Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11885–11889, December 1993
Immunology

Tyrosine kinase activity of CD4-associated p56^{lck} may not be required for CD4-dependent T-cell activation

(T-cell signaling)

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Communicated by Baruj Benacerraf, September 24, 1993

ABSTRACT **The lymphoid-specific tyrosine kinase p56^{lck} (Lck) is critical for the development and activation of T lymphocytes, and Lck kinase activity has been implicated in both T-cell antigen receptor/CD3- and CD4-mediated signaling. CD4-dependent T-cell activation has been demonstrated to be dependent upon the association of CD4 with Lck. To examine the role of the kinase activity of Lck in CD4-dependent T-cell activation, we have generated several kinase-deficient mutants of Lck. When transfected into CD4$^+$ murine T-cell hybridoma cells, these mutants cause ≈90% diminution in CD4-associated Lck kinase activity. Specifically, upon CD4 crosslinking there is decreased Lck autophosphorylation and decreased phosphorylation of an exogenous substrate. When CD4 is crosslinked to the T-cell antigen receptor–CD3 complex, decreased phosphorylation of associated substrates is also observed. In spite of this striking inhibition of Lck kinase function, cells expressing the kinase-deficient mutants demonstrate normal or enhanced CD4-dependent antigen responsiveness. These data demonstrate that the level of Lck kinase activity does not correlate with its CD4-associated function and suggest that the kinase activity of Lck may not be required for CD4-mediated signaling.**

T-cell antigen receptor (TCR) stimulation by antibody or antigen results in rapid tyrosine phosphorylation of several proteins, including the src family protein-tyrosine kinases p56^{lck} (Lck) and p59^{fyn} (Fyn) (1). Inhibition of tyrosine kinase activity during TCR/CD3 stimulation by herbimycin prevents the release of intracellular calcium, inositol phosphate generation, and interleukin 2 (IL-2) production (2), indicating a pivotal role for tyrosine kinase activity in TCR-mediated T-cell activation. The ability of phorbol esters to bypass herbimycin inhibition suggests that tyrosine kinases function early in the signaling cascade and furthermore suggests that protein-tyrosine phosphorylation initiates the T-cell signaling cascade. This conclusion is supported by the finding that phospholipase Cγ1 (PLCγ1) is tyrosine phosphorylated during T-cell stimulation and that phosphorylation of PLCγ1 on tyrosine (but not serine or threonine) results in increased enzymatic activity (3–5), indicating that tyrosine kinase activation occurs prior to activation of PLCγ1.

The importance of Lck tyrosine kinase activity in T-cell activation is emphasized by the finding that cells expressing a mutant form of Lck, lacking kinase activity, demonstrate a profound inhibition of tyrosine phosphorylation, release of calcium, and IL-2 production in response to stimulation by anti-CD3 antibodies (6). Interestingly, T cells that lack Fyn do not show a similar inhibition of response to anti-CD3 antibodies (7, 8), suggesting that Lck tyrosine kinase activity is required for T-cell activation. This is supported by work from several laboratories indicating that transfection of T cells with a constitutively active form of Lck (9, 10) results in enhanced IL-2 production in response to antigen (11, 12). Taken together, these data indicate that the kinase activity of Lck is required for TCR/CD3-mediated T-cell activation.

Lck associates with CD4 (13, 14), a T-cell coreceptor that binds major histocompatibility complex (MHC) class II molecules. In many cases, expression of CD4 is able to enhance T-cell responsiveness to antigen (15). The CD4-dependent increase in antigen responsiveness requires the association of CD4 with Lck (16, 17). Additionally, stimulation of T cells with anti-CD3 or anti-TCR antibodies is enhanced by crosslinking CD4 with the TCR/CD3 complex (18, 19). Antibody crosslinking of CD4 alone results in increased kinase activity and phosphorylation of Lck and in increased tyrosine phosphorylation of several substrates, including CD3ζ (20–22), suggesting that CD4 signaling is mediated by Lck.

While association with Lck is necessary for CD4-mediated antigen responsiveness, it has not been clearly demonstrated that the kinase activity of Lck plays a role in CD4-mediated T-cell activation. Lck contains several functional domains in addition to the kinase domain, including the src-homology (SH) domains 2 and 3. For other proteins, SH2 and SH3 domains have been demonstrated to mediate interprotein associations. To investigate the role of Lck kinase activity in CD4 signaling, we have generated several mutants of Lck that lack the kinase domain. Expression of these mutants in a murine T-cell hybridoma, which shows CD4-dependent IL-2 production in response to antigen, results in a marked diminution in CD4-associated Lck kinase activity. Expression of these mutants exerts a dominant negative effect, thus diminishing the kinase activity of wild-type endogenous Lck. However, in spite of an ≥90% reduction in inducible Lck kinase activity, cells expressing these kinase-deficient mutants do not demonstrate a reduction in CD4-dependent antigen or antibody responsiveness. These results suggest that although the kinase activity of Lck is required for TCR/CD3 signaling, it may not be necessary for CD4-dependent antigen responsiveness.

MATERIALS AND METHODS

Generation and Expression of LckΔ126 and LckΔ176 Mutants. LckΔ126 and LckΔ176 cDNAs were generated by the polymerase chain reaction, using a common 5′ primer spanning nucleotides −20 to −4 of the murine Lck cDNA (including a *Sal* I restriction site 5′ of the Lck-derived sequences) and unique 3′ primers spanning nucleotides +451 to

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Abbreviations: TCR, T-cell antigen receptor; Lck, p56^{lck}; Fyn, p59^{fyn}; mAb, monoclonal antibody; RAMG, rabbit anti-mouse immunoglobulin antibody; PAS, protein A-Sepharose; IL-2, interleukin 2; MHC, major histocompatibility complex; MBP, myelin basic protein; SH, src homology.

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471 for LckΔ126 (including a *Bam*HI restriction site 3′ of the Lck-derived sequences) or nucleotides +611 to 629 for LckΔ176 (including an *Eco*RI restriction site 3′ of the Lck-derived sequences). The resulting fragments were subcloned into the pFNeo expression vector, which contains the neomycin-resistance gene for drug selection. Constructs were confirmed by DNA sequencing. pFNeoLckΔ176 and pFNeo-LckΔ126 were linearized with the restriction enzyme *Xmn* I and electroporated into a CD4$^+$ T-cell hybridoma, BY155.16 (described in ref. 15). Transfectants were selected in medium containing G418 at 3.0 mg/ml for ≈2 weeks. G418-resistant clones were expanded and screened for expression of LckΔ176 or LckΔ126 by immunoblotting, as described below.

Immunoprecipitation and Immunoblotting. To prepare whole cell lysates, 1×10^6 cells were lysed by boiling in 100 μl of Laemmli sample buffer containing 10% 2-mercaptoethanol for 10 min; the lysates were cleared by centrifugation at 17,000 × *g* for 15 min. The samples were resolved in SDS/15% PAGE gels and transferred to nitrocellulose (Schleicher & Schuell) using a Bio-Rad semi-dry transfer apparatus; immunoblotting was performed as described below.

For immunoprecipitations, unless otherwise indicated, 2.5×10^7 cells were lysed by incubation for 20 min at 4°C in a buffer containing 0.5% Triton X-100, 150 mM NaCl, 20 mM Tris (pH 8.0), 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride, leupeptin (10 μg/ml), and aprotinin (10 μg/ml). Lysates were cleared by centrifugation at 17,000 × *g* for 2 min at 4°C, and then 1.8 μg of the anti-CD4 monoclonal antibody (mAb) Leu3a (Becton Dickinson) plus 35 μl of protein A-Sepharose (PAS) were added. The lysates were incubated with antibody plus PAS for 2 hr at 4°C with constant rocking; the PAS pellets were then washed once with phosphate-buffered saline, twice with a buffer containing 500 mM LiCl and 50 mM Tris (pH 7.6), and once with water. Samples were resolved on SDS/10% PAGE gels or as otherwise indicated.

For immunoblotting, samples were transferred to nitrocellulose using a Bio-Rad semidry blotting apparatus. The nitrocellulose membranes were blocked by incubation for 1 hr at room temperature in TBST (10 mM Tris/150 mM NaCl/0.05% Tween) containing 5% (dry) milk. The membranes were washed three or four times in TBST and then incubated for 1 hr at room temperature in TBST with antiserum directed against the N terminus of Lck (the generous gift of J. Bolen, Bristol-Myers Squibb) diluted 1:200 in TBST. After three or four washes with TBST, the membranes were incubated for 1 hr with horseradish peroxidase-labeled donkey anti-rabbit immunoglobulin antibody (Amersham) diluted 1:5000 in TBST. Finally, the membranes were washed extensively (up to 10 times) in TBST and developed using Amersham enhanced chemiluminescence (ECL) reagents.

***In Vitro* Immune Complex Kinase Assay.** A total of 1×10^7 cells were resuspended in 500 μl of RPMI 1640 and incubated on ice with 500 ng of the anti-TCR mAb F23.1 (purified from culture supernatant) and/or 500 ng of Leu3a, as indicated. After 10 min, 5 μg of rabbit anti-mouse immunoglobulin antibody (RAMG; Fisher) was added, and the cells were incubated for another 10 min on ice. The cells were stimulated by warming to 37°C for 2 min and then washed once in 1 ml of phosphate-buffered saline plus 400 mM sodium orthovanadate, 10 mM sodium fluoride, and 10 mM sodium pyrophosphate. Cells were then lysed by incubation for 20 min on ice in buffer A [150 mM NaCl, 50 mM Tris (pH 7.6), 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride, leupeptin (10 μg/ml), and aprotinin (10 μg/ml)] containing 1% Brij-96 (Sigma). The lysates were cleared by centrifugation at 17,000 × *g* for 2 min, 25 μl of PAS was added, and the samples were rocked for 1 hr at 4°C. PAS pellets were washed once with lysis buffer, twice with buffer A containing 0.1% Brij 96, and once with buffer A. The PAS pellets were then incubated in kinase buffer [20 mM Tris (pH 7.4), 10 mM $MnCl_2$, leupeptin (2 μg/ml), and aprotinin (2 μg/ml)] plus 10 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP and 2 μg of myelin basic protein (MBP; Sigma) for 2 min at 30°C. Samples were resolved in SDS/10% PAGE, transferred to poly(vinylidene difluoride) membranes, and exposed to film. Samples on poly(vinylidene difluoride) membranes were counted using a Betagen (Waltham, MA) Betascope 603 blot analyzer.

IL-2 Production. For antigen stimulation, a total of 5×10^4 CD4$^+$ parent (BY155.16), CD4$^+$LckΔ126$^+$, or CD4$^+$ LckΔ176$^+$ cells were cultured for 20 hr at 37°C (5% CO_2) in the presence or absence of various numbers of irradiated (6000 rads) Daudi cells in 1 ml of RPMI 1640 containing 10% fetal bovine serum (15), as indicated in the figure legends. For antibody stimulation, a total of 1×10^5 hybridoma cells were cultured for 20 hr at 37°C in the presence or absence of anti-TCR mAb (F23.1) at 10 ng/ml and/or anti-CD4 mAb (Leu3a) at 250 ng/ml plus 1×10^7 microspheres coated with goat anti-mouse immunoglobulin antibody (Kirkegaard & Perry Laboratories). Supernatants were harvested, titrated by serial 2-fold dilutions, and assayed for IL-2 by their ability to stimulate proliferation of the IL-2-dependent cell line CTLL-20, as described (15). Data are presented as units of IL-2 per ml as defined by comparison with the stimulation of CTLL-20 by 1 unit of human recombinant IL-2 per ml. Proliferation was measured by [^{3}H]thymidine incorporation.

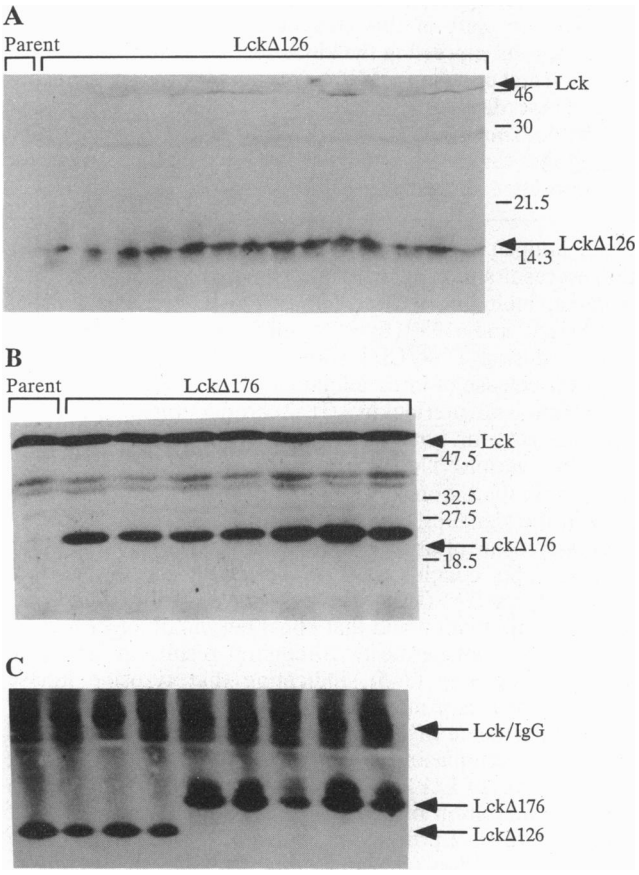

FIG. 1. Expression of LckΔ126 (*A*) and LckΔ176 (*B*). Cell lysates of CD4$^+$ T-cell hybridomas were resolved in SDS/15% PAGE and immunoblotted with an anti-Lck antiserum as described in *Materials and Methods*. (*C*) Association of LckΔ126 and LckΔ176 with CD4. The anti-CD4 mAb Leu3a was used for precipitation of CD4 from parent cells or cells expressing LckΔ126 or LckΔ176. Samples were resolved in SDS/10% PAGE and immunoblotted with anti-Lck antiserum. Each lane represents a different cell line.

Cell lines analyzed for IL-2 production were chosen for their ability to respond equivalently to plate-bound anti-CD3 mAb (145-2c11), to ensure that differences in CD4-dependent antigen or antibody responses were not due to variations in maximal cell responsiveness.

RESULTS

Expression of LckΔ126 and LckΔ176. To examine the role of the kinase domain of Lck in CD4-dependent T-cell activation, two mutants of Lck (LckΔ126 and LckΔ176) that lack the entire kinase domain of Lck were generated. LckΔ176 also has part of the SH2 domain deleted, whereas the SH2 domain is completely deleted in LckΔ126. These mutants were expressed in the murine T-cell hybridoma BY155.16, which responds weakly to human HLA-DR$^+$ Daudi stimulator cells, producing small amounts of IL-2. Expression of human CD4 in this T-cell hybridoma greatly enhances IL-2 production in response to Daudi cells (15). However, expression of CD4 molecules with mutations at either Cys-420 or Cys-422, which are unable to associate with Lck, does not enhance antigen responsiveness (22), indicating that in these cells CD4-dependent responses require CD4 signaling via Lck.

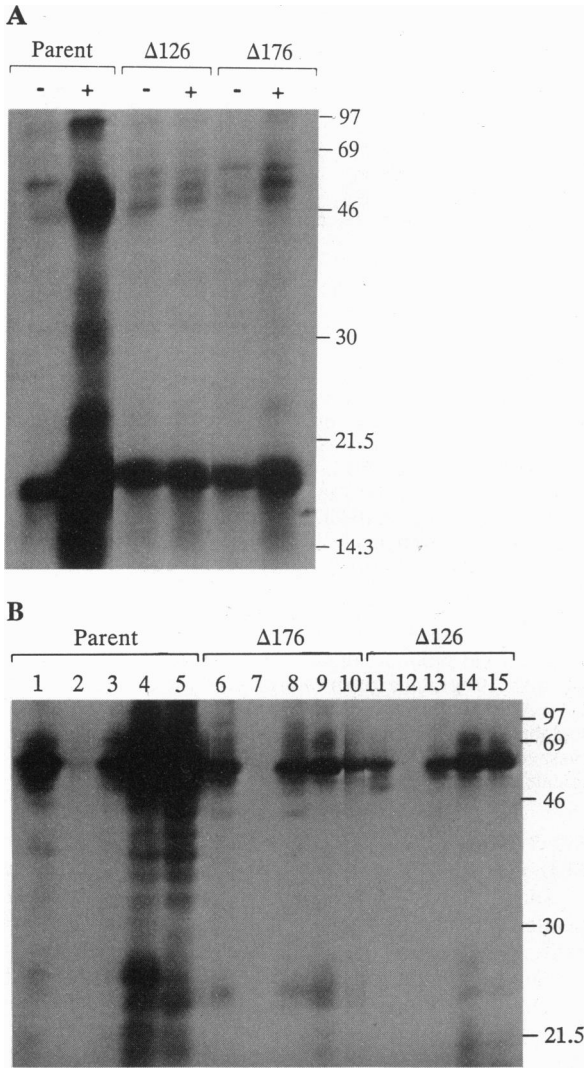

FIG. 2. Lck kinase activity of cells expressing LckΔ126 or LckΔ176. (*A*) Activation of Lck by crosslinking with anti-CD4 mAb. Cells were incubated with RAMG alone (−) or with anti-CD4 mAb followed by RAMG (+). Cells were lysed in Brij 96, immunoprecipitated, and incubated with [γ-^{32}P]ATP in an *in vitro* kinase assay as described in *Materials and Methods*. Lck runs as a phosphoprotein at 56 kDa; MBP was added to the assay and runs at ≈18 kDa. (*B*) Activation of tyrosine kinase activity by antibody-mediated crosslinking of TCR and CD4. Cells were incubated with RAMG alone (lanes 1, 6, and 11), with anti-TCR mAb followed by RAMG (lanes 2, 3, 7, 8, 12, and 13), with anti-TCR plus anti-CD4 mAb followed by RAMG (lanes 4, 9, and 14), or with anti-CD4 mAb only followed by RAMG (lanes 5, 10, and 15). Cells were lysed in Brij 96, and anti-CD4 mAb (250 ng) was added to lanes 1, 3, 6, 8, 11, and 13 before precipitation with PAS. This allowed an assessment of the basal level of Lck kinase activity associated with CD4 (lanes 1, 6, and 11) or of Lck kinase activity following stimulation via the TCR (lanes 3, 8, and 13). Precipitates were incubated with [γ-^{32}P]ATP as described in *Materials and Methods*.

Table 1. Quantification of the incorporation of ^{32}P into Lck or MBP

	Antibody crosslinking	Parent	LckΔ176	LckΔ126
Lck auto-phosphorylation	−	4,641	3,973	8,742
	+	77,466	9,542	7,846
MBP phosphorylation	−	24,562	27,102	29,312
	+	436,027	49,213	39,285

^{32}P incorporation from [γ-^{32}P]ATP was determined by counting on a Betagen Betascope 603 blot analyzer. The data are expressed as total counts obtained over a 30-min period. The samples used in this table are the same as those used in Fig. 2*A*.

LckΔ126 or LckΔ176 were transfected into cells expressing wild-type CD4, and whole-cell lysates were screened by immunoblotting with an antiserum that recognizes the N terminus of Lck (Fig. 1 *A* and *B*). Cell lines expressing levels of LckΔ126 or LckΔ176 that appeared to be equal to or greater than endogenous Lck were chosen for further study. All clones chosen expressed levels of CD4 and CD3 equivalent to the levels expressed by the parent cell line (data not shown). LckΔ126 and LckΔ176 association with CD4 was assessed by immunoprecipitation with anti-CD4 mAb (Leu3a) followed by immunoblotting with anti-Lck antiserum, to ensure that the mutant Lck molecules retained the ability to associate with CD4 (Fig. 1*C*). As expected, deletion of the kinase domain did not affect Lck association with CD4, since both LckΔ126 and LckΔ176 coprecipitated with CD4.

Effect of LckΔ126 and LckΔ176 on Endogenous Lck Kinase Activity. The effect of expression of LckΔ126 and LckΔ176 on the level of kinase activity associated with CD4 was examined. *In vitro* kinase assays were performed after antibody-mediated CD4 crosslinking as a means of stimulating Lck activity. Expression of LckΔ126 or LckΔ176 resulted in an ≥90% decrease in inducible Lck autophosphorylation and

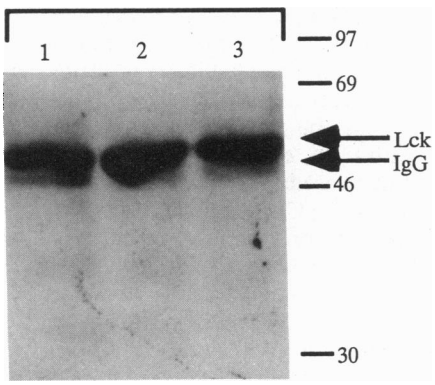

FIG. 3. Association of endogenous Lck with CD4 in cells expressing LckΔ126 and LckΔ176. Cells were lysed and CD4 was immunoprecipitated with anti-CD4 mAb, resolved in SDS/10% PAGE, and immunoblotted with anti-Lck antiserum. Lane 1, untransfected, CD4$^+$ parent; lane 2, LckΔ176-expressing cells; lane 3, LckΔ126-expressing cells.

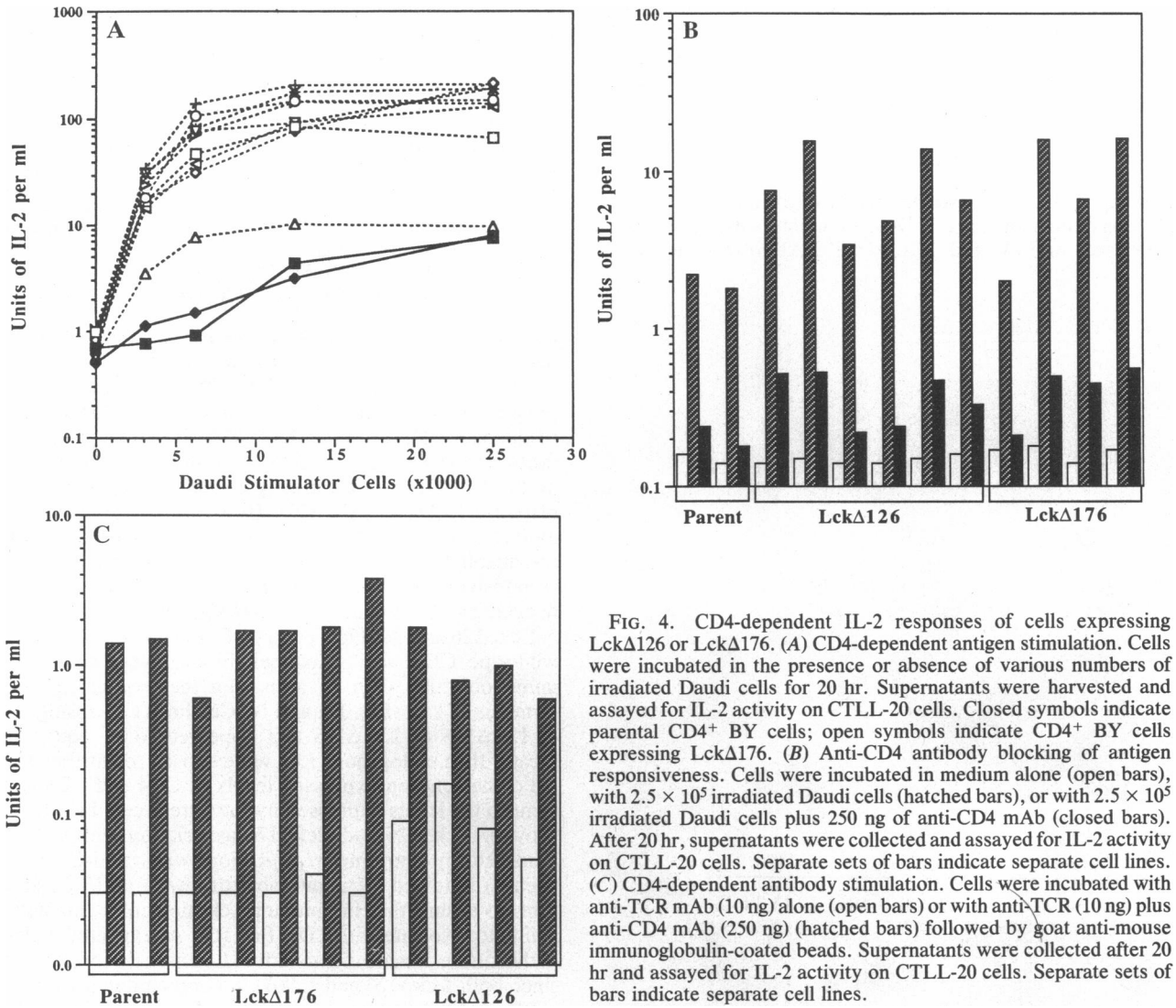

FIG. 4. CD4-dependent IL-2 responses of cells expressing LckΔ126 or LckΔ176. (*A*) CD4-dependent antigen stimulation. Cells were incubated in the presence or absence of various numbers of irradiated Daudi cells for 20 hr. Supernatants were harvested and assayed for IL-2 activity on CTLL-20 cells. Closed symbols indicate parental $CD4^+$ BY cells; open symbols indicate $CD4^+$ BY cells expressing LckΔ176. (*B*) Anti-CD4 antibody blocking of antigen responsiveness. Cells were incubated in medium alone (open bars), with 2.5×10^5 irradiated Daudi cells (hatched bars), or with 2.5×10^5 irradiated Daudi cells plus 250 ng of anti-CD4 mAb (closed bars). After 20 hr, supernatants were collected and assayed for IL-2 activity on CTLL-20 cells. Separate sets of bars indicate separate cell lines. (*C*) CD4-dependent antibody stimulation. Cells were incubated with anti-TCR mAb (10 ng) alone (open bars) or with anti-TCR (10 ng) plus anti-CD4 mAb (250 ng) (hatched bars) followed by goat anti-mouse immunoglobulin-coated beads. Supernatants were collected after 20 hr and assayed for IL-2 activity on CTLL-20 cells. Separate sets of bars indicate separate cell lines.

phosphorylation of an exogenous substrate, MBP (Fig. 2*A* and Table 1). To examine the effect of these mutants on phosphorylation of substrates associated with the TCR/CD3 complex, *in vitro* kinase assays were performed after stimulation by antibody-mediated crosslinking of the TCR and CD4. In the $CD4^+$ parent cells, ligation of the TCR with CD4 (but not of the TCR alone) resulted in the phosphorylation of multiple proteins of 20–25 kDa and 56–70 kDa. These phosphoproteins were not detected in assays using cells expressing LckΔ126 or LckΔ176. These data suggest that these mutants suppress the kinase activity of endogenous Lck associated with CD4, resulting in diminished autophosphorylation and decreased phosphorylation of substrates associated with the TCR/CD3 complex (Fig. 2*B*).

The diminished tyrosine kinase activity of endogenous Lck could be due to competition of LckΔ126 or LckΔ176 with endogenous Lck for binding to CD4. Therefore, we assessed the level of endogenous, full-length Lck associated with CD4 by immunoprecipitation with anti-CD4 antibody followed by immunoblotting with anti-Lck antiserum. Expression of LckΔ126 or LckΔ176 did not result in a detectable loss in the association of full-length Lck and CD4 (Fig. 3). These data suggest that LckΔ126 and LckΔ176 may act as dominant-negative mutants, inhibiting Lck kinase activity of endogenous wild-type Lck. A similar effect has been seen with the catalytically inactive form of Fyn (23).

Effect of Diminished CD4-Associated Lck Kinase Activity on CD4-Dependent T-Cell Stimulation. Association between CD4 and Lck has been shown to be necessary for CD4-dependent antigen responses (16, 17), and expression of a constitutively active form of Lck has been shown to result in an enhanced antigen response (11, 12) and spontaneous IL-2 production (24). These data suggest that the kinase domain of Lck plays a role in CD4-dependent signal transduction. To determine the effect of diminished CD4-associated kinase activity on CD4-dependent activation, the ability of $CD4^+$ cells expressing the LckΔ126 and LckΔ176 mutants to produce IL-2 in response to Daudi stimulator cells was determined. In spite of a marked reduction in the level of CD4-associated kinase activity, cells expressing LckΔ126 and LckΔ176 demonstrated levels of IL-2 production in response to antigen that were equal to or greater than $CD4^+$ parental cell lines (Fig. 4*A*). These responses were CD4 dependent, since addition of anti-CD4 mAb inhibited responsiveness of all cells tested by ≥85% (Fig. 4*B*).

T-cell response to antigen is dependent on multiple receptor–ligand interactions. To confine the analysis to the effect of CD4 on the TCR/CD3 complex, we used antibody-mediated CD4 crosslinking to the TCR to stimulate IL-2 production. T-cell hybridomas were cultured with suboptimal concentrations of anti-TCR mAb and optimal amounts of anti-CD4 mAb, then goat anti-mouse immunoglobulin-coated beads were added to crosslink the TCR and CD4 (Fig. 4*C*).

$CD4^+$ cells expressing LckΔ126 and LckΔ176 responded as well as or better than parent $CD4^+$ cells to cross-linking with anti-CD4 and anti-TCR antibodies (Fig. 4C). Normal or enhanced responses were seen in >30 independent T-cell clones tested from four independent transfections.

DISCUSSION

We have examined the role of the kinase domain of Lck in CD4-dependent T-cell responses. Expression of mutants lacking the kinase domain of Lck caused an ≥90% reduction in inducible Lck kinase activity without diminishing CD4-dependent responses. It is possible that the residual low level of Lck activity is sufficient to permit signal transduction; however, these data demonstrate that the level of CD4-dependent enhancement in antigen responsiveness does not correlate with the level of Lck kinase activity. Furthermore, the data suggest that although association of Lck with CD4 is required for CD4-dependent T-cell activation (16, 17), the kinase domain of Lck plays a limited role in CD4-mediated signaling.

Although the kinase activity of Lck associated with CD4 was diminished in cells expressing LckΔ126 and LckΔ176, the association of CD4 with endogenous Lck was not noticeably affected. Thus, it is possible that the role of Lck in CD4 signaling is largely performed by other domains of Lck. It is interesting to note that the CD4-dependent responsiveness of most cells expressing LckΔ126 and LckΔ176 is in fact greater than that of the untransfected parent cells, suggesting that LckΔ126 and LckΔ176 contribute to CD4-mediated T-cell responsiveness. This enhancement could result from the expression of additional SH3 domains contributed by LckΔ126 and LckΔ176, since both mutants contain intact SH3 domains. SH3 domains have been shown to mediate association between proteins by binding to proline-rich sequences (25). For example, the association of the adaptor protein Grb2 with the guanine nucleotide-releasing factor Sos1 is dependent on the SH3 domain of Grb2 binding to a proline-rich sequence within Sos1 (26–28). Recently, it has been demonstrated that the SH3 domain of Fyn binds phosphatidylinositol 3-kinase (29). Thus, it is possible that phosphatidylinositol 3-kinase is shuttled into the TCR/CD3 complex via Lck SH3 binding.

The ability of LckΔ126 and LckΔ176 to inhibit Lck kinase activity is intriguing, since the expression of these mutants does not dissociate wild-type endogenous Lck from CD4. It is possible that these mutants act as competitive inhibitors by binding a protein(s) necessary for the activation of Lck kinase activity. One such protein could be CD45, a protein-tyrosine phosphatase that has been shown to increase the tyrosine kinase activity of Lck when CD4 and CD45 are coclustered (30, 31). Another possibility is that for Lck kinase activation, CD4–Lck complexes must form dimers containing two CD4 and two Lck molecules. Receptor dimerization has been demonstrated for several receptor tyrosine kinases, and it has been suggested that kinase activation requires transphosphorylation (32). Cells expressing LckΔ126 and LckΔ176 would be less efficient at forming dimers between two functional Lck molecules. With the elucidation of the crystal structure of MHC class II, it has been noted that MHC class II $\alpha\beta$ dimers crystallize in pairs such that the two CD4-binding sites are in the closest possible proximity (33). MHC class II dimers may, therefore, facilitate the dimerization of CD4–Lck complexes. It has been previously shown that upon activation CD4 coassociates with the TCR/CD3 complex (17, 34, 35). Therefore, two TCRs may bind to a MHC class II dimer, resulting in complexes that would contain two CD4 molecules.

In conclusion, we have demonstrated that the expression of kinase-deficient Lck results in diminished activity of Lck associated with CD4, without causing a significant displacement of endogenous Lck from CD4. Additionally, we have shown that this decrease in kinase activity did not result in diminished CD4-dependent responsiveness but, in fact, resulted in enhanced CD4-dependent responses. These data suggest that domains of Lck other than the kinase domain play a role in CD4-dependent responses, either directly or through association with other molecules. Though the kinase activity of Lck associated with CD4 may not be necessary for CD4-dependent activation, Straus and Weiss (6) have provided convincing data that cellular Lck kinase activity is required for T-cell signaling.

We gratefully acknowledge Drs. J. Hutchcroft, B. Bierer, and P. D. Kassner for their helpful discussions and encouragement. This work was supported by National Institutes of Health Grant AI17258 (to S.J.B.) and Hoffman–La Roche.

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