Regulation of Casein Kinase 2 by Direct Interaction with Cell Surface Receptor CD5*

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The transmembrane protein CD5, expressed on all T cells and the B1 subset of B cells, modulates antigen receptor-mediated activation. We used the yeast two-hybrid system to identify proteins that interact with its cytoplasmic domain and play a role in CD5 proximal signaling events. We found that the β subunit of the serine/threonine kinase casein kinase 2 (CK2) interacts specifically with the cytoplasmic domain of CD5. Co-immunoprecipitation experiments showed activation-independent association of CK2 with CD5 in human and murine B and T cell lines and murine splenocytes. The interaction of CK2 holoenzyme with CD5 is mediated by the amino terminus of the regulatory subunit β. CK2 binds and phosphorylates CD5 at the CK2 motifs flanked by Ser^459 and Ser^461. Cross-linking of CD5 leads to the activation of CD5-associated CK2 in a murine B-lymphoma cell line and a human T-leukemia cell line and is independent of net recruitment of CK2 to CD5. In contrast, CK2 is not activated following cross-linking of the B cell receptor complex or the T cell receptor complex. This direct regulation of CK2 by a cell surface receptor provides a novel pathway for control of cell activation that could play a significant role in regulation of CD5-dependent antigen receptor activation in T and B cells.

CD5 is a 67-kDa glycoprotein that belongs to the cysteine-rich macrophage scavenger receptor family of proteins expressed on all thymocytes and T cells and a subset of B cells, described as B1a B cells (CD5 B cells) (1–4). It is expressed on T cells very early in development, before the expression of the TCR-CD3 complex, and during progressive stages of thymocyte development, the level of CD5 expression increases, suggesting a role in thymocyte biology (5). In cells of B-lineage, the onset of CD5 expression is not well defined, but it is expressed in all Abelson transformed lines, which represent the pre-B stage (6, 7). Proposed counter-receptors for CD5 include the B cell-specific CD72, gp35–37, which is expressed on activated splenocytes and activated T cell clones, and the Ig V_H framework region (8–11). The functional significance of these candidates in context with CD5 activation has not been established.

CD5 is physically associated with the antigen receptor complex in both T and B cells and modulates intracellular signals initiated by both TCR and BCR (12–14). The conserved cytoplasmic domain of CD5 contains four tyrosines and several sites for serine and threonine phosphorylation (15–20). Two of the tyrosines form an imperfect immunoreceptor tyrosine activation motif (21, 22). The serine/threonine sites include four CK2-dependent serine phosphorylation sites and a protein kinase C-dependent threonine phosphorylation site. TCR cross-linking leads to rapid tyrosine phosphorylation followed by serine/threonine phosphorylation of CD5 (14, 23, 24). In contrast, CD5 ligation leads to tyrosine kinase activation and tyrosine phosphorylation of several substrates but only to serine phosphorylation of its own cytoplasmic domain (25, 26). CD5 can associate with p56^ck and Zap70, but it is unknown if these tyrosine kinases are involved in tyrosine phosphorylation of CD5 in cells (27, 28).

Mitogenic CD5 antibodies cooperate with antibodies to CD28 to induce proliferation in mature T cells in the absence of TCR-CD3 stimulation (29–31). CD5 ligation synergizes with CD3 stimulation to increase intracellular calcium, interleukin-2 secretion, and interleukin-2 receptor expression (32–35) and is involved in both TCR-dependent and -independent activation of diacylglycerol production (36). These results suggest that in mature T cells, CD5 functions as a co-stimulatory molecule of T cell activation. In contrast, CD5 appears to attenuate TCR-CD3-induced signals in thymocytes (37). Single positive thymocytes from CD5-deficient mice exhibit enhanced proliferation to TCR-CD3-induced signals, with hyperphosphorylation of Vav and phospholipase C-γ, and enhanced intracellular calcium mobilization. In mature B1a B cells, CD5 appears to function as a negative regulator of BCR-induced signals (38). The basis of these opposing effects of CD5 signaling in immature and in mature thymocytes is unclear.

To define the molecules that may interact with CD5 and play a role in CD5-proximal signaling, we used the yeast two-hybrid system. The entire 94-amino acid cytoplasmic domain of human CD5, Y378-L471, was fused to the GAL4 binding domain (BD) and used as a “bait” to screen a GAL4 activation domain (AD) DNA library prepared from Epstein-Barr virus-transformed human peripheral blood lymphocytes. Using this approach, we show that casein kinase 2 (CK2), a serine/threonine kinase, interacts specifically with the cytoplasmic domain of CD5. The interaction of CK2 with CD5 is constitutive in human and mouse cell lines and murine splenocytes and is mediated by the regulatory β subunit of the tetrameric CK2 holoenzyme. We have mapped the CK2 binding and phosphorylation sites on CD5 to the two carboxyl-terminal CK2 motifs and have demonstrated CD5-dependent activation of CK2 in both B and T cell lines. This recruitment of a novel signaling pathway by

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¶The abbreviations used are: TCR, T cell receptor; BCR, B cell receptor; CK2, casein kinase 2; mAb, monoclonal antibody; BD, binding domain; AD, activation domain; UT, untranslated.
CD5 is likely to have significant implications for CD5-dependent regulation of TCR- and BCR-induced activation.

**EXPERIMENTAL PROCEDURES**

**Cell Lines, Tissue, and Reagents**—Murine B-lymphoma cell lines CH12 (gift from Dr. John F. Kearney) and the human T-leukemia cell line Jurkat (gift from Dr. Louis B. Justement) were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (Life Technologies, Inc.). Murine spleens were obtained from 6-8-week-old Balb/c mice (The Jackson Laboratory, Bar Harbor, ME). Anti-mouse CD5 mAb (clone 53–73–3), anti-mouse CD19 mAb (clone HIB19) were purchased from Pharmingen (San Diego, CA). Polyclonal anti-CD5 rabbit serum was used to generate the peptide sequence, TASHVDNEYSQPPR, in the CD5 cytoplasmic domain in a gift from Drs. Greg Appleyard and Bruce Wilkie (39). Goat anti-rabbit Ig, F(ab")2 fraction, and peroxidase-conjugated goat anti-rabbit IgG were from Jackson ImmunoResearch Laboratories (West Grove, PA). Protein A-agarose were obtained from Life Technologies, Inc., and SuperSignal chemiluminescence substrate was obtained from Pierce. Anti-mouse CD5 antibody was conjugated to agarose using the EDC kit from Pierce. The CD5-derived peptide DNSSDSYDILHQAQR containing the CD5 cytoplasmic domain residues 456–471, was obtained from Bio Synthesis (Lewisville, TX), and the standard CK2 substrate peptide RRREEEEE was obtained from Research Genetics (Huntsville, AL). Purified CK2 was purchased from Amersham Pharmacia Biotech.

**Yeast Two-hybrid Screen**—To generate the GAL4 binding domain-CD5 cytoplasmic domain (CD5) fusion, we amplified by polymerase chain reaction the cDNA representing the 94-amino acid cytoplasmic domain (Tyr378–Leu471) from the CD5 cDNA clone, pT2–2 (15) using sense primer 5'-GCTGATGCTCGACGACGGTGACTATGATCTG-3' and antisense 5'-AATGGATCCCTGACAACTCCGGCGACGGTGACTATGATCTG-3' primers and cloned the product into the pGBT9 vector (CLONTECH). After determining the accuracy of the nucleotide sequence by fluorescent dye terminator sequencing (ABI, Foster City, CA), the construct was transformed into the HF7c yeast strain as suggested by the manufacturer (CLONTECH Matchmaker 230). The CD5-CK2 was screened for nonspecific activation of the GAL4 promoter by co-transforming and in association with a co-transformed irrelevant AD-cDNA construct. The CD5-CK2 construct was used to screen an AD-cDNA library made with mRNA from Epstein-Barr virus-transformed pooled human peripheral blood lymphocytes in the GAL4 promoter directly and in association with a co-transformed fraction, and peroxidase-conjugated goat anti-rabbit IgG were from Jackson ImmunoResearch Laboratories (West Grove, PA). Protein A-agarose were obtained from Life Technologies, Inc., and SuperSignal chemiluminescence substrate was obtained from Pierce. Anti-mouse CD5 antibody was conjugated to agarose using the EDC kit from Pierce. The CD5-derived peptide DNSSDSYDILHQAQR containing the CD5 cytoplasmic domain residues 456–471, was obtained from Bio Synthesis (Lewisville, TX), and the standard CK2 substrate peptide RRREEEEE was obtained from Research Genetics (Huntsville, AL). Purified CK2 was purchased from Amersham Pharmacia Biotech.

**Mapping Studies and Constructs**—The generation of AD-CCK2α, AD-CCK2β, AD-CCK2α–215, AD-CCK2β–132, and AD-CCK2β–213–215 constructs has been described previously (41, 42). To generate BD-CD5 deletion mutants, we used the Seamless Cloning Kit from Stratagene. This system makes use of the type II restriction enzyme EcoRI to generate restriction-site independent deletions. The six primers used to generate all the deletions are as follows: 5'-TCTCTCTCTGACACCCACACGCTCC-3', 2) 5'-AGCTCCACTGTCCTGACACCCACACGCTCC-3', 3) 5'-TCTCTCTCTGACACCCACACGCTCC-3', 4) 5'-TCTCTCTCTGACACCCACACGCTCC-3', 5) 5'-TCTCTCTCTGACACCCACACGCTCC-3', and 6) 5'-TCTCTCTCTGACACCCACACGCTCC-3'.

**In Vitro Kinase Assay—**

**Immunoprecipitation and Western Blot Analysis—**

**TABLE 1**

| AD-CCK2β clone 11–11+ | Growth in plates | Lac Z expression |
|-----------------------|------------------|-----------------|
| BD-CD5               | +                | +               |
| BD lamin C<sup>a</sup>| +                | +               |
| BD<sup>b</sup>       | +                | +               |

<sup>a</sup> Human lamin C<sub>96–230</sub> in pGBT9-pLAM5<sup>a</sup>.  
<sup>b</sup> Gal4 BD vector pGBT9.

**TACATCGCGGCGAGTTGTCAGGCTG-3' (antisense) for the S459G mutant, 5'-GACACATCTGGCAGAGCTGACTGCTG-3' (sense) and 5'-CAGATAGATGTCACCTCAGGAGGAGTTCGTC-3' (antisense) for the S451G mutant, and 5'-CTGCTACATATGGGGCCGAGGTTGATGTC-3' (antisense) for the S459G, S451G double mutant. To generate the pThioHis-CD5 fusion protein, the CD5 cytoplasmic domain cDNA was amplified using sense primer 5'-ATCGAACCTCACGCGAGGTTGATGTC-3' and antisense primer 5'-GACAACTCCGGCGACGGTGACTATGATCTG-3'. Site-specific mutants S459A, S461A (single and double mutants) were constructed as described above for BD-CD5 Ser → Gly mutants except that the codons were changed to reflect Ser → Ala mutations. The absence of polymerase chain reaction-introduced artifacts and the presence of desired nucleotide changes were established by bidirectional sequencing using dye terminator chemistry.

Fusion proteins were prepared as described by Frangioni and Neel (43), and His<sub>6</sub>-containing fusion proteins were purified with nickel-agarose beads (Probond, Invitrogen). The beads were washed with 20 mM phosphate buffer, 500 mM NaCl at pH 5.5 to remove nonspecifically absorbed proteins, and the bound proteins were eluted with 20 mM phosphate buffer, 500 mM NaCl, pH 4.0. After equilibration to pH 7.0 using 20 mM Tris, pH 8.0, the fusion proteins were analyzed by silver stain of SDS-polyacrylamide gel electrophoresis, and preparations containing one band of the appropriate molecular weight were used for subsequent experiments. Protein concentration was quantitated using the Bio-Rad Protein Assay. Fusion proteins were prepared as described by Frangioni and Neel (43), and His<sub>6</sub>-containing fusion proteins were purified with nickel-agarose beads (Probond, Invitrogen). The beads were washed with 20 mM phosphate buffer, 500 mM NaCl at pH 5.5 to remove nonspecifically absorbed proteins, and the bound proteins were eluted with 20 mM phosphate buffer, 500 mM NaCl, pH 4.0. After equilibration to pH 7.0 using 20 mM Tris, pH 8.0, the fusion proteins were analyzed by silver stain of SDS-polyacrylamide gel electrophoresis, and preparations containing one band of the appropriate molecular weight were used for subsequent experiments. Protein concentration was quantitated using the Bio-Rad Protein Assay. Fusion proteins were prepared as described by Frangioni and Neel (43), and His<sub>6</sub>-containing fusion proteins were purified with nickel-agarose beads (Probond, Invitrogen). The beads were washed with 20 mM phosphate buffer, 500 mM NaCl at pH 5.5 to remove nonspecifically absorbed proteins, and the bound proteins were eluted with 20 mM phosphate buffer, 500 mM NaCl, pH 4.0. After equilibration to pH 7.0 using 20 mM Tris, pH 8.0, the fusion proteins were analyzed by silver stain of SDS-polyacrylamide gel electrophoresis, and preparations containing one band of the appropriate molecular weight were used for subsequent experiments. Protein concentration was quantitated using the Bio-Rad Protein Assay.
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**RESULTS**

**CK2β Associates with CD5 Cytoplasmic Domain**—We used the yeast two-hybrid system to identify proteins that interact with the cytoplasmic domain of CD5. We fused the entire 94 amino acid cytoplasmic domain of human CD5 to the GAL4 BD to generate BD-CD5 and used it as a bait to screen an AD-cDNA library made from human peripheral blood lymphocytes. From a screen of $6 \times 10^6$ co-transformants, we obtained 536 yeast colonies that grew on histidine-deficient plates, of which 510 were positive for LacZ expression by filter assay. We determined the nucleotide sequence of 10 randomly selected AD-cDNA isolated from His"LacZ" yeast colonies, and a BLAST analysis showed that 6 of the 10 were identical to the β-subunit of human CK2 (Fig. 1). In each of the six AD-CK2β clones, the in frame fusion with the GAL4-AD occurred in the 5'-untranslated (UT) region, and five of these six clearly represented independent clones because they had different lengths of 5'-UT amino acids. The interaction of AD-CK2β was specific to CD5 cytoplasmic domain as BD-lamin C or BD alone did not interact with CRKβ (Table I). A polymerase chain reaction-based assay using primers that specifically amplify a 300-base pair fragment within the coding region of CK2β and performed directly on yeasts derived from growth-positive yeast colonies revealed that of the remaining 500 colonies, 245 (48%) were AD-CK2β.

**Interaction of CK2 with CD5 in Mammalian Cells**—To determine whether CK2 associates with CD5 in mammalian cells, we performed co-immunoprecipitation experiments. 1% Nonidet P-40 lysates from 2 x 10^7 murine B-lymphoma cell lines CH12 and NY031, murine splenocytes, or the Jurkat human T cell line were immunoprecipitated with anti-CD5 or control mAb. Western blots of these immunoprecipitates probed anti-CK2α antibody showed that CK2α co-immunoprecipitated readily with CD5 specifically in each of these tissues (Fig. 2). As determined by comparison to whole cell lysates, the amount of CK2α associated with CD5 was less than 1% of total CK2, a very abundant cellular kinase (data not shown).

**The Interaction of CK2 with CD5 Is Mediated by the β Subunit**—We did not isolate any clone in the yeast two-hybrid screen that contained either of the two catalytic subunits of CK2, α, or α′. Due to the divergence of sequence homology in human and yeast CK2β, it seemed unlikely that human CK2β could complex with yeast CK2α to mediate the restoration of the GAL4 transactivation activator. Therefore, the yeast two-hybrid screen suggested that CK2 may interact with CD5 via its regulatory β subunit. However, to directly test whether the interaction of CK2 with CD5 is mediated by its catalytic domains, we tested the ability of BD-CD5 to interact with AD-CK2α or AD-CK2α′ in the yeast two-hybrid system along with the AD-CK2β clone 15-15 obtained from our library screen. We found that only co-transformants of BD-CD5 and AD-CK2β (clone 15-15) grew on histidine-deficient plates and expressed LacZ. AD-CK2α and AD-CK2α′ did not interact with BD-CD5 (Table II). The lack of interaction between BD-CD5 and either AD-CK2α or AD-CK2α′ is unlikely to be a construct artifact, because these constructs have been shown previously to be functional and have the capability to interact with CK2β (41, 42). Based on these data, we conclude that the interaction of CK2 to CD5 is mediated by the β subunit. The Interaction of CK2β with CD5 Is Mediated by Its Amino Terminus—The observation that each of the six completely sequenced AD-CK2β clones isolated from library were in frame fusions at the 5'-UT region suggested to us that CK2β might be interacting with CD5 via its amino terminus. To test this possibility directly, we compared the ability of the full-length AD-CK2β clone 15-15, which has in frame fusion in the 5'-UT region, the full-length clone AD-CK2β-215, which lacks a "linker" region, and deletion constructs of AD-CK2β constructs to interact with BD-CD5 (Fig. 3). Yeast containing BD-CD5 and AD clone 15-15, AD-CK2β-215, or AD-CK2β-132, but not AD-CK2β-133-215, grew in the absence of histidine and expressed LacZ (Fig. 3). Interestingly, the growth on histidine-deficient plates of yeast containing BD-CD5 and AD clone 15-15 was most rapid. In the LacZ assay, this co-trans-
formant also developed the most intense blue color in the shortest time (30 min versus 3 h) compared with co-transformants containing AD-CCKβ2–215 and AD-CCKβ2–132. This observation suggests that the “linker” contributed by the 5′-UT region facilitated the interaction between AD-CCKβ fusion and BD-CD5, most probably by making the amino terminus more accessible. From these results, we conclude that the amino terminus of CCKβ mediates the interaction with CD5 cytoplasmic tail.

Mapping of CCK Binding Site on CD5—The cytoplasmic tail of CD5 has four putative serine phosphorylation sites, Ser415, Ser423, Ser459, and Ser461, all of which have the consensus motif [(S/T)XX(D/E)] for phosphorylation by CCK2 (47, 48). To determine which of these motifs are involved in interaction with CCK2β, we generated a panel of BD-CD5 deletion constructs in which we had deleted one or more of these motifs and tested for their ability to interact with AD-CCK2β in the yeast two-hybrid assay (Fig. 4A). We found that deletion of the motif at Ser415 and Ser423 independently or together did not affect the ability of CCK2β to interact with CD5. In contrast, the interaction of CCK2β with CD5 was completely absent in the three constructs that included Ser459 and Ser461 and the non-CCK2 site Ser458. These data show that the interaction of CCK2β with CD5 is limited to the two overlapping CCK2 motifs that include Ser459 and Ser461 (458SSDSDYD464).

To determine whether Ser459 and Ser461 were required for interaction with CCK2β, we constructed wild-type BD-CD5 constructs containing S459G and S461G single and double substitutions and tested these constructs for their ability to interact with AD-CCK2β (Fig. 4B). The analysis showed a quantitatively decreased yet persistent interaction with AD-CCK2β compared with “wild type” BD-CD5, indicating that the presence of serine was not an absolute necessity for CCK2β binding to CD5 and that residues flanking the CCK2 motif can by themselves contribute to binding to CCK2β.

CK2 Phosphorylates CD5 at Ser459 and Ser461—To determine whether CCK2 can phosphorylate CD5, we performed an in vitro kinase assay with purified CD5 using a 16-amino acid synthetic CD5-peptide (456DNSSDSDYDLHGAQRL471) that included Ser459 and Ser461 CCK2 motifs and compared its ability to be phosphorylated with CCK2 standard substrate peptide (RRREEETEEE) (45). After normalizing for equivalent moles of peptide, we determined that the 32P incorporation in CD5-peptide was approximately 1.5-fold greater than CCK2 control peptide (Fig. 5A). Because there is only one available phosphorylation site in the CCK2 standard peptide, the greater phosphorylation may indicate that both the CCK2 sites in CD5-peptide are phosphorylated.

To confirm that Ser459 and Ser461 are phosphorylated by CCK2 and to determine whether other serine or threonine sites are phosphorylated following CCK2 binding to CD5, we constructed ThioHisCD5 WT cytoplasmic tail fusion protein and S459A and S461A single and double mutant fusion proteins. In an in vitro kinase assay with purified CCK2 and these fusion proteins as substrates, we observed essentially no phosphorylation of S459A,S461A double mutant and markedly reduced phosphorylation of both the S459A and the S461A single mutant fusion proteins, compared with wild type fusion protein (Fig. 5B). Adjusted for protein concentration by densitometric analysis, PhosphorImager analysis showed that the phosphorylation of S459A mutant fusion protein was 2-fold lower than that of S461A fusion protein. Overall these results indicate that both S459A and S461A are sites of CCK2 phosphorylation, with Ser459 being preferred. Notably, in the in vitro kinase assay under conditions of high concentrations of substrate and purified kinase, neither Ser415 nor Ser423 was phosphorylated.
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Cross-linking of CD5 Activates CK2—We tested the possibility that CD5 may function as a regulator of CK2 activity because CK2 interacts with CD5 constitutively via its regulatory subunit in the absence of demonstrable phosphorylation. Using CK2 standard peptide as substrate, we determined the CK2 activity in CD5 immunoprecipitates from the B lymphoma line, CH12, following stimulation with anti-CD5 mAb or control antibody. We found that CD5-associated CK2 activity increased 9-fold following stimulation with anti-CD5 antibody compared with CK2 activity from control antibody-treated cells (Fig. 6A). The activation of CD5-associated CK2 was not due to net recruitment of CK2 to CD5 because the amount of CK2 protein co-immunoprecipitated was the same in anti-CD5 stimulated and control antibody-treated cells (Fig. 6B). The CK2 activity in control antibody immunoprecipitates was not altered by CD5 stimulation and did not differ from that in anti-CD5 immunoprecipitates of control antibody-treated cells (Fig. 6A). Activation of CK2 was not limited to B-lineage cells because CK2 activity in anti-CD5 immunoprecipitates from the human T-leukemia cell line Jurkat was increased by approximately 10-fold following cross-linking of CD5 compared with control antibody-treated cells (Fig. 6C). This effect in Jurkat cells was also not due to net recruitment of CK2 to CD5 (Fig. 6D).

To determine whether the kinase activity measured in the immunoprecipitates is CK2-dependent, we used heparin, a specific inhibitor of CK2 in in vitro kinase assays (49). The CK2 activity in anti-CD5 immunoprecipitates following stimulation of CH12 cells with anti-CD5 was completely inhibited when heparin at 10 μg/ml was added to the kinase assay (Fig. 7). Taken together, these data show that the activation of CK2 is specific to CD5 stimulation. These results show that the activity of CK2 associated with CD5 can be regulated by cross-linking of the receptor and that this property is not restricted to one specific cell type or species.

CK2 Activation Is Specific to CD5 Cross-linking—CD5 associates with the antigen receptor in both T and B cells, and therefore it is possible that the activation of CK2 is mediated by associated BCR or TCR molecules. To determine whether the activation of CK2 is specific to CD5 stimulation, CH12 cells were stimulated with anti-CD5, anti-μ, or control antibody, and the CK2 activity was determined in anti-CD5 immunoprecipitates. We observed that CK2 activity was enhanced only in immunoprecipitates from anti-CD5 stimulated cells (Fig. 8). The CK2 activity in anti-CD5 immunoprecipitates from anti-μ stimulated cells was not different from control antibody treated cells. To determine whether the lack of observable CK2 activation following anti-μ stimulation can be explained by decreased association of CK2 with CD5, we immunoprecipitated CD5 from anti-μ stimulated cells and compared the amount of co-immunoprecipitated CK2 with that co-immunoprecipitated with CD5 from anti-CD5 stimulated cells. The amount of CK2 associated with CD5 was same in anti-CD5 stimulated and anti-μ stimulated cells, showing that the lack of CK2 activation following BCR cross-linking was not due to net decrease in CK2 associated with CD5 (Fig. 6B). Similarly, TCR cross-linking of Jurkat cells with anti-CD3ε antibody did not activate CD5 associated CK2, whereas CD5 stimulation did (Fig. 8B). The lack of CK2 activation following TCR cross-linking was also not due to change in net CK2 association with CD5 (Fig. 6D). Taken together, these data show that the activation of CK2 is specific to CD5 stimulation.

DISCUSSION

In this study, we have shown that the serine/threonine kinase CK2 interacts specifically with CD5. The direct association of CK2 with a cell surface receptor is particularly intriguing because this kinase is involved in regulating intermediate to distal events of signaling in the cytosol and nucleus (50, 51). This report, the first to demonstrate the localization of CK2 to the cell membrane in association with a cell surface receptor, suggests that CK2 may also play a role in the regulation of membrane proximal signaling events.

The holoenzyme CK2 consists of catalytic subunits α and α’ and a regulatory subunit, β, in the tetrameric configuration αβα’/β2, αα’β2, or α’β2 β2 (51). The α and α’ subunits are highly homologous to each other but are products of different genes (50). The kinase is a major regulator of cell growth, cell division, and signal transduction pathways, and the wide range of substrates phosphorylated by CK2 includes transcription factors, protein synthesis factors, nucleic acid synthesis proteins, polymerases, and signal transduction proteins. The conservation of CK2 through phylogeny suggests that it is a critical enzyme in cell regulation, and indeed, the disruption of the catalytic subunits in Saccharomyces cerevisiae confers lethality (52).

CK2 can interact with its substrates either in its holoenzyme form nucleus (50, 51) or as individual subunits, as illustrated by the interaction of CK2α with PPP2A and the interaction of CK2β with the serine/threonine kinase Mos (53, 54). Given the association of CK2β with the CD5 cytoplasmic domain in the yeast two-hybrid assay, we can conclude the holoenzyme form of CK2 interacts with the cytoplasmic domain of CD5 in intact cells based on the co-immunoprecipitation of CK2α with CD5 and the presence of...
CK2 kinase activity in CD5. The interaction, however, is mediated by the regulatory \( b \) subunit as neither the \( a \) nor the \( a_9 \) catalytic subunits associates with CD5 directly. Our mapping experiments localize the site of interaction with CD5 to the amino terminus of CK2 \( b \), as was found with the nuclear protein Nopp140 (55). This presumably allows the carboxyl terminus to be available for interaction with the catalytic subunits (42). Our data do suggest the possibility that CK2 \( b \) can interact with CD5 in the absence of \( a/a_9 \). If CD5 does interact with free CK2\( b \) in vivo, it will be most likely under conditions where this subunit is in excess, which may occur in some neoplastic cells (56). Alternatively, substrates such as the serine/threonine kinase Mos that interact exclusively with the CK2 \( b \) subunit at the carboxyl terminus of CK2\( b \) might compete with the catalytic \( a/a_9 \) subunits to form novel multisubunit complexes with kinase activities (54). At present, there is no evidence for this intriguing possibility in live cells.

We have identified that of the four CK2 motifs in CD5 cytoplasmic domain, the kinase interacted with and phosphorylated the two distal motifs, Ser459 and Ser461. A recent report indicated that Ser 459 and Ser 461 are phosphorylated on CD5 (36), and our data suggest that the kinase responsible is CK2. It is notable that the phosphorylation was very specific to Ser459 and Ser461, because Ser 458 was not phosphorylated by CK2. The continued, albeit reduced, interaction of CK2 \( b \) with the CD5 Ser 459 \& Ser 461 double mutant indicates that CK2 binding may be influenced by but is not absolutely dependent on phosphorylation. In that context, it is interesting to note that the CK2 phosphorylation site and binding site in the CD5 cytoplasmic domain are the same, in spite of the fact that the interaction is mediated by the CK2 \( b \) and not by the catalytic subunits CK2\( a/a' \). Because the crystal structure for CK2 holoenzyme is not known, we can only speculate that CK2\( b \) interacts with residues proximal to Ser459 and Ser461 but not directly with them, in a configuration that allows these sites to be available for phosphorylation by CK2\( a \), as supported by the observation that Ser459 and Ser461 are not absolutely required for binding.

The constitutive association of CK2 with CD5 in cell lines and primary cells and its ability to associate in a phosphorylation-independent manner suggested to us that CD5 may function as a regulator of CK2 activity. In fact, our data indicate that the CK2 associated with CD5 is relatively inactive in unstimulated cells and is activated 9–10-fold in the absence of net recruitment following ligation of CD5. This activation is very specific to CD5 stimulation because ligation of TCR or BCR did not cause this effect, even though CD5 clearly associ-
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ates with these receptor complexes (12–14). This observation is particularly notable because the mechanisms that regulate CK2 under physiological conditions are poorly understood (50, 51). Although other stimulators of CK2 activity have been reported, those data have not been consistent. The ability to separate activated CK2 from inactive CK2 in the form of complexes with CD5 will be beneficial for studies to define the mechanism that regulates the kinase activity of CK2.

Another potential mechanism of CD5-dependent regulation of CK2 may be based on the association and dissociation of α and β subunits of CK2. The β subunit has a cyclin-like “destruction box” in its amino terminus, which may be involved in ubiquitin-mediated proteolysis by the proteasome pathway (57). Therefore, the binding of β to CD5 may protect it from this degradation pathway. This may have specific relevance in nonplastic cells that have elevated levels of CK2 and express excess β in relation to the α/α catalytic subunits (58). Interestingly, nonplastic cells also express higher level of CD5.

The association with and activation of CK2 by CD5 is likely to have significant implication on the regulation of TCR- and BCR-induced activation. We hypothesize that CK2, following activation by CD5, translocates and phosphorylates molecules associated with the antigen receptors in both T and B cells. The effect of this on TCR/BCR signaling will depend on the substrate, because phosphorylation of a substrate by CK2 can lead to its positive or negative regulation (46, 59–65). In support of this hypothesis, Simarro et al. (36) have recently reported that the integrity of distal region of CD5 cytoplasmic domain, the site of CK2 binding and activation, was required for both TCR-dependent and TCR-independent diacylglycerol synthesis. The TCR-dependent diacylglycerol synthesis is most likely mediated by phospholipase C-γ, which has 24 conserved CK2 phosphorylation sites. Similarly, several other molecules that are involved in TCR/BCR/CD5 proximal events of CD5 signaling contain sites for CK2-dependent phosphorylation, and studies are under way to address whether they are inducibly phosphorylated by CK2 are in progress.

In summary, this study is the first to demonstrate the activation of CK2 by direct association with a cell surface receptor. The ability to separate inactive CK2 from active CK2 will facilitate studies to define the properties that regulate its kinase activity. The findings presented here have the potential to expand the role of CK2 as regulator of membrane proximal signals in addition to previously described intermediate and distal events.

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