Human Homologue of the Drosophila Discs Large Tumor Suppressor Binds to p56^ck Tyrosine Kinase and Shaker Type Kv1.3 Potassium Channel in T Lymphocytes*

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Human homologue of the Drosophila discs large tumor suppressor protein (hDlg) belongs to a newly discovered family of proteins termed MAGUKs that appear to have structural as well as signaling functions. Consistent with the multi-domain organization of MAGUKs, hDlg consists of three copies of the PDZ (PSD-95/Discs large/Discs large O-1) domain, an SH3 motif, and a guanylate kinase-like domain. In addition, the hDlg contains an amino-terminal proline-rich domain that is absent in other MAGUKs. To explore the role of hDlg in cell signaling pathways, we used human T lymphocytes as a model system to investigate interaction of hDlg with known tyrosine kinases. In human T lymphocyte cell lines, binding properties of hDlg were studied by immunoprecipitation, immunoblotting, and immune complex kinase assays. Our results show that protein tyrosine kinase activity is associated with the immunoprecipitates of hDlg. Immunoblotting experiments revealed that the immunoprecipitates of hDlg contain p56^ck, a member of the Src family of tyrosine kinases. The specificity of the interaction is demonstrated by the lack of p59^shc, a member of the Src family of tyrosine kinases. The presence of distinct protein modules including the PDZ domain, SH3 domain, and guanylate kinase-like domain (3, 4). hDlg is a peripheral membrane protein associated with the membrane cytoskeleton presumably via its protein 4.1-binding domain (5, 6). The PDZ domains of hDlg have been shown to interact with the carboxyl termini of several proteins including Shaker-type K^+ channels and adenomatous polyposis coli tumor suppressor protein (7, 8). Unlike other MAGUKs, hDlg contains a proline-rich amino-terminal domain with two potential SH3 domain binding sites (1, 9). The presence of these consensus binding sites suggests that hDlg participates in signaling pathways by forming protein complexes via the SH3 domains of other proteins.

The Shaker-related channel Kv1.3 plays a critical role in modulating the membrane potential of T lymphocytes (10, 11). Many structurally dissimilar peptide and nonpeptide blockers of the Kv1.3 channel inhibit mitogen-induced [3H]thymidine incorporation and interleukin-2 production by T cells in vitro (12–17) and immune responses in vivo (18). These antagonists are thought to chronically depolarize the T cell membrane, reduce calcium entry via calcium-activated release calcium channels in the plasma membrane, and consequently inhibit the calcium signaling pathway essential for lymphocyte activation (10, 11). Due to its restricted tissue distribution (19) and distinct mechanism of action, Kv1.3 is widely recognized as a therapeutic target for novel immunosuppressive drugs that may prove useful for transplantation therapy as well as for the treatment of autoimmune disorders (16, 18).

The stimulation of Fas receptor leads to rapid tyrosine phosphorylation of Kv1.3 channel and dramatic inhibition of potassium channel current in Jurkat T cells (20). The Fas-induced tyrosine phosphorylation of Kv1.3 channel is not observed in Jurkat cells lacking p56^ck (JCaM1), suggesting that Kv1.3 channel is phosphorylated by p56^ck tyrosine kinase in vivo (20). It is noteworthy here that the Src tyrosine kinase phosphorylates human Kv1.5 channel and suppresses its channel current in the transfected human embryonic kidney cells (21). A proline-rich motif within the cytoplasmic domain of Kv1.5 channel has been identified as the binding site for the SH3 domain of Src tyrosine kinase (21). In contrast, the cytoplasmic domain of Kv1.3 channel does not appear to conform to known SH3 binding consensus motifs, which may facilitate its binding to p56^ck tyrosine kinase. Therefore, the mechanism by which p56^ck is recruited to Kv1.3 channel in T lymphocytes remains unknown.

In this study, we report that hDlg binds independently to p56^ck tyrosine kinase and Kv1.3 channel in human T lymphocytes. Our results suggest a mechanism by which hDlg could recruit p56^ck to the cytoplasmic domain of Kv1.3 channel in human T lymphocytes.

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**hDlg is the closest human homologue of the Drosophila discs large tumor suppressor protein (1, 2). It belongs to a rapidly expanding family of proteins termed MAGUKs (membrane-associated guanylate kinases). MAGUKs are characterized by the presence of distinct protein modules including the PDZ domain, SH3 domain, and guanylate kinase-like domain (3, 4).**

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**hDlg Interaction with p56^{ck} and Kv1.3 Channel in T Lymphocytes**

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Antibodies—**Human T cell leukemia cell line Jurkat J77 was maintained in RPMI 1640 supplemented with 10% fetal calf serum, 1.0 mM sodium pyruvate, 4.0 mM t-glutamine, and necessary supplements. Polyclonal antibodies against hDlg were generated by injecting a unique amino-terminal peptide (DRSKPSEPIQPVN) of hDlg in rabbits. Serum was affinity-purified by chromatography on a column of protein A-Sepharose coupled with the immunizing peptide. A mouse monoclonal antibody (Rw2–8C8) and a control monoclonal antibody (Enn69–11c5) were kindly provided by Dr. Ellis Reinherz of the Dana Farber Cancer Institute (Boston, MA). Baculovirus-expressed human p56^{ck} was a gift from Dr. M. Eck of the Children’s Hospital, Harvard Medical School (Boston, MA). The properties of the baculovirus-produced p56^{ck} have been published previously (22). Anti-phosphotyrosine monoclonal antibodies (4G10), anti-phosphotyrosine antibodies (4G10), anti-p56^{ck} (3A5) and anti-p59^{fyn} (Fyn15) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

**Generation of Glutathione S-Transferase Fusion Proteins—**GST fusion proteins of hDlg were generated using standard procedures (GST Gene Fusion System, Pharmacia Biotech Inc.). GST-hDlg protein corresponds to the full-length hDlg including insertions I-1 and I-3 (1). Gene Fusion System, Pharmacia Biotech Inc.). GST-hDlg protein was resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and immunoblotted with the appropriate antibodies. Blots were developed by ECL (Amersham Corp.). For lipid kinase assay, the GST-hDlg fusion proteins were probed with a bacterially affinity-purified antibody (1).

**Immunoprecipitation, Immunoblotting, and Lipid Kinase Assay—**Cells were lysed in lysis buffer (1% Triton X-100, 0.5% Nonidet P-40, 50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.5% Nonidet P-40, 1 mM NaF, 1 mM sodium orthovanadate, 2.0 mM phenylmethylsulfonyl fluoride, 10 μg/ml each of aprotinin, leupeptin, and pepstatin) for 30 min at 4 °C, and the lysates were cleared by centrifugation at 15,000 rpm for 30 min. The supernatant of the lysates were precleared with 50 μl of protein A-Sepharose CL-4B (Pharmacia) for 2 h at 4 °C and then used for immunoprecipitation or pull-down assay with GST fusion proteins. For immunoprecipitation, lysate was incubated with an appropriate antibody for 4 h in 4 °C and then with protein A-Sepharose for 1 h. For pull-down assay with GST fusion proteins, the lysate was incubated with GST fusion protein attached to glutathione-Sepharose beads for 4 h in 4 °C. The precipitated beads were washed six times with the lysis buffer and once with PBS and solubilized in SDS sample buffer. The proteins were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and immunoblotted with the appropriate antibodies. Blots were developed by ECL (Amersham Corp.). For lipid kinase assay, the GST-fused hDlg-expressing transfected lysates were washed six times with lysis buffer, four times with PI 3-kinase buffer (25 mM MOPS, pH 7.0, 5.0 mM MgCl₂, 1.0 mM EDTA, 1.0 mM NaF, 1.0 mM Na₃VO₄, 2.0 mM phenylmethylsulfonyl fluoride, 10 μg/ml each of aprotinin, leupeptin, and pepstatin) for 30 min at 4 °C, and the lysates were cleared by centrifugation at 15,000 rpm for 30 min. The supernatant of the lysates was preincubated with 50 μl of protein A-Sepharose CL-4B (Pharmacia) for 2 h at 4 °C and then used for immunoprecipitation or pull-down assay with GST fusion proteins. For immunoprecipitation, lysate was incubated with an appropriate antibody for 4 h in 4 °C and then with protein A-Sepharose for 1 h. For pull-down assay with GST fusion proteins, the lysate was incubated with GST fusion protein attached to glutathione-Sepharose beads for 4 h in 4 °C. The precipitated beads were washed six times with the lysis buffer and once with PBS and then resolved by SDS-polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membrane and immunoblotted with anti-phosphotyrosine antibody (4G10). Blots were developed using an ECL kit (Amersham Corp.).

**In Vitro Binding of Recombinant p56^{ck} and GST-hDlg Fusion Protein—**2.0 μg of purified baculovirus-expressed p56^{ck} (residues 53–509 of human p56^{ck}) (22) was incubated with either GST or GST-hDlg fusion proteins bound to 10 μl of glutathione Sepharose beads in 250 μl of lysis buffer at 4 °C for 3 h. The beads were washed six times with lysis buffer and once with PBS and then resolved by SDS-polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membrane and immunoblotted with anti-p56^{ck} mAb (3A5).

**Surface Plasmon Resonance Measurements—**A BIAcore biosensor instrument (Pharmacia Biosensor) was used to detect binding interactions between purified p56^{ck} and NVH-terminal segment of hDlg. Purified p56^{ck} was immobilized on the surface of a CM5 sensor chip by amine coupling (24). Approximately 530 resonance units of immobilized p56^{ck}, which corresponds to ~0.5 ng protein/mm² surface was obtained. Purified hDlg(N) protein, which was cleaved from GST-NT fusion protein using a thrombin-specific cleavage site, was injected in HBS (10 mM HEPES, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.02% surfactant P20); continuous flow buffer. After each protein injection, the p56^{ck}–immobilized surface was regenerated with two short pulses of 0.03% SDS. The background nonspecific binding of hDlg(N) and the contribution of bulk solution in SPR signal were determined by injecting a hDlg(N) solution in HBS onto a blank CM5 sensor chip surface activated with NHS/EDC and blocked with 1.0 M hydroxylamine hydrochloride.

**RESULTS**

**Protein Tyrosine Kinase Activity Is Associated with the Immunoprecipitates of hDlg—**A functional role of MAGUKs has been suggested in signaling pathways. However, no evidence exists to support this hypothesis in a mammalian system. The presence of two potential tyrosine phosphorylation sites in hDlg (located between the protein 4.1-binding domain and guanylate kinase-like domain) suggests that hDlg may be tyrosine phosphorylated. We used a phosphotyrosine-immunoblotting antibody to test the hDlg protein phosphorylation of hDlg in J77 cells. Immunoprecipitation of p56^{ck} served as a positive control. As expected, p56^{ck} immunoprecipitated from nonactivated J77 cells was tyrosine phosphorylated (Fig. 1A, lane 1). Incubation of p56^{ck}-immune complexes with ATP further enhanced tyrosine phosphorylation of p56^{ck} (Fig. 1A, lane 2). In contrast, hDlg protein immunoprecipitated from nonactivated J77 cells was not tyrosine phosphorylated (Fig. 1A, lane 3).
Fig. 1. Immune complex in vitro kinase assay and anti-phenosphotyrosine Western blot analysis. Nonactivated J77 cells (5 × 10⁶ cells/lane) were lysed in lysis buffer. A, immunoprecipitations were performed either with anti-p56<sup>lck</sup> (lanes 1 and 2) or anti-hDlg (lanes 3 and 4). Immune complexes were incubated with 0.5 mM ATP (lanes 2 and 4) and transferred to nitrocellulose, and blots were analyzed using anti-phenosphotyrosine monoclonal antibodies (4G10). The position of phenosphotyrosylated hDlg is marked with an asterisk (lane 4). B, lysates from nonactivated J77 cells were incubated with glutathione beads containing GST-hDlg fusion protein. After extensive washing of beads with the lysis buffer, beads were incubated with ATP and analyzed using anti-phenosphotyrosine monoclonal antibodies (4G10). The position of phenosphotyrosylated hDlg is shown with an asterisk in lane 2. Note that the tyrosine phosphorylated bands are detectable only after incubation of beads with ATP.

J77 cells was not tyrosine phosphorylated (Fig. 2A, lane 3). Similarly, the hDlg protein immunoprecipitated from CD3-activated J77 cells was also not tyrosine phosphorylated (data not shown). However, incubation of beads containing hDlg immune complexes with ATP resulted in the tyrosine phosphorylation of a 120-kDa protein (Fig. 1A, lane 4, asterisk). The hDlg immunoprecipitates also contained a tyrosine phosphorylated protein of 55–60 kDa that migrated just above the IgG band (Fig. 1A, lane 4). In addition, CD3-mediated activation of J77 cells did not modulate tyrosine phosphorylation of hDlg-associated proteins as detected by in vitro kinase assay (data not shown).

To determine whether the tyrosine phosphorylated 120-kDa protein was hDlg, bacterially expressed GST-hDlg fusion protein coupled to glutathione-Sepharose beads was incubated with J77 cell lysate. The GST-hDlg beads were then sedimented, incubated with ATP, and immunoblotted using anti-phenosphotyrosine antibodies. Again, two tyrosine phosphorylated proteins of 110 and 55–60 kDa were detected, indicating that bacterially expressed hDlg can be tyrosine phosphorylated by protein kinases that are present in J77 cells (Fig. 2A, lane 1). As shown in Fig. 2A, lane 2, 4, and 5, phenosphotyrosylated proteins were specifically associated with anti-p56<sup>lck</sup> but not with anti-hDlg.

Fig. 2. Coimmunoprecipitation of p56<sup>lck</sup> with hDlg. A, lysis buffer lysate from J77 cells (2 × 10⁶ cells/lane) was incubated with either protein A beads alone (lane 1) or protein A beads containing anti-hDlg (lane 2). After extensive washing of the beads, bound proteins were analyzed by Western blotting using an anti-p56<sup>lck</sup> monoclonal antibody (3A5). The locations of IgG band and p56<sup>lck</sup> are indicated by an arrow and an arrowhead, respectively (lane 2). B, J77 lysate was immunoprecipitated using normal rabbit serum (NRS) (lane 2) and anti-hDlg Ab (lane 3). Western blotting with anti-p59<sup>56kDa</sup> mAb (fyn15) did not detect any p59<sup>56kDa</sup> in hDlg immunoprecipitates. Lane 1 shows the presence of p59<sup>56kDa</sup> in J77 lysate. C, J77 lysate (5 × 10⁶ cells/sample) was immunoprecipitated with a control antibody (11c5) (lanes 1 and 2), anti-hDlg (lanes 3 and 4), and anti-PI 3-kinase (lanes 5 and 6). Lipid kinase activity was assayed in the presence and the absence of 0.5% Nonidet P-40 (NP40), which is known to inhibit the activity of PI 3-kinase. As shown in lane 3, no detectable amount of phosphatidylinositol phosphate was produced by hDlg immunoprecipitates.

Direct Binding of p56<sup>lck</sup> with the NH₂-terminal Segment of hDlg—Using GST fusion proteins of hDlg, a sedimentation assay was performed to determine the binding site of p56<sup>lck</sup> within hDlg. The GST-NT fusion protein contained amino acids 1–229 of hDlg including its proline-rich insertion I-1. The GST-hDlgNT fusion protein contained amino acids 201–926 of hDlg (Fig. 3). The GST-hDlg fusion protein was immobilized on glutathione beads and incubated with the lysate of nonactivated J77 cells. Proteins that bound to the fusion proteins were detected by immunoblotting using an anti-p56<sup>lck</sup> mAb. As shown in Fig. 4A, p56<sup>lck</sup> bound specifically to GST-hDlg but not to GST-hDlgNT fusion protein. Similar sedimentation assays using hDlg fusion proteins failed to coprecipitate p59<sup>56kDa</sup> further, supporting the specificity of the interactions (data not shown). To
pressed in Sf9 cells (22). Again, p56Lck
washing of the beads, bound p56
amino-terminal (GST-NT) fusion proteins of hDlg.
channel-clustering proteins
made in neuronal cells, imply that MAGUKs functioned as
3lysate (2
Shaker-related Kv1 family proteins directly in-
Lymphocytes—-
domain binding motif, (T/S)
confirm direct association of p56Lck with the NH2-terminal segment
of hDlg, GST-NT fusion protein, in addition to GST-hDlg and
GST-hDlgANT, was incubated with purified p56Lck expressed in Sf9 cells (22). Again, p56Lck specifically associated with GST-hDlg and GST-NT but not with the GST-hDlgANT fusion protein (Fig. 4B). Further evidence supporting direct association between p56Lck and hDlg was obtained from surface plasmon resonance measurements. The NH2-terminal segment of hDlg without GST (Fig. 3) specifically interacted with p56Lck immobilized onto a CM5 sensor chip surface (data not shown). Although concentration dependence was apparent in the binding isotherm, precise quantification of the binding was unsuccessful due to the weak nature of the binding interaction and the sensitivity limitations of the BIAcore biosensor instrument. We estimate the Kd value in the mM range for in vitro binding between p56Lck and recombinant NH2-terminal domain of hDlg. In summary, these results show that purified p56Lck directly binds to the proline-rich NH2-terminal domain of hDlg.

hDlg Forms a Stable Complex with the Kv1.3 channel in T Lymphocytes—Shaker-related Kv1 family proteins directly interact with hDlg, PDS-95, and Chapsyn-110 through a PDZ domain binding motif, (T/S)XXV (7, 26). These observations, made in neuronal cells, imply that MAGUKs functioned as channel-clustering proteins in vivo (27, 28). Because the Kv1.3 channel is expressed principally in lymphocytes (19) and because the carboxyl terminus of Kv1.3 contains a PDZ domain binding motif (TDV), we tested whether hDlg associated with the Kv1.3 channel in T lymphocytes. Using a vaccinia virus/T7 hybrid expression system (29), the Kv1.3 channel was expressed in human J77 and JCaM1 (p56Lck-deficient) T cells as an epitope-tagged fusion protein. The use of this expression system was necessitated because of the lack of antibodies that can distinguish Kv1.3 in cells. Immunoblot analysis revealed that the T7-tagged Kv1.3 protein migrated as a 64-kDa band (Fig. 5A), which is consistent with its predicted size (29). Interestingly, the expression of epitope-tagged Kv1.3 protein was significantly lower in J77 cells as compared with the p56Lck deficient-JCaM1 cells (Fig. 5, compare lanes 1 and 2), although the reasons for this differential expression remain an enigma. Immunoprecipitation of hDlg with a specific polyclonal antibody from both J77 and JCaM1 cells also coprecipitated the Kv1.3 fusion protein (Fig. 5A, lanes 5 and 6). In parallel experiments, the Kv1.3 protein was immunoprecipitated from JCaM1 cells with an anti-T7 monoclonal antibody, and subsequent immunoblotting with the anti-hDlg antibody revealed the presence of hDlg in the precipitate. Together, these results show that hDlg directly associates with the Kv1.3 protein in human T lymphocytes, and the binding is independent of the presence of p56Lck.

DISCUSSION

Activation of T lymphocytes and their elimination via apoptosis are key events required for the maintenance of immune homeostasis. These events are initiated by the transmission of extracellular signals to the cell interior via distinct transmembrane proteins (30, 31). A common theme in T cell activation and apoptosis is the immediate and rapid phosphorylation of multiple substrates by Src family tyrosine kinases p56Lck and p59Fyn (32–35). p56Lck is predominantly expressed in T lymphocytes associated with the cytoplasmic domains of CD4/CD8 co-receptors and plays a critical role in T cell activation and thymocyte development (32, 36). In addition to the T cell receptor signaling, p56Lck also mediates signaling through CD28, CD44, and interleukin-2 receptor (37–39). The data reported in this paper establish direct binding of p56Lck with the human homologue (hDlg) of the Drosophila discs large tumor suppressor protein.

The interaction between p56Lck and hDlg appears to be constitutive and independent of CD3/T cell receptor-mediated T cell activation. CD3 cross-linking of J77 cells does not appear to affect hDlg interactions with other T cell proteins as assessed by metabolic radiolabeling and immunoprecipitation techniques (data not shown). In vivo, hDlg is not tyrosine phosphorylated when immunoprecipitated from either unstimulated or activated J77 cells (Fig. 1). In contrast, incubation of hDlg immunoprecipitates with ATP induces tyrosine phosphorylation of hDlg (Fig. 1). Although we have not yet identified signaling pathways that can stimulate tyrosine phosphorylation of hDlg in vivo, the presence of two potential tyrosine phosphorylation sites located near the protein 4.1-binding domain of hDlg (25) may have important physiological consequences. Phosphorylation of these tyrosines and the effect of
channels is not resolved (20). The cytoplasmic domain of Kv1.3 terminal segment of hDlg (Figs. 3 and 4). Because the proline-rich sequences of hDlg. This proposal is further supported by the fact that the proline-rich sequences encoded in the alternatively spliced insertions I-1 in hDlg appear likely to mediate its specific interaction with the SH3 domain of p56\(^{lck}\), the expression of tissue-specific isoforms of hDlg will likely determine its binding function in a particular tissue. Moreover, the specificity of the hDlg-p56\(^{lck}\) interaction in a given cell type is supported by the fact that hDlg does not bind to other SH3 domain-containing proteins such as p59\(^{fyn}\) and PI-3 kinase in T lymphocytes.

This phosphorylation on hDlg-protein 4.1 binding, as well as its effect on the nuclear localization of hDlg, are currently under investigation.

The discovery of a ~56-kDa tyrosine phosphorylated protein in hDlg immunoprecipitates and in the GST-hDlg precipitated from J77 lysates prompted us to investigate whether hDlg is associated with p56\(^{lck}\), a Src-like tyrosine kinase. The recombinant p56\(^{lck}\) used in this study was engineered to encode the SH3, SH2, and protein kinase domains (amino acids 53–509) (22), and the p56\(^{lck}\) binding site was localized to the NH\(_2\)-terminal segment of hDlg (Figs. 3 and 4). Because the proline-rich domain present in the NH\(_2\)-terminal segment of hDlg contains two potential SH3 binding motifs (1, 9), it appears likely that the hDlg-p56\(^{lck}\) interaction is mediated by the direct binding of SH3 domain of p56\(^{lck}\) with the proline-rich sequences of hDlg. This proposal is further supported by the fact that the in vitro interaction between p56\(^{lck}\) and hDlg(N) is relatively weak consistent with the lower affinity of known SH3 domain-mediated interactions (40).

Our results provide evidence of the direct association of a tyrosine kinase with a member of the MAGUK family and may have important physiological consequences. hDlg may play a role in tyrosine phosphorylation of Kv1.3 channel in T cells (41). Although Kv1.3 channels are known to be phosphorylated by p56\(^{lck}\), the issue of how p56\(^{lck}\) is recruited to these ion channels is not resolved (29). The cytoplasmic domain of Kv1.3 channel lacks consensus proline-rich sequences that might bind the SH3 domain of p56\(^{lck}\) (19), in the way that Kv1.5 directly binds to the SH3 domain of Src tyrosine kinase (21). However, Kv1.3 channel contains the PDZ domain binding consensus sequence in its COOH terminus cytoplasmic tail and was shown to bind PDZ domain of hDlg as well as its close relative, PSD-95, in neuronal cells (7, 26). Our results provide the first evidence of the in vivo interaction between a MAGUK and Shaker type potassium channel in non-neuronal cells. Because of the lack of a specific antibody against Kv1.3 channel, we used T7 epitope-tagged Kv1.3 channel expressed in T lymphocytes. Immunoprecipitation of hDlg coprecipitated Kv1.3 channel, whereas immunoprecipitation of T7-tagged Kv1.3 channel coprecipitated hDlg (Fig. 5). It is relevant to note here that native Kv1.3 channel expressed in T lymphocytes migrates as a 65-kDa protein (42), and the immunoprecipitation of hDlg from metabolically radiolabeled J77 cells coprecipitates a 65-kDa protein that is likely to be Kv1.3 channel (data not shown). These results suggest that hDlg associates with native as well as epitope-tagged Kv1.3 channel expressed in T lymphocytes. In summary, our results are consistent with the possibilities for hDlg to form independent complexes with p56\(^{lck}\) and Kv1.3, although it is intriguing to consider the possibility that hDlg functions as an adaptor protein to bring p56\(^{lck}\) in proximity to Kv1.3 channel and allow the tyrosine phosphorylation to take place. We were, however, unable to verify the latter possibility because the expression of epitope-tagged Kv1.3 channel was very poor in J77 cells, and the endogenous Kv1.3 levels in these cells were below the level of detection by immunoprecipitation and immunoblotting assays.

The hDlg transcript and protein isoforms are ubiquitously distributed (1), whereas p56\(^{lck}\) expression is restricted largely to T cells (32). Although we could not detect binding of hDlg with p59\(^{fyn}\), it is possible that hDlg associates with other Src family tyrosine kinases in nonhematopoietic cells. Because the proline-rich sequences encoded in the alternatively spliced insertion I-1 in hDlg appear likely to mediate its specific interaction with the SH3 domain of p56\(^{lck}\), the expression of tissue-specific isoforms of hDlg will likely determine its binding function in a particular tissue. Moreover, the specificity of the hDlg-p56\(^{lck}\) interaction in a given cell type is supported by the fact that hDlg does not bind to other SH3 domain-containing proteins such as p59\(^{fyn}\) and PI-3 kinase in T lymphocytes. Our observation that the complete deficiency of p56\(^{lck}\) in JCaM1 cells does not affect binding of hDlg with Kv1.3 channel is also consistent with the proposed function of hDlg as an adaptor protein coupling Kv1.3 channel to p56\(^{lck}\) in T lymphocytes. The restricted expression of Kv1.3 channel in lymphocytes and brain (19) lends credence to our hypothesis that hDlg may link novel tyrosine kinases to potassium channel in lymphocytes as well as in neuronal cells.

The hDlg-mediated recruitment of tyrosine kinases to specific sites may couple these critical enzymes to cytoskeletal proteins such as p59\(^{fyn}\) and PI-3 kinase in T lymphocytes. Our observation that the complete deficiency of p56\(^{lck}\) in JCaM1 cells does not affect binding of hDlg with Kv1.3 channel is also consistent with the proposed function of hDlg as an adaptor protein coupling Kv1.3 channel to p56\(^{lck}\) in T lymphocytes. The restricted expression of Kv1.3 channel in lymphocytes and brain (19) lends credence to our hypothesis that hDlg may link novel tyrosine kinases to potassium channel in lymphocytes as well as in neuronal cells.

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