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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 αvβ3 (VLA-5), but not αvβ1 (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 dhesion 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 subunits, α4β1 (VLA-4) and α5β1, which recognize distinct domains of the matrix glycoprotein. αvβ3 binds the CS1 region within the 12th type III repeat of FN, in which the peptide sequence EILDV is critical (4–6); αvβ1 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 αvβ3 and αvβ1 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 adhesive-ness 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 pp125 FAK, 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 (25–28).

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 glycosy-calyx (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...
mAbs, have 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 $\alpha_\beta_2$, but not $\alpha_\beta_3$. 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 integrin-dependent 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 E6-1 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 J5.1L3.B3, expressing normal human CD45, and J45.01.C11, 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, 1-t-glutamine, and 10% FCS (HyClone, Logan, UT). Transfected cell lines were grown in this medium supplemented with 1.0 mg/ml (active) heparin (52). The catalytically inactive CD45 has been previously described and, after incubation, was washed by gently bringing each well up to and, after incubation, were washed three times in PBS and twice in adhesion buffer before use. Cells (5 x 10^5) were pelleted from suspension culture, washed twice in adhesion buffer containing RPMI medium and were gently spun into contact with substrate-coated plates at 1 x 10^5 cells/ml. Plates were then transferred to a 37°C water bath and, after incubation, were washed by gently bringing each well up to capacity (400 µl) with adhesion buffer using a multichannel pipette. Plates were sealed with acetic acid plate sealers (Dynatech) and centrifuged at 37°C for 8 min at room temperature in a swinging bucket rotor. The fluorescence of each well was determined and, after incubation, were washed three times in PBS and twice in adhesion buffer before use.


cDNAs and transfections

CD45 cDNAs (psi isoform) (46) and derivatives were expressed using the BSRoDEN vector (47). Chimeric constructs were follows. A chimeric mouse CD45 cDNA was digested with SalI and SpeI to generate a fragment encoding CD45 aa 420-1152 (48). This fragment was ligated to a fragment generated by PCR after digestion with SalI and SpeI that encoded human IFN-γ receptor α-chain aa 23–227 (49) (provided by Robert Schreiber, Washington University, St. Louis, MO), using the oligonucleotides 5’-CCAGCGACCGTCGACACCACCATGGCTCTCCTCTTT-3’ and 5’-TGGAAATCCAAAAGACTGTTTATATCTGTC-3’ for forward and reverse priming, respectively. This chimeric DNA encoded the extracellular domain of the human IFN-γ receptor α-chain and the transmembrane and cytoplasmic domains of mouse CD45; it was termed ψ/α1. To generate a similar construct that encoded the human CD44 transmembrane domain instead of the mouse CD45 transmembrane domain, the above construct was digested with SpeI and ClaI, and a fragment encoding the 22 aa of the CD44 transmembrane domain was isolated as a SpeI-ClaI fragment from a previously reported chimeric construct (45) and ligated. This chimeric DNA was termed ψ/α5/44/45. The catalytically inactive CD45 has been previously reported (29).

Generation of stably transfected cell lines

J45.01 or L3M cells were transfected by electroporation at 1000 µF and 300 V using an Electroporator II (Invitrogen, San Diego, CA). Transfected cells were selected in 2 mg/ml G418 (Genetecin, Life Technologies) containing RPMI medium. High expressing populations were isolated by FACS sorting.

Monovalent Abs

mAbs against human $\alpha_\beta_1$ and $\alpha_\beta_2$ were purchased from Upstate Biotechnology (Lake Placid, NY). mAbs against mouse $\alpha_\beta_3$ and $\alpha_\beta_4$ were 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 4.3 and anti-mouse CD45 13.2.3 were used as supernatants, and polyclonal rabbit anti-murine CD45AP was previously described (45). The anti-human IFNγ-α chain mAb GIB 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 $\alpha_\beta_1$ and $\alpha_\beta_4$ integrins were prepared and purified by chromatography on gelatin and heparin affinity purification as previously described (51). CBD is the 110-kDa chymotryptic FN fragment containing the binding site for $\alpha_\beta_1$ 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 $\alpha_\beta_1$, was prepared as previously described (4). The purity of FN fragments was verified by SDS-PAGE, and isolated binding domains were purified and used as supernatants, and polyclonal rabbit anti-murine CD45AP was prepared as previously described (4). To generate CD45 deficient L3M.46 were obtained from Sigma (St. Louis, MO) for development using standard protocols.

Analysis of coimmunoprecipitations

Cells (5 x 10^6) 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 perva nablate). Cells were lysed for 30 min at 4°C, and cell debris was pelleted at 10,000 x 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 were gently spun into contact with substrate-coated plates at 1 x 10^4 cells/ml. Lysates were washed three times with modified RIPA, solubilized in reducing SDS-sample buffer, and subjected to electrophoresis on an 8% SDS-polyacylamide gel. Samples were transferred to polyvinyldene difluoride membrane, and transferred proteins were detected with the CD45AP antiserum using protein G-Sepharose-HP (Bio-Rad, Hercules, CA) and enhanced chemiluminescence (Amer sham, Arlington Heights, IL) for development using standard protocols.
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 132/3 conjugated Sepharose or 3 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

α5β1-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 α5β1 and α4β1, 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 α5β1 and α4β1 were derived by FACS sorting (Table I). CD45-deficient J45.01 showed enhanced adhesion via α5β1 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, α4β1-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, α5β1 and α4β1 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, α4β1-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-α4β1 blocked binding of Jurkat and J45.01 to FN CS1 equivalently, and the addition of anti-α4β1 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). α5β1-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 (γ45/45) and in which in addition to this substitution the CD45 transmembrane domain was replaced by the CD44 transmembrane domain (γ44/45; Fig. 4A). Several stable populations with equivalent α5β1 expressions were derived for each transfected DNA, with similar results for all lines.

α5β1-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 (γ45/45; 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 CD45 transmembrane domain was substituted for CD45 (γ44/45; 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

### Table I. MCF of integrins α4 and α5 on CD45-sufficient and -deficient T lymphocyte lines and transfectants

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cell Surface Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No 1° Ab</td>
</tr>
<tr>
<td>L3 (CD45⁺)</td>
<td></td>
</tr>
<tr>
<td>L3M (CD45⁻)</td>
<td></td>
</tr>
<tr>
<td>γ45/45⁺</td>
<td></td>
</tr>
<tr>
<td>γ45/45⁻</td>
<td></td>
</tr>
<tr>
<td>Jurkat (CD45⁺)</td>
<td></td>
</tr>
<tr>
<td>J45.01 (CD45⁻)</td>
<td></td>
</tr>
<tr>
<td>J45.LB3 (Ref. 54)</td>
<td></td>
</tr>
<tr>
<td>J45.CH11 (Ref. 54)</td>
<td></td>
</tr>
</tbody>
</table>

*a SD of fluorescence for all populations <15% of MCF.

*b See text for details.

† Mean cell fluorescence of HLA A2 = 32.
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.
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,

![Image of Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** Integrin receptor-mediated adhesion of human T cell lines to FN CBD and FN CS1. The adhesion of Jurkat and J45.01 to FN CBD via $\alpha_5\beta_1$ (A) or FN CS1 via $\alpha_5\beta_1$ (B) was assessed in the presence or the absence of mAbs. Data are expressed as described in Fig. 1. Shown are the mean and SEM of three experiments, each performed in triplicate. Anti-VLA5 inhibited binding to CBD, and anti-VLA4 inhibited binding to CS1 ($p < 0.05$ in both cases).

![Image of Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Expression of CD45 or chimeric proteins in J45.01. Binding of parental Jurkat to FN CBD was compared with CD45-deficient Jurkat (J45.01), J45.01 reconstituted with wild-type CD45 (J45.LB3, low m.w. isom), and J45.01 expressing a chimeric molecule consisting of HLA-A2 extracellular and transmembrane domains with the CD45 cytoplasmic domain (J45.CH11). Only reconstitution with wild-type CD45 restores adhesion of the CD45-deficient cell line to parental levels. Data are expressed as described in Fig. 1. Shown is a representative experiment of five performed.

![Image of Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** Expression of wild-type and chimeric CD45 cDNAs in CD45-deficient murine T cells. A, Schematic of cDNAs expressed in J45.01 and L3M. B, Flow cytometric analysis of the expression of $\gamma$45/45 and $\gamma$44/45 cDNAs with the anti-IFN$\gamma$R Ab GIR-208. C, Flow cytometric analysis of wild-type and the DCS mutant compared with the parental L3 cell line with the anti-CD45 mAb 13/2.3. Cell populations were derived as stated in Materials and Methods. The negative control in C was a non-binding Ab directed against human $\alpha_v\beta_3$. 

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 α5β1-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 γ/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)a</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>

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

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

CD45AP and CD45 was analyzed by coimmunoprecipitation, wild-type CD45 (not shown) and the γ45/45 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.
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 

\[ p56^{-} \]

Src deficiency slows focal contact formation (61), and pp125 

\[ FAK \]

deficiency impairs cell motility, probably by inhibiting turnover of focal adhesions required for movement (34). The small GTPases Rac, Rho, and cdc42 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 \( \alpha_{5}\beta_{1} \)-mediated adhesion to FN. In both cell lines, CD45 limited adhesion. \( \alpha_{5}\beta_{1} \), the other lymphocyte FN receptor, is apparently not regulated by CD45 in these cell lines. The reason for the difference between the regulation of \( \alpha_{5}\beta_{1} \) and \( \alpha_{5}\beta_{1} \) 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 CD45AP 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.

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