of CD45AP and CD45 was analyzed by coimmunoprecipitation, wild-type CD45 (not shown) and the $\gamma_\delta/45/45$ chimera expressing the CD45 transmembrane domain coimmunoprecipitated with CD45AP, while the chimera expressing the CD44 transmembrane domain did not (Fig. 7). Thus, there is a correlation between the ability of the transfected molecule to restore normal regulation of adhesion and its ability to associate with CD45AP.

Discussion

It has become increasingly clear that signal transduction and adhesion are closely related phenomena in many cells. Integrin-mediated adhesion is required for a proliferative response to growth

Table II. Requirement for tyrosine phosphatase activity of CD45 in regulation of adhesion

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Variant</th>
<th>% of Maximum Adhesion (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3</td>
<td>L3P</td>
<td>21.2 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>L3M.WT</td>
<td>27.4 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>L3M.DCS</td>
<td>57.3 ± 4.9</td>
</tr>
<tr>
<td>Jurkat</td>
<td>Jurkat</td>
<td>55.9 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>J45.WT</td>
<td>59.2 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>J45.DCS</td>
<td>85.7 ± 4.0</td>
</tr>
</tbody>
</table>

*Data are normalized to adhesion of given species’ CD45-deficient cell lines, J45.01, or L3M, defined as 100% adhesion.

*The difference between wild-type (WT) and DCS reconstitution, within species, was significant ($p < 0.05$) using Bonferroni’s multiple comparison test.

CD45AP was analyzed by coimmunoprecipitation, wild-type CD45 (not shown) and the $\gamma_4/45$ chimera expressing the CD45 transmembrane domain coimmunoprecipitated with CD45AP, while the chimera expressing the CD44 transmembrane domain did not (Fig. 7). Thus, there is a correlation between the ability of the transfected molecule to restore normal regulation of adhesion and its ability to associate with CD45AP.

FIGURE 5. Adhesion of murine and human lymphocyte cell lines expressing chimeric CD45 to ligands. L3M (A) and J45.01 (B) were transfected with cDNA encoding the chimeric cDNAs illustrated in Fig. 4. Cells were assessed for the ability to attach to the FN CBD or the nonspecific ligand human serum albumin (HSA). Shown are the mean and SEM of three independent experiments, each performed in quadruplicate. The CD45 transmembrane domain was required for chimeric molecule inhibition of adhesion in both L3M and J45.01 ($p < 0.05$ for both cell types).

FIGURE 6. Ag receptor signal transduction in CD45-deficient cell lines. J45.01 transfected with $\gamma_4/45$, $\gamma_4/44/45$, wild-type human CD45 (A), wild-type murine CD45 (B), or the DCS mutant that abolishes tyrosine phosphatase activity were assessed for Ag receptor signal transduction. TCR was cross-linked with a clonotypic anti-TCR mAb (C305), and intracytoplasmic Ca$^{2+}$ mobilization was measured as described in Materials and Methods. Shown are representative tracings of three tracings performed for each transfected cell line.

FIGURE 7. Association of CD45AP requires CD45 transmembrane domain. The $\gamma_4/45$ and $\gamma_4/44/45$ chimeras stably transfected into L3M were immunoprecipitated with anti-human IFN-$\gamma$R $\alpha$-chain (49). Western blots of immunoprecipitates resolved by 10/15% discontinuous SDS-PAGE were separated at the discontinuity; the top was developed with anti-mouse CD45 cytoplasmic domain, and the bottom was developed with anti-CD45AP.
factors in diverse cell types. In the absence of adhesion, these growth factors induce an abortive signal. The adhesion-dependent signal depends on the requirement for integrin-mediated adhesion in the activation of tyrosine kinase cascades. Although integrins themselves have no kinase activity, their cytoplasmic tails associate with several tyrosine kinases, directly or indirectly (32, 57–60). Moreover, tyrosine-phosphorylated proteins accumulate at integrin-dependent focal contact sites, where actin cytoskeleton meets the plasma membrane. At the same time, tyrosine kinase cascades affect adhesion. For example, pp60^Sce deficiency slows focal contact formation (61), and pp125FAK deficiency impairs cell motility, probably by inhibiting turnover of focal adhesions required for movement (34). The small GTPases Rac, Rho, andcdc42 all affect the interaction of cytoskeleton with integrins and subsequent downstream integrin-dependent signaling (62–65).

Our work demonstrates distinct adhesion abnormalities in both mouse and human CD45-deficient lymphocytes in α3β1-mediated adhesion to FN. In both cell lines, CD45 limited adhesion. α3β1, the other lymphocyte FN receptor, is apparently not regulated by CD45 in these cell lines. The reason for the difference between the regulation of α3β1 and α3β1 is uncertain. There are multiple differences in the functions of these two integrins, including their association with cytoskeleton, promotion of migration, and recruitment to focal contacts (66–68). Thus, it is likely that different mechanisms exist for regulation of the functions of these two integrins.

CD45 is a large, highly glycosylated molecule that is the most abundant protein on the lymphocyte plasma membrane. Previous work has suggested that cross-linking CD45 can induce homotypic lymphocyte adhesion (41, 42, 44). This has led to speculation that its role in adhesion might simply be steric, because of its extension from the membrane and its large negative charge that might repel other surfaces (35, 69). It is important to note that our data rule out this hypothesis as the primary function of CD45 in regulation of adhesion. CD45 can be expressed at much lower than wild-type levels, and its extracellular domain can be replaced entirely without disturbing its regulation of adhesion.

Our data clearly distinguish the minimal domain required for CD45 regulation of Ag receptor signaling from its role in adhesion. Normal Ag receptor signaling can be restored by a membrane-anchored CD45 phosphatase domain alone (70). However, adhesion requires both the phosphatase domain and the transmembrane domain of CD45. While some property of the CD44 transmembrane and cytoplasmic domains can target molecules away from microvilli (71), this is unlikely to be the basis of the difference between the CD44 and CD45 transmembrane domains in the regulation of adhesion, as a chimeric molecule containing the HLA-A2 transmembrane domain also fails to restore normal regulation of adhesion (Fig. 3). Instead, the CD45 transmembrane domain plays an active role in regulation of adhesion. This may be because of its association with CD45AP, a transmembrane protein with no previously described function. Restoration of normal CD45 regulation of adhesion correlated with CD45 coimmunoprecipitation with CD45AP, already known to be a property of the CD45 transmembrane domain. The cytoplasmic domain of CD45AP contains a WW motif, which has been implicated in protein-protein association in cytoskeletal proteins (72). It is interesting to speculate that CD45 association with cytoskeleton may be indirect, mediated via its interaction with CD45AP.

CD45 phosphatase activity also is required for regulation of adhesion. Previous work from our laboratories has shown that the activities of the Src family kinases Lyn and Hck are deregulated in CD45-deficient macrophages, and studies from several laboratories have implicated CD45 in the regulation of the Src family kinases Lck and Fyn in lymphocytes (29, 73). However, we have not found CD45-dependent differences in Lck or Fyn activity in adherent Jurkat or L3 cells (data not shown). In addition, no CD45-dependent differences in ZAP-70 activity or ERK1/2 activation were found during adhesion of these cell lines (data not shown). Instead, our data suggest the hypothesis that CD45 regulates adhesion through appropriate localization of its tyrosine phosphatase activity. Previous studies have shown that CD45 is present at integrin-dependent adhesion sites (29). It is likely that CD45 association with the cytoskeleton, perhaps through CD45AP, is critical in localization of its phosphatase activity to adhesion sites, where it dephosphorylates substrates important in the maintenance of adhesion. This new paradigm extends the role for CD45 in lymphocyte biology and may be relevant to the mechanism by which transmembrane tyrosine phosphatases regulate adhesion in a variety of cell types.

Acknowledgments
We thank Drs. Ellen Cahir-McFarland, Andy Chan, Gary Koretzky, and Robert Schreiber for gifts of cells, plasmids, and reagents, and Drs. Scott Blystone and Jennifer Green for helpful critique of the manuscript.

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