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Immunology

Intimate association of Thy-1 and the T-cell antigen receptor with the CD45 tyrosine phosphatase

(T200/leukocyte common antigen/T-cell activation/chemical cross-linking)

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ABSTRACT **Immunoprecipitation of Thy-1 from Triton X-100 detergent lysates of surface-iodinated and chemically cross-linked T cells precipitated at least five major and discrete bands. Four of these bands were identified as Thy-1, CD45 (a transmembrane tyrosine phosphatase), a major histocompatibility complex-encoded class I molecule, and β_2-microglobulin. Similar analyses revealed that CD45 was coprecipitated from lysates of cross-linker-treated cells by antibodies to the T-cell antigen receptor (TCR). The same pattern of coprecipitated bands was observed when digitonin was used to lyse untreated cells. Immunoprecipitation of Thy-1 or the TCR from lysates of cross-linked T cells precipitated CD45 tyrosine phosphatase activity. Calculations based upon the amounts of coprecipitated enzymatic activity or TCR ζ chain indicate that a substantial fraction of Thy-1 and TCR complexes can be cross-linked to CD45. These data support a model in which the dependence of Thy-1 signaling on TCR coexpression is due to their common interaction with a tyrosine phosphatase and provide a possible structural basis for the influence of CD45 on TCR-mediated signaling.**

T-cell activation is initiated by the interaction of certain cell surface molecules with their respective ligands or with specific antibodies. The best understood of these molecules is the TCR, a highly organized multisubunit complex with both ligand-binding and signal-transducing elements (1). A growing number of plasma membrane molecules of unknown function can also initiate signals leading to cellular activation events (2). Among the best characterized of these molecules is Thy-1. Interestingly, Thy-1 is not normally found as a transmembrane molecule but is anchored to plasma membrane lipids by a glycosyl-phosphatidylinositol linkage (3). For this molecule, then, the transfer of information from the outside of the cell to the inside most likely involves the participation of one or more additional signal-transducing elements. Many of the T-cell responses elicited by Thy-1 require or are enhanced by coexpression of the TCR (for review, see ref. 1), leading to the speculation that Thy-1 may physically associate with the TCR.

We have explored the question of whether activating T-cell surface molecules physically interact in the plasma membrane by analyzing coprecipitation patterns after using either homobifunctional chemicals to cross-link cell surface proteins or nondisruptive detergents to lyse the cells. These studies demonstrate that a substantial fraction of Thy-1 and TCR molecules are physically associated with CD45, a transmembrane tyrosine phosphatase, and support the view that physical interactions among these molecules are involved in the initiation or modification of transmembrane signaling pathways.

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MATERIALS AND METHODS

Mice. C3H/HeJ mice were obtained from the Developmental Therapeutics Program, NCI, Frederick, MD.

Cells. 2B4.11 is a pigeon cytochrome *c*-specific T-cell hybridoma (4). Initially $CD4^+$, $CD8^-$, its surface phenotype is now Thy-1^+, $CD45^+$, TCR^+, $CD4^-$, $CD8^-$, and $CD2^-$. BW5147 (BW^+) and ASL1.1($ASL1.1^+$) are CD45-bearing T-cell tumors (5). BW5147($T200^-$a) (BW^-) and ASL1.1($T200^-$b) ($ASL1.1^-$) are their respective CD45-negative mutants (5).

Antibodies and Reagents. The following purified monoclonal antibodies (mAbs) were used: G7, specific for a nonpolymorphic Thy-1 determinant (6); 145-2C11 (2C11), an anti-CD3-ε (7); M1/89.18.7.HK, an anti-CD45 (8); and FD441.8, an anti-lymphocyte function-associated antigen 1 (anti-LFA-1) (9). Rabbit anti-β_2-microglobulin (anti-β_2m) antiserum was the gift of David Margulies(National Institutes of Health). Other reagents used were as follows: rabbit anti-mouse (RαM) antibodies (Dako, Santa Barbara, CA), disuccinimidyl suberate (DSS; noncleavable, span of 11.4 Å), dithiobis(succinimidylpropionate) (DSP; cleavable under reducing conditions; span of 12 Å) (Pierce), and [Val^5]angiotensin II (Sigma). Cross-linkers were dissolved in dimethyl sulfoxide.

Purification of Splenic T Cells. Unfractionated splenocytes were enriched for T cells (to ≥95% purity) by passage over nylon wool columns.

Radiolabeling, Cross-Linking, Immunoprecipitation, and Gel Electrophoresis. Cells were radiolabeled with $Na^{125}I$ (ICN) by using lactoperoxidase as described (10). Radiolabeled cells were resuspended in isotonic phosphate-buffered saline and incubated with dimethyl sulfoxide alone (mock cross-linked) or with 0.2 mM DSS, 0.2 mM DSP, or 0.2 mM bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone (BSOCOES). Cells were incubated for 30–90 min at 37°C. Results were identical when cross-linking was performed on cells incubated on ice. Cells were then lysed in 0.5% Triton X-100/50 mM Tris·HCl, pH 7.6/300 mM NaCl (lysis buffer). Lysates were precleared with RαM and then immunoprecipitated with specific mAbs. After precipitation, beads were washed and boiled in sample buffer [10% (vol/vol) glycerol/2% (wt/vol) SDS/0.25 M Tris·HCl, pH 6.8] to which 1% 2-mercaptoethanol was added when indicated. The eluted material was analyzed by SDS/PAGE (15% polyacrylamide unless otherwise noted) using AcrylAide as the cross-linker (FMC). For reprecipitation experiments, after immunoprecipitation from lysates of radioiodinated and DSP-cross-linked 2B4.11 cells, beads were incubated in buffer containing 0.1% Triton X-100 plus 0.5% 2-mercaptoethanol. After 5 min, 200 μl of washing buffer (lysis buffer with 0.1% Triton

Abbreviations: MHC, major histocompatibility complex; β_2m, β_2-microglobulin; TCR, T-cell antigen receptor; mAb, monoclonal antibody; LFA-1, lymphocyte function-associated antigen 1; RαM, rabbit anti-mouse; DSS, disuccinimidyl suberate; DSP, dithiobis(succinimidylpropionate).

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X-100) was added, the beads were centrifuged, supernatant was removed, and the proteins released from the cross-linked complexes were reprecipitated.

Tyrosine Phosphatase Assay. LSTRA cells membranes were used as a source of the tyrosine kinase pp56^{lck} to catalyze the transfer to ^{32}P to substrate, [Val^5]angiotensin II (11). The tyrosine phosphatase assay was performed as described (Y. Minami, J. Lippincott-Schwartz, L. C. Yuan, and R. D. Klausner, personal communication). Briefly, cells were lysed in a 0.5% Nonidet P-40 in 50 mM Tris·HCl, pH 7.4/300 mM NaCl/leupeptin (0.1 mg/ml)/aprotinin (0.1 mg/ml)/0.25 mM phenylmethylsulfonylfluoride, for 60 min on ice. The postnuclear fraction was immunoprecipitated with specific antibodies, no antibody, or RαM only. The beads were washed, and, at time 0, 10 μl of substrate was added, and the reaction proceeded at 30°C as indicated and was terminated by the addition of 5% (wt/vol) activated charcoal suspended in 20 mM Hepes (pH 7.4). Radioactivity in supernatants was measured in a β-scintillation counter.

Western Blot Analysis of TCRζ. Immunoblot analysis of the TCRζ chain after immunoprecipitation was performed as described (12).

RESULTS

Thy-1 Can Be Chemically Cross-Linked to a Limited Set of T-Cell Surface Molecules. The G7 mAb precipitated an easily detectable Thy-1 band of ≈28 kDa from the detergent lysate of mock-cross-linked 2B4.11 T hybridoma cells (Fig. 1*A*, lane 1). After cross-linking with DSS and precipitation with G7, a variable number of high molecular mass bands representing multimers or aggregates were observed. When DSP-cross-linked cell lysates were immunoprecipitated with G7 and resolved after reduction/cleavage, five major and discrete bands were observed at 180, 70, 42, 28, and 12 kDa. Purified splenic T cells were also analyzed for cell surface molecular associations (Fig. 1*B*). G7 precipitated a major band of 28 kDa from mock-cross-linked cells. As noted (6), a faint band of 45–50 kDa could sometimes be detected under these conditions. The G7 co-precipitation pattern from DSP- and DSS-cross-linked splenic T-cell lysates was similar to that for 2B4.11 cells except that the 70-kDa band was not observed. We tentatively conclude that this band represents viral glycoprotein gp70, typically found on *in vitro*-propagated cell lines (13). Identical patterns were found with the bis[2-(succinimidooxycarbonyloxy)ethyl] sulfone cross-linker, which is cleaved at high pH rather than by reduction (data not shown).

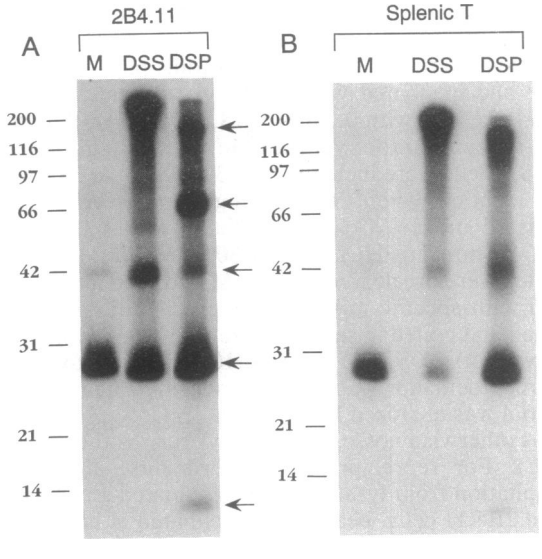

FIG. 1. Effect of chemical cross-linking on SDS/PAGE analysis of radiolabeled T-cell plasma membranes. 2B4.11 T cells (*A*) or purified murine (C3H/HeJ) splenic T cells (*B*) were iodinated and then subjected to mock cross-linking with dimethyl sulfoxide alone (lane M) or chemically cross-linked with either DSS or DSP, as indicated. Cell lysates were immunoprecipitated with G7 and analyzed by SDS/PAGE under reducing conditions. Arrows in *A* denote the major bands precipitated by G7 after cross-linking and cleavage. Molecular masses on the left are in kDa.

The same set of bands was detected when G7 was used to immunoprecipitate the lysates of DSP-cross-linked 2B4.11 variants, which bear little or no cell surface TCR, MA5.8 (14), and 21.2.2 (15) (data not shown). Therefore, none of the major species coprecipitated with Thy-1 are components of the TCR. Furthermore, the 42- and 12-kDa bands have been identified as major histocompatibility complex (MHC) class I and β_2m, respectively; G7 immunoprecipitation of lysates from DSP-cross-linked R1.1 cells (MHC class I/β_2m positive), but not the R1E variant cell (MHC class I/β_2m negative) (16), brought down bands of 180, 70, and 28 kDa but not 42 and 12 kDa (data not shown).

Chemical Cross-Linking of CD45 to Thy-1. It seemed possible that the high molecular mass band represented CD45, also known as leukocyte common antigen or T200 and which is found on most lymphocytes and hematopoietic cells (17). To address this, two sets of Thy-1$^+$, CD45$^+$ wild-type cells and their Thy-1$^+$, CD45$^-$ mutants (5) were analyzed. Anti-CD45 precipitated a 180-kDa band from lysates of DSP-cross-linked ASL1.1$^+$ cells; the same antibody precipitated nothing from the CD45$^-$ variant. The material precipitated by G7 from DSP-treated ASL1.1$^+$ cells displayed the same pattern by SDS/PAGE as 2B4.11 cells (Fig. 2*A*, note arrows).

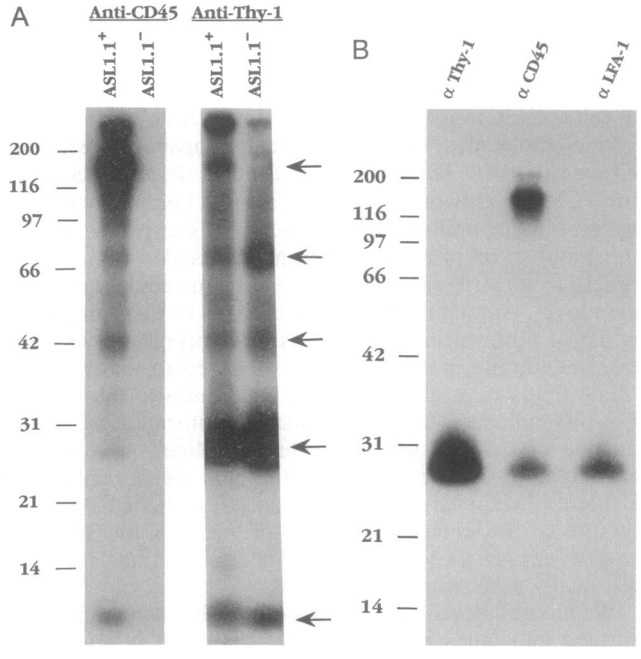

FIG. 2. Analysis of chemical cross-linking patterns of CD45$^+$ and CD45$^-$ T-cell lines. (*A*) The CD45$^+$ cell ASL1.1$^+$ and its CD45$^-$ variant, ASL1.1$^-$, were surface labeled with ^{125}I, cross-linked with DSP, and immunoprecipitated with M1/89.18.7.HK or G7; the precipitated material was analyzed by reducing SDS/PAGE. Arrows indicate the location of the major bands coprecipitated by G7 from DSP-cross-linked cells. The four lanes were from the same gel but were exposed to x-ray film for different periods of time (M1/89.18.7.HK precipitate, 48 hr; G7 precipitate, 96 hr) to obtain adequate exposure of the major bands. (*B*) After cross-linking with DSP and immunoprecipitation with G7, cross-linked proteins were eluted from the beads. Supernatants were reprecipitated with G7 (lane αThy-1), M1/89.18.7.HK (lane αCD45), or FD441.8 (lane αLFA-1).

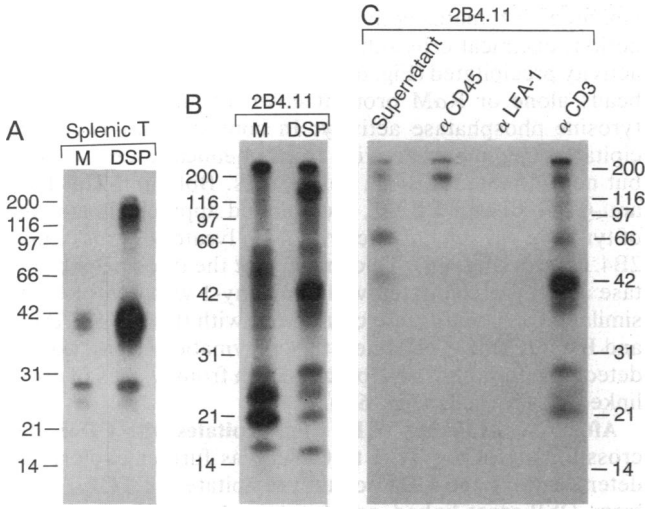

FIG. 3. Analysis of proteins chemically cross-linked to CD3. Radioiodinated purified C3H/HeJ splenic T cells (*A*) or 2B4.11 T cells (*B*) were immunoprecipitated with 2C11. Prior to lysis the cells were either mock-cross-linked (lanes M) or cross-linked with DSP, as indicated. SDS/PAGE was performed under reducing conditions. (*C*) Radioiodinated and DSP-cross-linked 2B4.11 cells were lysed and immunoprecipitated with 2C11. Some of the precipitated material was analyzed by reducing SDS/PAGE (lane αCD3). The majority of the beads were treated with 2-mercaptoethanol to reduce the cross-linker, and an aliquot of the supernatant was analyzed (lane supernatant). The remaining supernatant was then subjected to reprecipitation with either M1/89.18.7.HK or FD441.8 (lanes αCD45 or αLFA-1, respectively).

Notably, G7 coprecipitated the same set of bands from the $CD45^-$, $ASL1.1^-$ cells with the exception of the 180-kDa species. Similar results were obtained with BW^+ cells and its $CD45^-$ variant (data not shown).

To confirm the identification of the 180-kDa band, proteins from radiolabeled 2B4.11 cells were precipitated and eluted from the beads by cleavage of the cross-linker, and the supernatants were subjected to a second immunoprecipitation (Fig. 2*B*). In addition to the 28-kDa Thy-1 band (present in all lanes, probably due to leaching of G7 from the original beads), anti-CD45 specifically precipitated a 180-kDa band. No such band was seen when either anti-Thy-1 or anti-LFA-1 was used. These data clearly identify the high molecular mass species coprecipitated with Thy-1 as CD45 and are consistent with studies in which cocapping of Thy-1 and class I molecules with CD45 was demonstrated (18) or in which anti-T200 and anti-Thy-1 mAbs were found to cross-block (19).

Cross-Linking of CD45 to the TCR. We considered the possibility that CD45 might be an intermediary molecule that interacted with both Thy-1 and the TCR. After cell surface iodination and DSP cross-linker treatment of purified splenic T cells, 2C11 immunoprecipitates were analyzed by reducing SDS/PAGE (Fig. 3*A*). From lysates of mock cross-linked cells, 2C11 precipitated the CD3-ε and -δ chains as well as a relatively broad band near 42 kDa representing the α and β chains. A similar band pattern was observed with material from chemically cross-linked cells, except that the αβ band was enhanced in intensity and a new band of ≈180 kDa was observed. When a similar experiment was performed with 2B4.11 cells (Fig. 3*B*), 2C11 precipitated all of the CD3 chains as well as TCR ζ. Although visible, the αβ heterodimer was not well resolved on this gel. 2C11 precipitation after treatment with DSP yielded a well-defined αβ band of 42–45 kDa as well as an intense band of ≈180 kDa. To confirm the identity of the 180-kDa species, lysates from DSP-cross-linked 2B4.11 cells were immunoprecipitated with 2C11, the cross-linker was cleaved, and the supernatant was reprecipitated (Fig. 3*C*). Reprecipitation with anti-CD45, but not anti-LFA-1, specifically brought down the 180-kDa band.

To obtain better resolution of the high molecular mass band, reducing SDS/PAGE was performed with low-density gels (Fig. 4*C*). Anti-CD45, anti-Thy-1, and anti-CD3-ε immunoprecipitated the identical 180-kDa band from 2B4.11 cells. An anti-TCRα mAb coprecipitated the same band (data not shown). Anti-LFA-1 precipitated only the expected LFA-1 α and β chains (160–170 and 90–95 kDa, respectively). By using DSP-treated purified splenic T cells, anti-CD45 precipitated three distinct CD45 species, with bands at 180–210 kDa (Fig. 4*A*). Precipitation with anti-Thy-1 or anti-CD3 brought down the identical high molecular mass bands (Fig. 4 *A* and *B*). In contrast, anti-LFA-1 precipitated the LFA-1 heterodimer but none of the larger CD45 bands. We have obtained similar coprecipitation results with another T-cell tumor line, YAC-1. Unlike 2B4.11 cells, YAC-1 bears a cell surface interleukin 2 receptor α chain. Immunoprecipitation of DSP-cross-linked cell lysates with anti-interleukin 2 receptor mAbs does not coprecipitate CD45 (data not shown).

Coprecipitation in the Absence of Chemical Cross-Linking. To determine if these molecular associations could be detected in the absence of cross-linking, radiolabeled 2B4.11 cells were solubilized in lysis buffer containing 1% digitonin, a relatively nondisruptive detergent (Fig. 5). In addition to a 28-kDa band, anti-Thy-1 coprecipitated bands corresponding to MHC class I/β_2m, gp70, and CD45 molecules. Two uncharacterized bands of ≈90 and 116 kDa were also observed. 2C11 precipitated three faint bands corresponding to the chains of the CD3 complex as well as an easily detectable

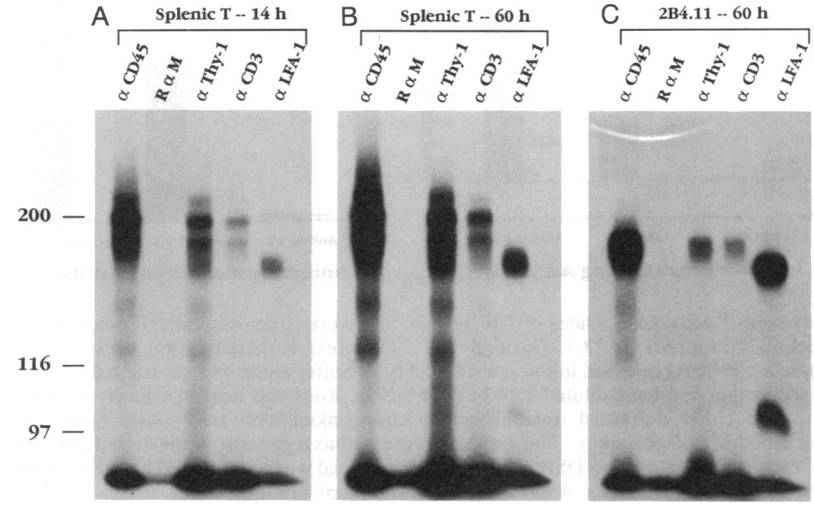

FIG. 4. Analysis of proteins chemically cross-linked to CD45. Purified splenic T cells (>98% TCR^+) (*A* and *B*) or 2B4.11 cells (*C*) were radioiodinated and cross-linked with DSP. Cell lysates were immunoprecipitated with RαM, M1/89.18.7.HK (lane αCD45), G7 (lane αThy-1), 2C11 (lane αCD3), or FD441.8 (lane αLFA-1). The precipitated material was analyzed by SDS/PAGE on a 6% acrylamide/AcrylAide gel under reducing conditions. (*A* and *B*) Same gel exposed to x-ray film for different periods of time.

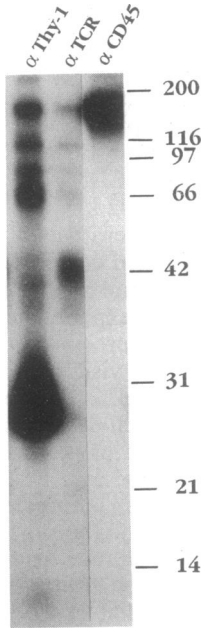

FIG. 5. Coprecipitation of CD45 in the absence of chemical cross-linking. 2B4.11 cells were labeled with ^{125}I and cross-linked with DSP. Cells were solubilized in lysis buffer containing 1% digitonin, immunoprecipitated, washed, and analyzed by SDS/PAGE. α, Anti-.

band corresponding to TCRαβ at 40–45 kDa. In addition, the higher molecular mass bands coprecipitated with Thy-1 (including the 180-kDa CD45 band) were easily visible. Precipitation from lysates of digitonin-solubilized cells with anti-LFA-1 did not bring down CD45 (data not shown).

Precipitation of Tyrosine Phosphatase Activity. CD45 is a tyrosine phosphatase (20, 21). We therefore determined if this enzymatic activity could be coprecipitated with Thy-1 or the TCR. Lysates from 2B4.11 cells that were untreated or cross-linked with DSP were immunoprecipitated with anti-CD45, anti-Thy-1, or anti-CD3 (Fig. 6). Anti-CD45 precipitated large amounts of tyrosine phosphatase activity. To adjust the activity to levels comparable to that coprecipitated by G7 (so that they could be directly compared in the same assay), anti-CD45 immunoprecipitations were performed on 2B4.11 lysates diluted 1:10 in lysis buffer. Anti-CD45 precipitated enzymatic activity that was linear over a 1-min period; chemical cross-linking did not affect the amount of activity precipitated (Fig. 6*A*). Nonspecific precipitation with beads alone or RαM brought down negligible amounts of tyrosine phosphatase activity. In contrast, anti-Thy-1 precipitated enzymatic activity from chemically cross-linked, but not untreated, 2B4.11 cell lysates. Both anti-Thy-1 and anti-CD3, but not LFA-1, precipitated appreciable amounts of tyrosine phosphatase activity from lysates of cross-linked 2B4.11 cells (Fig. 6*B*). To confirm that the tyrosine phosphatase activity precipitated with anti-Thy-1 was that of CD45, similar experiments were carried out with the BW^+ ($CD45^+$) and BW^- ($CD45^-$) cell lines; this enzymatic activity was not detected after anti-Thy-1 precipitation from lysates of cross-linked $CD45^-$ cells (Fig. 6*C*).

After Cross-Linking TCRζ Coprecipitates with CD45. The cross-linking of the TCR to CD45 was further explored by determining if anti-CD45 could precipitate the TCR ζ chain from DSP-cross-linked cell lysates. Anti-CD3-ε and anti-CD45, but not anti-LFA-1, coprecipitated TCRζ from DSP-cross-linked cells (Fig. 7). By using quantitative densitometry, it was determined that between 19% (Fig. 7*A*) and 37% (Fig. 7*B*) of the amount of TCRζ precipitated by anti-CD3 was coprecipitated with anti-CD45. These figures are comparable to estimates derived from the precipitation of tyrosine phosphatase activity from lysates of from DSP-treated cells with anti-CD3, anti-Thy-1, or anti-CD45 antibodies; 35–85% of TCRs, and 10–20% of Thy-1 molecules, were estimated to have been cross-linked to CD45 (data not shown).

DISCUSSION

CD45 is a transmembrane protein for which differences in the composition of the extracellular portions are generated as a result of glycosylation and the alternative mRNA splicing of three 5′ exons (22). The highly conserved cytoplasmic portion of CD45 is composed of two domains that are homologous to a placental tyrosine phosphatase and that have been shown to have intrinsic tyrosine phosphatase activity (20, 21). Numerous studies report an effect of anti-CD45 antibodies on lymphocyte function *in vitro*. mAbs against CD45 block cytotoxic T-cell function and natural killer cytotoxic activity and, under certain circumstances, the proliferation of B cells (23–26). Anti-CD45 antibodies also can enhance the proliferative response of T cells to lectins and anti-CD2 and anti-CD3 mAbs (27–30). It has been suggested that CD45 can

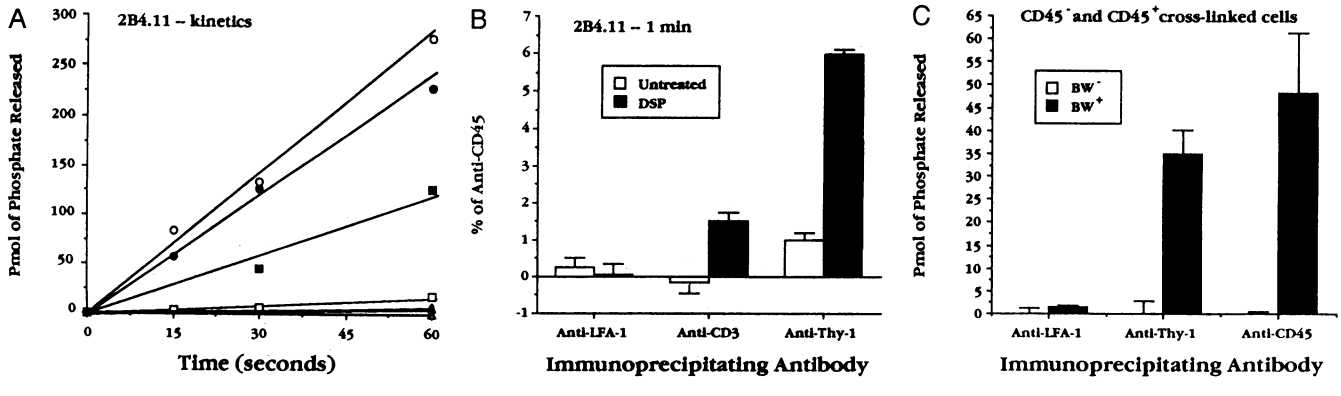

FIG. 6. Coprecipitation of tyrosine phosphatase activity from T cells. (*A*) Lysates of 2B4.11 cells that were either untreated (open symbols) or cross-linked with DSP (solid symbols) were immunoprecipitated with M1/89.18.7.HK (circles), G7 (squares), RαM (triangles), or beads alone (diamonds) and tyrosine phosphatase activity was quantitated. ^{32}P (cpm) released in the presence of lysis buffer alone was subtracted to obtain Δcpm. M1/89.18.7.HK precipitation was performed on lysates that had been diluted 1:10 in lysis buffer. RαM and beads alone are clustered near zero at 60 sec. (*B*) Lysates of 2B4.11 cells that were either untreated (open bars) or cross-linked with DSP (solid bars) were immunoprecipitated and tyrosine kinase activity was determined in a 60-sec assay. The data are expressed as a percent of the activity brought down by M1/89.18.7.HK (anti-CD45). (*C*) BW^+ and BW^- cells were treated with DSP and lysed, and material was precipitated by the indicated antibodies and assayed for tyrosine kinase activity in a 60-sec assay. SD values of duplicate determinations are indicated.

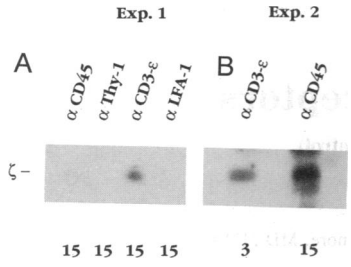

FIG. 7. 2B4.11 cells [cells ($\times 10^{-6}$) per lane, as indicated under each lane] were cross-linked with DSP, lysed, and immunoprecipitated. Immunoprecipitates were resolved by SDS/PAGE, transferred to nitrocellulose, and immunoblotted with an anti-TCRζ antiserum. Two independent experiments are shown. The 16-kDa TCRζ band is indicated. (*A* and *B*) Experiments 1 and 2, respectively.

modulate T-cell signal transduction by interacting with other cell surface molecules (31, 32).

The observation that a tyrosine phosphatase is physically associated with a substantial fraction of cell surface TCRs may have profound implications for T-cell biological responses. Activation by the TCR or Thy-1 is known to increase the activity of one or more tyrosine kinases, resulting in phosphorylation of, among other proteins, the TCR ζ chain (33). In light of the close proximity of these molecules, it is possible that the TCR or Thy-1 modulate the enzymatic activity of CD45 or perhaps the accessibility of critical subtrates. The removal of phosphates from tyrosyl residues could have pleotropic effects on T-cell function. For example, increases in CD45 enzymatic activity could enhance tyrosine kinase activity by dephosphorylation of tyrosine kinase regulatory tyrosine residues (34, 35), whereas decreases in phosphatase activity could lead to exaggerated tyrosine kinase-mediated effects. Alternatively, the removal of phosphates from some tyrosine substrates could antagonize the effects of tyrosine kinases involved in activation pathways. Indeed, CD45 might regulate complex biological functions by promoting all of these effects.

Although we were unable to detect a direct Thy-1–TCR interaction, the mutual association of CD45 with Thy-1 and the TCR may provide a simple mechanistic explanation for how the latter two molecules "talk" to each other. It is not known if all three molecules can be found in the same multimeric complex or if Thy-1 and the TCR associate with CD45 independently. If the former is true, then cross-linking of Thy-1 may be tantamount to cross-linking the TCR, thereby generating the characteristic changes in inositol phospholipid hydrolysis, Ca^{2+} levels, and protein kinase C activation. In either case, a functional association between Thy-1 and the TCR might be due, at least in part, to the CD45-mediated removal of phosphates from the TCR ζ chain. Furthermore, direct interaction of Thy-1 with CD45 might explain why antibodies to Thy-1 can still elicit a subset of activation events (e.g., enhanced tyrosine kinase activity) in the absence of the TCR (36). It is also worth noting that CD2, another activating molecule that requires coexpression of the TCR to induce a complete cellular response, has been found to associate with CD45 on murine T cells (37). The possibility thus arises that the TCR and a number of activating cell surface molecules form functional associations whose signaling utilizes the CD45 tyrosine phosphatase. Pingel and Thomas (38) have reported that $CD45^-$ variants of a murine normal T-cell clone fail to proliferate when stimulated with antigen or anti-CD3 antibodies. Unfortunately, the $CD45^-$ T-cell variants used for our biochemical studies lack cell surface TCR (unpublished observation). In any case, the data in the current report provide a structural basis for the influence of CD45 on TCR-mediated signal transduction and cellular activation.

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