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Tyrosine kinase activity associated with the CD7 antigen: correlation with regulation of T cell integrin function*

Rapid up-regulation of the functional activity of integrin adhesion receptors is a hallmark of T cell activation. Monoclonal antibody engagement of the CD7 antigen on human T cells results in an increase in $\beta1$ and $\beta2$ integrin-mediated adhesion within minutes. This suggests that CD7 is capable of transducing intracellular signals, and is consistent with other indirect studies implicating CD7 as a signaling receptor on T cells. In this report, we have explored the intracellular mechanism by which CD7 modulates integrin functional activity. First, CD7-mediated up-regulation of T cell adhesion was found to be unique when compared to phorbol ester stimulation and CD3/T cell receptor cross-linking, based on differences in the kinetics of activation-dependent integrin-mediated adhesion and lack of increase in CD2 functional activity. Second, up-regulation of integrin activity mediated by CD7 cross-linking was completely inhibited by the tyrosine kinase inhibitor herbimycin A. Third, antiphosphotyrosine immunoblotting demonstrated that antibody engagement of CD7 results in a rapid but transient increase in tyrosine phosphorylation in human T cells. Finally, CD7 immunoprecipitates contain in vitro kinase activity, as demonstrated by phosphorylation of a predominant band of 80 kDa and multiple other bands. Phosphoamino acid analysis of the 80-kDa substrate revealed phosphorylation on tyrosine as well as serine and threonine residues. Together, our results suggest that CD7 is associated with tyrosine kinase activity and that this tyrosine kinase activity correlates with the ability of CD7 to regulate T cell integrin functional activity.

1 Introduction

T lymphocyte function requires the precise regulation of expression and function of cell surface receptors that mediate T cell adhesion to other cells and to components of the extracellular matrix. Intracellular signals generated by T cell activation represent one such mode of regulation. In fact, one of the earliest functional consequences of T cell activation is the rapid up-regulation of the functional activity of the integrin family of adhesion molecules [1–4]. Various stimuli, most notably phorbol ester stimulation and antibody cross-linking of the antigen-specific CD3/TCR, result within minutes in an increase in integrin functional activity without a change in the level of integrin surface expression. Such activation-dependent up-regulation of integrin activity has been demonstrated for T cell adhesion to various integrin ligands, including the LFA-1 ligand

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ICAM-1 [2, 4], the $\alpha4\beta1$ ligand VCAM-1 [5], the $\alpha4\beta1$ and $\alpha5\beta1$ extracellular matrix ligand fibronectin (FN) [3], and the $\alpha6\beta1$ ligand laminin [3]. Phorbol ester stimulation and CD3 cross-linking have also been shown to up-regulate the binding of the CD2 receptor to one of its counter-receptors, LFA-3 (CD58) [6], suggesting that activation results in increased activity of multiple different adhesion molecules. Rapid changes in adhesion receptor activity upon activation have been proposed to play an important regulatory role in both T cell recognition of foreign antigen and T cell localization in lymphoid organs and sites of inflammation [1, 7, 8].

We have previously demonstrated that antibody cross-linking of the CD7 antigen on human T cells can rapidly up-regulate T cell $\beta1$ and $\beta2$ integrin functional activity [9]. CD7 is a 40-kDa glycoprotein that is expressed on T cells and NK cells [10, 11]; a subpopulation of normal T cells lacking CD7 expression has also recently been reported [12]. In addition to our studies demonstrating a role for CD7 in regulating T cell adhesion, there is additional evidence that CD7 can play a role in T cell activation. First, antibody cross-linking of CD7 induces an increase in intracellular calcium [13]. Second, various studies have demonstrated both inhibitory and stimulatory effects of CD7-specific mAb on the proliferation and cytokine response of both α/β^+ T cells and γ/δ^+ T cells [14–19]. Furthermore, changes in CD7 expression on T cells or T cell subsets have been reported in various disease states, including HIV infection [20] and rheumatoid arthritis [21]. CD7 has also recently been implicated as a potential cofactor in syncytium formation induced by HIV-1 [22]. Clinically, CD7 has been used as a therapeutic target in the treatment of various diseases, including T cell leukemia [23,

24] and rheumatoid arthritis [25], and CD7-specific mAb have been proposed as a useful therapy for preventing renal allograft rejection [26]. CD7 also appears to be an adhesion regulator on NK cells, since antibody cross-linking of CD7 on NK cells has been reported to increase NK cell adhesion to FN [11].

Our interest in the role of CD7 in regulation of T cell adhesion led us to explore further the mechanism by which CD7 transduces signals. In this paper, we show that: (i) CD7 exhibits unique adhesion regulatory properties, as judged by kinetics of increased integrin function and lack of up-regulation of CD2 function; (ii) CD7-mediated up-regulation of integrin function appears to involve tyrosine kinases and (iii) tyrosine kinase activity is associated with the CD7 molecule.

2 Materials and methods

2.1 Isolation of human T cells

Resting peripheral CD4$^+$ T cells were isolated by negative magnetic immunoselection as previously described [3, 27]. Briefly, PBMC were isolated from either leukapheresis packs or buffy coats by Ficoll-Hypaque density-gradient centrifugation. Resting CD4$^+$ T cells were subsequently isolated from PBMC using Advanced Magnetics Particles (Advanced Magnetics, Inc., Cambridge, MA) and a cocktail of mAb consisting of: IVA12 (anti-HLA class II), FMC63 (anti-CD19), 3G8 (anti-CD16), NIH11b-1 (anti-CD11b), 63D3 (anti-CD14), B9.8.4 or 51.1 (both anti-CD8) and 10F7 (anti-glycophorin). The T cell populations were typically >97% CD3$^+$CD4$^+$ as assessed by flow-cytometric analysis.

2.2 Antibodies and other reagents

The following mAb were used as purified IgG: anti-CD7 mAb 3A1, anti-CD3 mAb OKT3, anti-LFA-3 mAb TS2/9 (all American Type Culture Collection, Rockville, MD), anti-CD7 mAb 8H8.1 (AMAC, Westbrook, ME), anti-ICAM-1 mAb 84H10. The following mAb were used as dilutions of ascites fluid: anti-HLA class II mAb IVA12, anti-CD14 mAb 63D3, anti-glycophorin mAb 10F7, anti-CD8 mAb 51.1, anti-CD2 mAb 35.1 (all ATCC), anti-CD19 mAb FMC63 (Dr. H. Zola, Flinders Medical Centre, Bedford Park, Australia), anti-CD16 mAb 3G8 (Dr. D. Segal, NIH, Bethesda MD), anti-CD11b mAb NIH11b-1 [28], anti-CD2 mAb 95-5-49 (Dr. R. Gress, NIH), anti-phosphotyrosine mAb PY20 (Dr. M. Kamps, University of California, San Diego, CA) and anti-CD8 mAb B9.8.4 (Dr. B. Malissen, Centre d'Immunologie de Marseille-Luminy, Marseilles, France).

ICAM-1 was purified by affinity chromatography using the anti-ICAM-1 mAb 84H10 from the Hodgkin's lymphoma cell line L428 as previously described [29]. The purity of the ICAM-1 preparation was established by silver staining and mAb blocking studies demonstrating LFA-1-specific adhesion of PMA-activated T cells to purified ICAM-1 (not shown). Recombinant LFA-3-Ig chimeric protein was kindly provided by Dr. P. Hochman (Biogen, Cambridge, MA) [30]. FN was purchased from the New York Blood Center

(New York, NY). PMA (Sigma Chemical Co., St. Louis, MO) was prepared as a stock solution in DMSO and diluted with PBS just before use. Herbimycin A was a kind gift from Dr. R. Jove (University of Michigan Medical School, Ann Arbor, MI) and genistein was purchased from GIBCO/BRL (Grand Island, NY).

2.3 Adhesion assays

Adhesion assays were performed essentially as described [9, 31]. Briefly, 96-well microtiter plates (NUNC ELISA plates for ICAM-1 and LFA-3-Ig, Costar 3596 plates for FN) were incubated overnight at 4 °C with the indicated concentrations of FN or purified ICAM-1 (diluted in PBS with Ca^{2+} and Mg^{2+}). For LFA-3-Ig, plates were first preincubated overnight at 4 °C with 50 µl of a 10-µg/ml stock of goat anti-human IgG (Organon Teknika, Malvern, PA) diluted in PBS with Ca^{2+} and Mg^{2+}, washed twice to remove unbound antibody and incubated overnight at 4 °C with the indicated concentrations of LFA-3-Ig diluted in Ca^{2+}/Mg^{2+}-containing PBS. Following ligand coating, plates were blocked with PBS/2.5% BSA for 1–2 h at 37 °C. Each well contained 50 000 ^{51}Cr-labeled CD4$^+$ T cells in a final volume of 0.1 ml PBS/0.5% human serum albumin (HSA). For PMA activation, cells were added to wells containing 10 ng/ml PMA. For CD3 and CD7 stimulation, cells were first incubated with saturating amounts of antibody for 30 min at 4 °C, washed twice, and added to wells containing 0.5–1 µg/ml goat anti-mouse Ig (Organon Teknika). Blocking antibodies were added to the wells at a concentration of 10 µg/ml. After 1 h settling at 4 °C, plates were rapidly warmed up to 37 °C for the indicated period of time, nonadherent cells washed off, and the percentage of bound cells determined by lysing the well contents with detergent and counting γ-emissions. All data are expressed as the mean percentage of cells binding in three replicate wells ± SEM. For the herbimycin A experiments, CD4$^+$ T cells were preincubated overnight at 37 °C at 5 × 10^6 cells/ml in RPMI/10% FCS containing 3 µg/ml of herbimycin A (stock solution in DMSO) or an appropriate amount of DMSO as a control before being tested in the adhesion assay as described above.

2.4 Antiphosphotyrosine immunoblotting

Antiphosphotyrosine immunoblotting was performed essentially as previously described [32]. Briefly, CD4$^+$ T cells were incubated with saturating amounts of the CD7-specific mAb 3A1 or 8H8.1 or the CD3-specific mAb OKT3 for 30 min at 4 °C in PBS/0.5% HSA. Cells were washed three times at 4 °C to remove unbound mAb, resuspended in 0.5 ml of ice-cold PBS/0.5% HSA containing 2.5 µg of goat anti-mouse IgG and incubated for 15 min at 4 °C. The cells were then rapidly warmed up to 37 °C for the indicated period of time and stimulation terminated by adding an equal volume of ice-cold 2 × lysis buffer (2% NP-40, 300 mM NaCl, 100 mM Tris-HCl, pH 8.0, 0.8 mM NaOVO$_4$, 10 mM EDTA, 20 mM iodoacetamide, 20 mM NaF, 20 mM sodium pyrophosphate, 2 mM PMSF). After a 15-min incubation on ice, insoluble material was removed by centrifugation for 30 min at 13 000 × g at 4 °C. Lysates were immunoprecipitated by a 2- to 3-h incubation with protein A Sepharose beads pre-coated with the antiphos-

photyrosine mAb PY20. Bound material was eluted in Laemmli sample buffer, boiled, separated by SDS-PAGE, and electrophoretically transferred to PVDF membrane. Antiphosphotyrosine Western blotting was conducted as described [32], with the exception that the antiphosphotyrosine mAb 4G10 was used and ^{125}I-labeled sheep anti-mouse IgG (ICN, Costa Mesa, CA) was used as the secondary detection reagent.

2.5 In vitro kinase assays

Resting CD4$^+$ T cells were lysed at 10^6 cells/ml in 0.3% CHAPS buffer (140 mM NaCl, 10 mM Tris-HCl, pH 8.0, 0.1% BSA, 0.02% Na azide, 0.2 mM NaOVO$_4$ and 10 mM NaF) for 15 min on ice. Insoluble material was removed by centrifugation for 30 min at 13 000 × g at 4 °C. Lysates were precleared with Pansorbin for 1 h before immunoprecipitation with protein A Sepharose beads pre-coated with either the anti-glycophorin mAb 10F7 as a control or the anti-CD7 mAb 3A1 (2–3-h incubation at 4 °C). Beads were then washed three times with CHAPS buffer and two times with TN buffer (150 mM NaCl, 50 mM Tris, pH 7.2) before being resuspended in 25 µl of kinase buffer (40 mM Pipes, pH 7.0, 10 mM MnCl$_2$) containing 5 µCi of γ-^{32}P-labeled ATP (NEN/Dupont, Wilmington, DE). In vitro kinase reaction was performed at 30 °C for 10 min and terminated by adding ice-cold TN buffer. Beads were washed once with TN buffer to remove uncoupled ATP. Bound material was eluted in Laemmli sample buffer, boiled for 5 min and proteins were subjected to SDS-PAGE. The gel was stained with Coomassie Blue and phosphorylated proteins detected by autoradiography.

2.6 Phosphoamino acid analysis

Following in vitro kinase reaction and SDS-PAGE (performed as described above), proteins were transferred to PVDF membrane and detected by autoradiography. The region of the membrane containing the p80 phosphorylated protein was identified, cut out, incubated in 100 µl 5.7 N HCl at 110 °C for 1 h and then lyophilized. Acid-hydrolyzed amino acids were resuspended in 10 µl pH 1.9 buffer (2.5% formic acid, 7.8% glacial acetic acid), and loaded onto a thin layer chromatography plate. The sample was subjected to two-dimensional electrophoresis as described [33] using a Hunter HTLE-7000 thin layer chromatography system and phosphoamino acids were detected by autoradiography.

3 Results

3.1 CD7 cross-linking induces rapid and prolonged adhesion of CD4$^+$ human T cells to FN and ICAM-1

Integrin functional activity on human T cells can be rapidly up-regulated by several different activation stimuli, most notably treatment with the phorbol ester PMA and antibody cross-linking of the CD3/TCR with CD3-specific mAb [2–4]. A distinguishing characteristic of these integrin regulatory signals is the kinetics of the increased adhesion. Both PMA treatment and CD3 cross-linking result in maximal integrin functional activity within 10 min of stimulation. However, PMA-induced adhesion remains

strong at later time points (60 min) while CD3-induced adhesion peaks at 10 min and returns to baseline levels within 60 min [2, 34] (Fig. 1). The kinetics of adhesion of human T cells to both the β1 integrin ligand FN and the LFA-1 integrin counter-receptor ICAM-1 following antibody cross-linking of CD7 have two distinct characteristics (Fig. 1). First, CD7-induced adhesion peaks at 20 min post-stimulation rather than at 10 min. Second, adhesion at later time points resembles the prolonged kinetics of PMA-stimulated cells. This kinetic analysis suggests that CD7-mediated up-regulation of T cell integrin function is distinct from other modes of up-regulating integrin activity, particularly CD3 cross-linking.
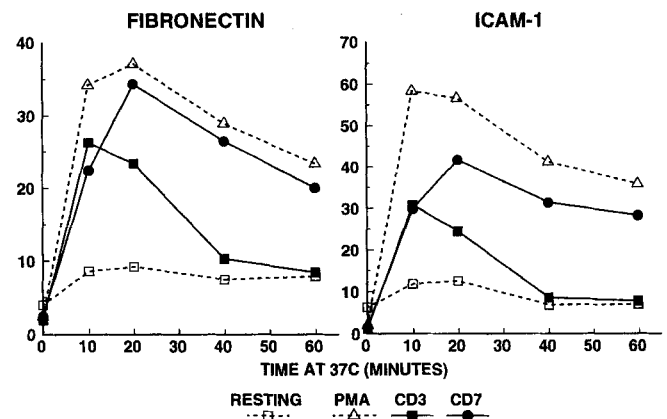

Figure 1. Kinetics of CD7-mediated up-regulation of adhesion to FN and ICAM-1. The adhesion of purified ^{51}Cr-labeled CD4$^+$ T cells to FN (left), applied at 1 µg/well, or ICAM-1 (right), applied at 20 ng/well, was assessed after incubation at 37 °C for the indicated times with no stimulation (open squares), or in the presence of 10 ng/ml PMA (open triangles), anti-CD3 mAb OKT3 cross-linked with goat anti-mouse IgG (solid squares) or anti-CD7 mAb 3A1 cross-linked with goat anti-mouse IgG (solid circles). Data are representative of a minimum of three experiments with different donors.

3.2 CD7 cross-linking specifically up-regulates integrin function

In addition to up-regulating integrin activity, PMA stimulation and antibody cross-linking of the CD3/TCR have also been shown to up-regulate CD2-mediated adhesion to LFA-3 [6]. In order to further distinguish the effects of CD7 stimulation on T cell adhesion, we have assessed the ability of CD7 cross-linking to modulate the adhesion of CD4$^+$ T cells to a LFA-3-Ig chimeric protein immobilized on plastic. Fig. 2 demonstrates that CD4$^+$ T cells bind to LFA-3-Ig and this binding can be specifically inhibited by CD2- or LFA-3-specific mAb. As previously reported, PMA stimulation, CD3 cross-linking, and CD7 cross-linking all up-regulate adhesion to FN and ICAM-1 (Fig. 3). While the basal level of T cell adhesion to LFA-3-Ig is higher than that seen with FN or ICAM-1, the overall level of binding to LFA-3-Ig increases further upon PMA stimulation or CD3 cross-linking. However, CD7 stimulation fails to increase T cell adhesion to LFA-3-Ig above the basal level observed with resting T cells.
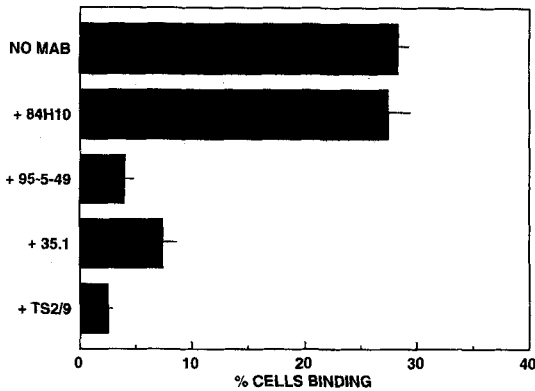
Figure 2. Adhesion of CD4⁺ T cells to purified LFA3-Ig. The adhesion of resting ^{51}Cr-labeled CD4⁺ T cells to purified LFA-3-Ig (immobilized at 2 μg/ml) after a 10-min incubation at 37 °C was assessed in the presence of: no mAb, the anti-ICAM-1 mAb 84H10, the anti-CD2 mAb 95-5-49, the anti-CD2 mAb 35.1 or the anti-LFA-3 mAb TS2/9 (all mAb used at 10 μg/ml). Binding of T cells to the negative control protein collagen was <7% and was not subtracted from the values shown. Data are representative of a minimum of three experiments with different donors.
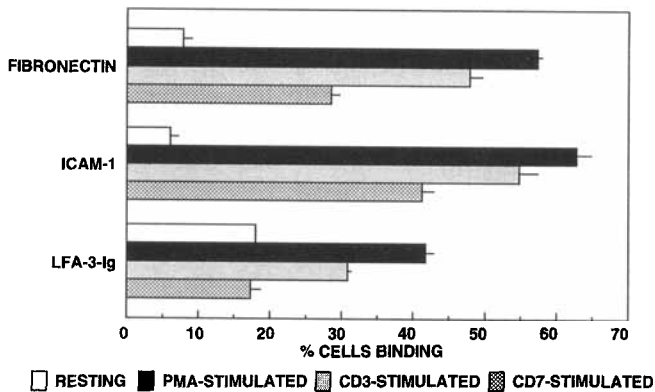

Figure 3. CD7 cross-linking up-regulates adhesion to FN and ICAM-1 but not LFA-3. The adhesion of purified ^{51}Cr-labeled CD4⁺ T cells to FN, ICAM-1 or LFA-3-Ig was assessed after incubation at 37 °C for 10 min with no stimulation (open bars), or in the presence of 10 ng/ml PMA (solid bars), anti-CD3 mAb OKT3 cross-linked with goat anti-mouse IgG (shaded bars) or anti-CD7 mAb 3A1 cross-linked with goat anti-mouse IgG (cross-hatched bars). FN was immobilized at 1 μg/well, ICAM-1 at 20 ng/well, and LFA-3-Ig at 4 μg/ml. Data are representative of three experiments with different donors.

3.3 CD7-mediated up-regulation of T cell integrin functional activity is inhibited by herbimycin A

Since many T cell surface receptors mediate signal transduction via activation of tyrosine kinases, we explored the potential role of tyrosine kinases in CD7-mediated up-regulation of T cell integrin activity. Pretreatment of CD4⁺ T cells with the tyrosine kinase inhibitor herbimycin A results in complete inhibition of CD7-mediated up-regulation of T cell adhesion to FN (Fig. 4). Similar inhibition was observed with the tyrosine kinase inhibitor genistein (data not shown). Up-regulation of T cell integrin activity mediated by antibody cross-linking of the CD3/TCR was also inhibited by herbimycin A pretreatment, consistent with the role of tyrosine kinases in CD3/TCR signaling [35].
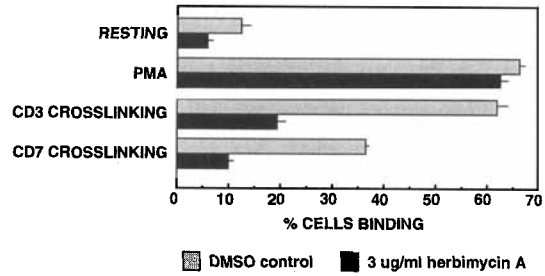

Figure 4. Inhibition of CD7-mediated up-regulation of T cell adhesion to FN by the tyrosine kinase inhibitor herbimycin A. Adhesion of purified ^{51}Cr-labeled CD4⁺ T cells to FN (applied at 1 μg/well) was assessed after a 10-min incubation at 37 °C using the activation conditions described in the legend to Fig. 1. Before the adhesion assay was conducted, T cells were either preincubated for 16 h at 37 °C with 3 μg/ml herbimycin A (solid bars) or with an equivalent amount of DMSO as a control (shaded bars). Data are representative of a minimum of four experiments with different donors. Similar inhibitory effects were observed with genistein treatment of T cells (not shown).

Herbimycin A does not nonspecifically inhibit integrin function, since herbimycin A pretreatment does not inhibit PMA-induced up-regulation of T cell adhesion to FN (Fig. 4). Thus, these results suggest a role for tyrosine kinases in CD7-mediated up-regulation of integrin functional activity.
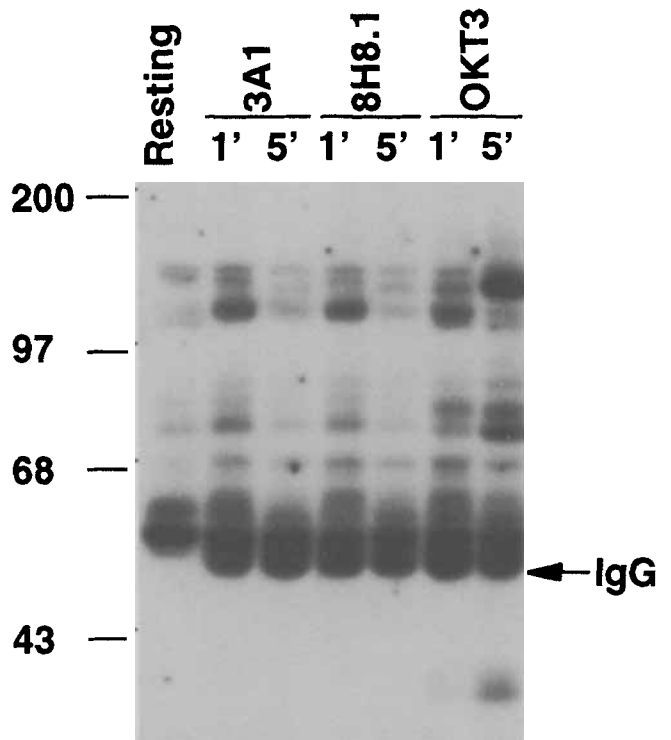

Figure 5. CD7 cross-linking induces tyrosine phosphorylation in CD4⁺ T cells. CD4⁺ T cells were incubated with saturating concentrations of either the CD7-specific mAb 3A1 or 8H8.1 or the CD3-specific mAb OKT3, washed and activated for either 1 or 5 min at 37 °C in the presence of goat anti-mouse IgG. Cells were solubilized in detergent, immunoprecipitated with the anti-phosphotyrosine mAb PY20, run out on a 7.5% SDS-polyacrylamide gel, transferred to PVDF membrane and immunoblotted with the anti-phosphotyrosine mAb 4G10 and ^{125}I-labeled sheep anti-mouse IgG.

3.4 CD7 cross-linking induces tyrosine phosphorylation in human T cells

To further investigate the role of tyrosine kinases in CD7-mediated signaling, we performed anti-phosphotyrosine immunoblotting. Antibody cross-linking conditions in these anti-phosphotyrosine immunoblotting experiments were identical to those used to assess CD7-mediated up-regulation of integrin activity in adhesion assays (Figs. 1, 3 and 4). As shown in Fig. 5, antibody cross-linking of CD7 on CD4$^+$ T cells results in tyrosine phosphorylation of several substrates within 1 min. This phosphorylation diminishes rapidly, returning to baseline levels of tyrosine phosphorylation within 5 min of stimulation. When comparing the tyrosine phosphorylation pattern and kinetics of phosphorylation between CD7 and CD3 stimulation, several differences were noted (Fig. 5). First, a smaller number of substrates appear to be phosphorylated by CD7 stimulation compared to CD3 cross-linking. Second, CD3 cross-linking also results in tyrosine phosphorylation within 1 min of stimulation. However, in contrast to CD7 cross-linking, engagement of CD3/TCR results in more prolonged tyrosine phosphorylation, with clear evidence of phosphorylation of additional substrates at 5 min that are not observed upon similar stimulation via CD7.
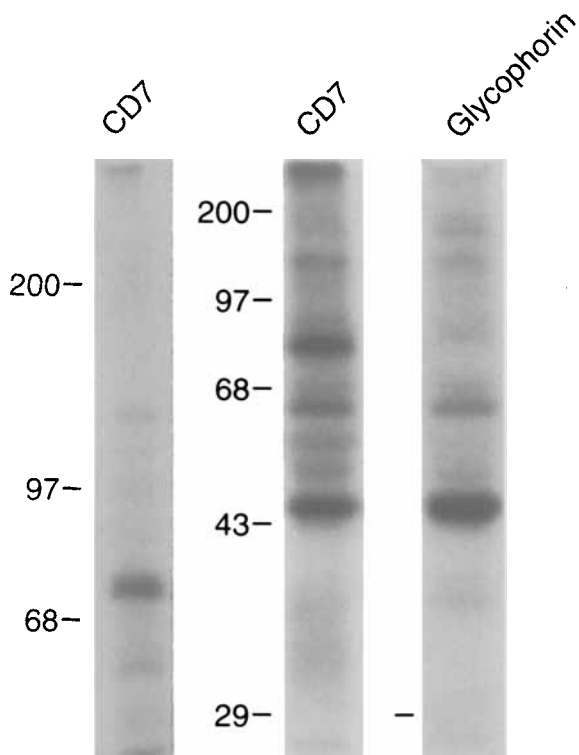
3.5 Immunoprecipitates of CD7 contain *in vitro* kinase activity

Additional biochemical evidence was obtained for a physical association between CD7 and tyrosine kinases. Detergent lysates of CD4$^+$ T cells were immunoprecipitated with the CD7-specific mAb 3A1 and the presence of kinases in the immunoprecipitate was assessed with an *in vitro* kinase assay (Fig. 6). Several phosphorylated bands are found following this reaction, notably a predominant band of ~ 80–85 kDa (designated p80) and weaker bands at 40–50 kDa and 30–38 kDa (Fig. 6). Additional phosphorylated bands are seen, but they are also found in control immunoprecipitates using the anti-glycophorin mAb 10F7. Although the results shown in Fig. 6 use lysates solubilized in 0.3% CHAPS, similar results have been obtained using lysis buffers containing 1% NP-40 (not shown).

To verify that the phosphorylation observed *in vitro* is due to activation of tyrosine kinases, we performed phospho-amino acid analysis on the predominant phosphorylated protein, p80. Fig. 7 shows that p80 is phosphorylated on tyrosine residues as well as serine and threonine. Thus, these results demonstrate that CD7 immunoprecipitates contain associated tyrosine kinase activity *in vitro*.
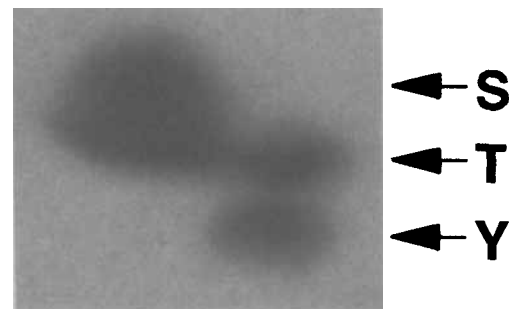

Figure 6. CD7 immunoprecipitates isolated from CD4$^+$ T cell lysates contain *in vitro* kinase activity. CD4$^+$ T cells were lysed in 0.3% CHAPS lysis buffer and lysates containing 10 × 10^6 cell equivalents were immunoprecipitated with either the CD7-specific mAb 3A1 or the glycophorin-specific mAb 10F7 as a negative control. The pellets containing the immunoprecipitating beads were washed with TN buffer, resuspended in kinase buffer and 5 µCi of [γ-^{32}P] ATP was added. The tubes were incubated for 10 min at 30 °C, washed with TN buffer and the samples subjected to SDS-PAGE (7.5% for the left lane, 12% for the middle and right lanes). The gels were dried and exposed for 24 h to X-ray film before developing.

Figure 7. Phosphoamino acid analysis of p80 protein. *In vitro* kinase assays were performed as described in the legend to Fig. 6. The p80 protein was excised out of the PVDF membrane and subjected to phosphoamino acid analysis as described in Sect. 2.6. Serine (S), threonine (T) and tyrosine (Y) residues are indicated.

4 Discussion

The results presented here provide several new insights into the role of CD7 in regulating T cell integrin function and the biochemical mechanism by which this regulation is mediated. First, CD7-mediated up-regulation of T cell integrin activity is clearly distinct from PMA stimulation and CD3 cross-linking. Analysis of the kinetics of induced adhesion to FN and ICAM-1 demonstrate that CD7 cross-linking induces rapid but prolonged integrin-mediated adhesion. The increase in adhesion observed upon CD7 stimulation peaks at a slightly later time (20 min *versus* 10 min) than seen with either PMA stimulation or CD3 stimulation. Furthermore, unlike CD3 stimulation, CD7 cross-linking results in prolonged adhesion over time, similar to that seen with PMA stimulation. Analysis of T cell adhesion to the CD2 ligand LFA-3 also reveals

differences between CD7 stimulation and other modes of activation that modulate T cell adhesion. Thus, while PMA stimulation and CD3 cross-linking have both been shown to up-regulate T cell adhesion to LFA-3 [6], CD7 cross-linking fails to increase T cell adhesion to this non-integrin ligand. Consequently, CD7 triggering induces changes in T cell adhesion that are clearly distinct from PMA stimulation and antibody cross-linking of the CD3/TCR. These data are consistent with recent studies demonstrating a loss of CD7-mediated up-regulation of adhesion in T cell lines that are still responsive to CD3- and PMA-stimulation [36].

Second, our data suggest that CD7 cross-linking results in activation of tyrosine kinases and that these kinases may be involved in up-regulating integrin activity. Treatment of T cells with the tyrosine kinase inhibitor herbimycin A results in complete inhibition of CD7-mediated up-regulation of adhesion to FN. Antiphosphotyrosine immunoblotting also demonstrates that anti-CD7 antibody cross-linking conditions that result in increased integrin activity also result in tyrosine phosphorylation. The kinetics and pattern of phosphorylation in CD7-stimulated T cells differ from that found in CD3-stimulated T cells, again emphasizing potential differences in the signaling mediated by CD3 and CD7. Finally, *in vitro* kinase assays and phosphoamino acid analysis demonstrated tyrosine kinase activity in CD7 immunoprecipitates, with tyrosine phosphorylation of a predominant band of 80 kDa (p80). Since the cytoplasmic domain of CD7 does not appear to have intrinsic tyrosine kinase activity [10], our results imply a physical association between CD7 and an unidentified tyrosine kinase.

Our studies on the role of CD7 in regulating T cell integrin function are consistent with other studies that have provided evidence for CD7-mediated signal transduction. Early experiments demonstrated that antibody cross-linking of CD7 on human T cells results in increases in intracellular calcium [13]. In addition, CD7-specific mAb can enhance CD3-dependent T cell proliferation when co-immobilized with CD3-specific mAb [14]. CD7-specific mAb have also been found to augment the proliferation of PBMC induced by antigen, mitogen or CD3-specific mAb [16]. Other studies have demonstrated inhibitory effects of CD7-specific mAb on T cell proliferation [19]. CD7 mAb alone have also been reported to specifically induce increases in intracellular calcium and cytokine production in γ/δ^+ T cells, but not in α/β^+ T cells [15]. Thus, there may be lineage-specific differences in the signal transduction capabilities of the CD7 molecule. Furthermore, as we initially reported with human T cells, antibody cross-linking of CD7 on human NK cells has also been reported to up-regulate NK cell adhesion to FN [11]. It will be of interest to determine whether CD7 expressed on different cell types, or during different stages of T cell differentiation, has differences in associated tyrosine kinase activity.

Since CD7 is clearly a signaling molecule with regulatory effects on various aspects of T cell activation, particularly adhesion receptor function, differences in CD7 expression on normal T cell subsets [12] or changes in CD7 expression during disease [20, 21] may result in altered functional responses by these cells. The true impact of these changes in CD7 expression on various T cell subsets in normal individ-

uals and in various diseased states remains to be elucidated. Furthermore, it is now clear that CD7 can generate signals when appropriately engaged by antibody. Therefore, attempts to use CD7 as a molecular target for delivery of immunotoxins or other therapeutic agents must now take into account the potential functional effect of antibody engagement of CD7 on the targeted cell population.

In summary, we have demonstrated that: (i) CD7-mediated up-regulation of integrin function on human T cells is distinct from other modes of up-regulating integrin activity; (ii) CD7 is functionally and physically associated with tyrosine kinases and (iii) CD7-associated tyrosine kinase activity is important in CD7 regulation of integrin function. Thus, these results provide important insights not only into the role of CD7 in regulation of T cell adhesion, but also provide more direct evidence for CD7-mediated signal transduction. Further progress in understanding the role of CD7 in T cell function will now require not only the identification of CD7-associated tyrosine kinases and relevant substrates but also a more complete understanding of the consequences of CD7 stimulation for T cell function.

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