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Alterations in Tyrosine Protein Phosphorylation Induced by Antibody-Mediated Cross-Linking of the CD4 Receptor of T Lymphocytes

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Accumulating data suggest that the CD4 T-cell surface antigen transduces an independent intracellular signal during antigen-mediated T-cell activation. CD4 is physically associated with the internal membrane tyrosine protein kinase p56^{lck} and can mediate, after antibody-mediated cross-linking, the rapid enzymatic activation of Lck, implying that CD4 signalling may involve changes in tyrosine protein phosphorylation. In this report, we describe that cross-linking of CD4 results in a series of rapid changes in intracellular tyrosine protein phosphorylation. The most prominent CD4-induced tyrosine phosphorylation change involved p56^{lck}, which became extensively phosphorylated on the carboxy-terminal tyrosine residue 505 and, to a lesser extent, tyrosine residue 394 within 1 min of CD4 cross-linking. These data establish that the CD4 receptor of T lymphocytes can transduce an intracellular signal resulting in tyrosine protein phosphorylation and strongly suggest that this property of CD4 is mediated through p56^{lck}.

The CD4 T-cell surface antigen is a 55-kilodalton (kDa) integral membrane glycoprotein recognizing nonpolymorphic class II major histocompatibility complex (MHC) determinants expressed on antigen-presenting cells (APCs). CD4 is thought to enhance physical interactions between T cells and APCs as well as transduce an independent intracellular signal during antigen-mediated T-cell activation. Although the precise contribution of this CD4-derived signal to T-cell activation has remained somewhat unclear (with evidence suggesting both positive and negative functions), accumulating data suggest that CD4 can mediate intracellular events through its ability to interact with a cytoplasmic signal transducer(s) (for a review, see reference 2a).

lck is a member of the *src* family of tyrosine protein kinase genes which is predominantly expressed, among nontransformed cell populations, in T lymphocytes (10, 13). Although its gene product, p56^{lck}, lacks extracellular and transmembrane domains required for direct interactions with the extracellular milieu, a fatty acid modification of its amino terminus (myristylation) allows stable association with the inner aspect of the plasma membrane (9, 10, 18, 19). This subcellular localization is thought to indicate that p56^{lck} (as well as the other members of the Src family) may function as a signal transducer and/or amplifier of cell-to-cell communication (7).

Direct evidence for this view is provided by the observations that Lck is physically associated with CD4 (12, 16) and that antibody-mediated cross-linking of CD4 (used to mimic CD4-class II MHC interactions) results in a rapid and specific increase in Lck-associated tyrosine kinase activity (17). These findings suggest that p56^{lck} may mediate a CD4-triggered signal during antigen stimulation and imply that CD4-induced signalling events may involve the phosphorylation of cellular substrates on tyrosine residues. Preliminary evidence supporting this model has been provided by the observation that CD4 cross-linking induces an increase in tyrosine phosphorylation of the zeta subunit of the T-cell receptor complex (17).

To further characterize the nature of CD4-related signalling events in T lymphocytes, we have evaluated the effects of CD4 stimulation on intracellular tyrosine protein phosphorylation. Using antiphosphotyrosine immunoblotting, we have found that CD4 cross-linking induces the tyrosine phosphorylation of a number of cellular products, in parallel with the Lck enzymatic activation described previously (17). The most prominent CD4-specific tyrosine phosphorylation "substrate" is a 56-kDa product which becomes maximally phosphorylated within 1 min of CD4 cross-linking. Further studies have identified this product as p56^{lck} and demonstrated that the enhanced phosphorylation primarily involves the carboxy-terminal tyrosine residue 505 (Y-505). Together, these data provide additional evidence that CD4 transduces an intracellular signal involving alterations of p56^{lck} and that results in the phosphorylation of a number of cellular products on tyrosine residues.

MATERIALS AND METHODS

Cells. The CD4$^+$ murine T-cell clone C8 has been described previously (3). Cells were grown and prepared according to established protocols (15).

Treatments. After being washed in cold medium, cells were incubated for 30 min on ice in the presence of saturating concentrations of various monoclonal antibodies (MAbs) (6, 15). After the excess antibodies were washed off, the cells were incubated for variable periods of time at 37°C with the relevant second-step antibody (rabbit anti-rat [RAR] or rabbit anti-hamster [RAH] immunoglobulin G [IgG; Organon-Teknika]) as described before (16, 17). For metabolic labeling experiments, $^{32}P_i$ was added to all media and buffers prior to cell lysis (see below). Fab fragments of MAb GK1.5 (20) were used as described before (17) and were kindly provided by Ada Kruisbeek, National Cancer Institute. The structural integrity of the Fab fragments used in our experiments was confirmed by reduced and nonreduced sodium

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dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (our unpublished data). Their functional integrity was confirmed by fluorescence-activated cell sorter analysis, demonstrating that essentially all MAb GK1.5-binding sites were saturated by the Fab preparation (our unpublished data). Further evaluation of the Fab fragment functional integrity by testing the effect of their cross-linking on tyrosine protein phosphorylation was not exhaustively persued, since preliminary experiments revealed that such a regimen may not readily mimic the biological effects of bivalent antibody-mediated cross-linking (i.e., ability to block antigen-mediated T-cell activation).

Antiphosphotyrosine antibody immunoblotting. Following the various treatments, 20×10^6 to 50×10^6 T cells were immediately lysed in boiling 2× SDS buffer (100 mM Tris [pH 8.0], 4% SDS, 40 mM EDTA, pH 8.0) supplemented with 200 μM sodium orthovanadate, 20 μg/ml each of leupeptin and aprotinin, and 100 mM sodium fluoride. After heating at 100°C for 5 min, DNA was sheared by passing through 20- and 25-gauge needles. Samples buffer was then added to lysate volumes corresponding to equivalent cell numbers, and the polypeptides resolved on 8% SDS–PAGE gels. Transfer to nitrocellulose filters was performed as described before (14). After incubation in blocking buffer (10 mM Tris [pH 7.5], 2.5 mM EDTA [pH 8.0], 50 mM NaCl, 5% bovine serum albumin, 0.1% Tween-20, 0.01% NaN_3) for 1 h, the filters were exposed to the mouse antiphosphotyrosine MAb PY-20 (purchased from ICN Immunobiologicals) (used at 2 μg/ml in blocking buffer) for 1.5 h (5). After being washed five to six times in saline buffer, the filters were incubated for 1 h with ^{125}I-labeled sheep anti-mouse IgG (Amersham), washed again, and then subjected to autoradiography (14). Quantitation of the intensity of the detected bands was performed by cutting the bands and counting in a gamma counter.

Metabolic labeling. Metabolic labeling was performed for 4 h with 1 mCi of $^{32}P_i$ (carrier free; Amersham) per ml as described previously (15). Similar results have been obtained for labeling periods of 18 h (15). In cross-linking experiments, the same concentration of labeled phosphate was added to all media and washing solutions prior to cell lysis. Phosphoamino acid analysis and peptide mapping studies with cyanogen bromide and trypsin were performed according to standard protocols (14, 15).

Immunoprecipitations. All immunoprecipitations were performed as described previously (16). When SDS buffer lysates were used, 4 volumes of TNE lysis buffer (50 mM Tris [pH 8.0], 1% Nonidet P-40, 2 mM EDTA [pH 8.0]) supplemented with the same protease and phosphatase inhibitors were added to SDS lysates to avoid SDS precipitation. Subsequent antiphosphotyrosine and Lck immunoblotting were performed as described above (14).

RESULTS

Effects of CD4 cross-linking on tyrosine protein phosphorylation in T lymphocytes. We have previously demonstrated that antibody-mediated cross-linking of the CD4 T-cell surface antigen results in a rapid and specific increase in tyrosine protein kinase activity of CD4-bound Lck molecules (17), suggesting that CD4 stimulation may result in tyrosine phosphorylation changes mediated through $p56^{lck}$. Consistent with this view is the finding that one of the invariant subunits of the T-cell receptor complex (zeta) becomes phosphorylated on tyrosine residues following CD4 cross-linking (17).

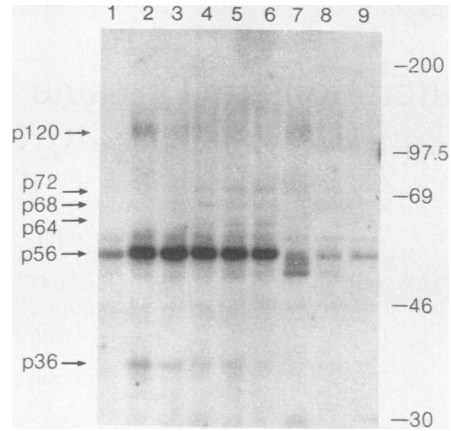

FIG. 1. Analysis of the effects of surface antigen cross-linking on phosphotyrosine-containing proteins expressed in $CD4^+$ T lymphocytes (C8 clone). Cells were treated and lysed in boiling SDS buffer as described in Materials and Methods. The expression of phosphotyrosine-containing proteins was examined by immunoblotting with the murine antiphosphotyrosine MAb PY-20 (5). Lanes: 1, RAR IgG alone (30 min); 2 to 6, anti-CD4 MAb GK1.5 plus RAR IgG for 1, 5, 10, 15, and 30 min, respectively; 7, anti-Thy1 MAb G7 (30 min); 8, anti-CD3 (epsilon) MAb 145-2C11 plus RAH IgG (30 min); 9, Fab fragments of anti-CD4 MAb GK1.5 (30 min). It should be noted that signalling through Thy1 (which results in T-cell activation) can be efficiently induced by treatment with MAb G7 alone without a second-step antibody (6). Exposure, 3 h. Positions of molecular mass markers are shown to the right (in kilodaltons).

To evaluate further the possibility that changes in tyrosine phosphorylation are implicated in CD4-related signalling events, we analyzed the extent of tyrosine protein phosphorylation in mature murine T lymphocytes by using antiphosphotyrosine immunoblotting assays (Fig. 1). Untreated resting $CD4^+$ murine T cells (C8 clone) were found to contain a single major phosphotyrosine-containing protein migrating at approximately 56 kDa on SDS-PAGE gels (Fig. 1, lane 1). In addition, these cells expressed a less prominent species of approximately 60 kDa. Interestingly, these two products had the same apparent molecular masses as the major phosphotyrosine-containing proteins detected in membrane phosphorylation assays of T lymphocytes (corresponding to $p56^{lck}$ and $p60^{fyn}$; A. Veillette, unpublished).

Treatment of the $CD4^+$ T cells with anti-CD4 MAb GK1.5 and RAR IgG resulted in a rapid increase (within 1 min) in the tyrosine phosphorylation of products migrating at 120 (p120) (which actually represents a doublet), 56 (p56), and 36 (p36) kDa (lane 2). A slower increase in the phosphorylation of a series of products of apparent molecular masses between 64 and 72 kDa was also detected (lanes 3 to 6). Whereas the elevated p56 tyrosine phosphorylation was maintained for at least 30 min after CD4 cross-linking, the phosphorylation of p120 and p36 decreased rapidly over the same time interval.

Cross-linking of Thy1 (lane 7) induced the tyrosine phosphorylation of products migrating at 120, 54, 52, 28, and, to a lesser extent, 64 to 72 kDa without significantly altering the detectability of p56 and p36. CD3 cross-linking (lane 8) stimulated the tyrosine phosphorylation of 120-kDa and, to a lesser extent, 68- to 72-kDa products. No changes were observed after treatment with anti-T200 (CD45) antibodies (data not shown), monovalent Fab fragments of MAb GK1.5 (lane 9), or RAR IgG (data not shown).

Identification of $p56^{lck}$ as the major CD4 tyrosine protein phosphorylation substrate. Although we observed above that

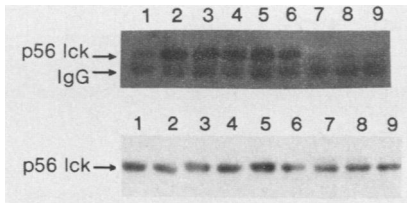

FIG. 2. Effects of surface antigen cross-linking on tyrosine phosphorylation of p56^{lck}. Lck proteins were immunoprecipitated from the cell lysates described in Fig. 1 with a specific rabbit anti-Lck antiserum (14). Antiphosphotyrosine or anti-Lck immunoblotting was subsequently performed as described in Materials and Methods. Lanes: 1, RAR IgG alone (30 min); 2 to 6, anti-CD4 MAb GK1.5 plus RAR IgG for 1, 5, 10, 15, or 30 min, respectively; 7, anti-Thy1 MAb G7 (30 min); 8, anti-CD3 MAb 145-2C11 plus RAH IgG (30 min); 9, Fab fragments of anti-CD4 MAb GK1.5 (30 min). Top panel, Antiphosphotyrosine immunoblotting. Bottom panel, Anti-Lck immunoblotting. Exposures were (top panel) 24 and (bottom panel) 12 h.

cross-linking of several T-cell surface antigens (Thy1, CD3, and CD4) induced intracellular tyrosine protein phosphorylation changes, we found that CD4 cross-linking specifically modified at least two distinct cellular products (p56 and p36). This finding indicated that CD4 stimulation is coupled to a specific tyrosine protein phosphorylation signal. To evaluate the nature of this signal, we attempted to characterize further the cellular products specifically modified by CD4 cross-linking. As already noted, the most prominent of these product (p56) had the same apparent molecular mass as Lck. To test the possibility that this 56-kDa species represents p56^{lck}, Lck immunoprecipitates were obtained from the lysates studied above (Fig. 1) and examined for their phosphotyrosine content by using antiphosphotyrosine immunoblotting (Fig. 2, top panel). This assay revealed that CD4 cross-linking induced a rapid increase in Lck tyrosine phosphorylation, with the same kinetics as the increased p56 phosphorylation detected in total-cell lysates (Fig. 1). In contrast, no increase in Lck tyrosine phosphorylation was seen upon cross-linking of Thy1 (lane 7), CD3 (lane 8), or T200 (CD45) (data not shown). These CD4-induced changes in Lck tyrosine phosphorylation were not due to increasing levels of p56^{lck}, since the abundance of Lck in these SDS lysates was not found to be affected by CD4 cross-linking (Fig. 2, bottom panel). This last result is contrary to our previous report of diminishing p56^{lck} levels in nonionic detergent lysates of T lymphocytes following CD4 cross-linking (15, 16), implying that p56^{lck} was lost from the detergent-soluble cellular compartment in those earlier assays. The exact basis for this discrepancy remains to be established.

CD4 cross-linking results in increased phosphorylation of the carboxy-terminal tyrosine residue 505 of p56^{lck}. These results indicate that one of the salient features of CD4-mediated signalling events is a rapid alteration in the state of tyrosine phosphorylation of p56^{lck}. Since, by analogy with pp60$^{c\text{-}src}$ and p56^{lck} expressed in cells of fibroblastic lineage (1, 11; for a review, see A. Veillette and J. B. Bolen, *in* C. Benz and E. Liu, ed., *Oncogenes*, in press), the degree of tyrosine phosphorylation of p56^{lck} expressed in T lymphocytes may reflect and possibly regulate its catalytic activity, additional studies were performed to identify the tyrosine residue(s) modified upon CD4 cross-linking. The CD4$^+$ T cells were metabolically labeled with $^{32}P_i$ and incubated with different antibody regimens, and the effects of these treatments on Lck phosphorylation were evaluated by immunoprecipitation (Fig. 3). Consistent with the results obtained with antiphosphotyrosine immunoblotting, CD4 cross-linking was found to induce a rapid (within 1 min, two- to threefold) increase in Lck phosphorylation (lane 5). A small increase was also seen after treatment with bivalent anti-CD4 antibody alone (lane 3). Similar observations have been made with antiphosphotyrosine immunoblotting (data not shown). However, since treatment with monovalent Fab fragments of this antibody did not alter the levels of Lck tyrosine phosphorylation (Fig. 1, lane 9), it is likely that the change induced by intact MAb GK1.5 reflected the ability of the antibody preparation to induce a certain degree of CD4 cross-linking. This is consistent with our previous observa-

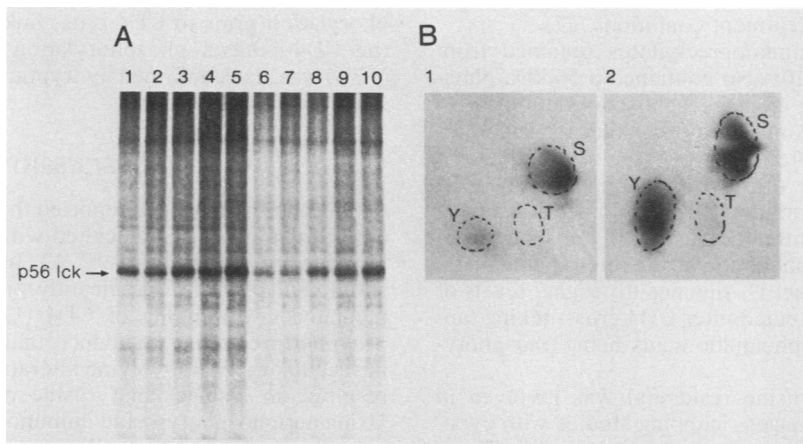

FIG. 3. Effects of anti-CD4 antibodies on Lck phosphorylation: metabolic labeling studies. (A) Immunoprecipitations. Cells were labeled with 1 mCi of $^{32}P_i$ per ml for 4 h and subsequently treated with various antibody regimens as described in Materials and Methods. Cross-linking time was 1 min. Total Lck and CD4-bound Lck were obtained from detergent lysates as described before (17). Lanes: 1 to 5, anti-Lck immunoprecipitates; 6 to 10, anti-CD4 (MAb GK1.5) immunoprecipitates; 1 and 6; untreated control; 2 and 7, RAR IgG alone; 3 and 8, MAb GK1.5 alone; 4 and 9, MAb GK1.5 (with RAR IgG added to lysate); 5 and 10, MAb GK1.5 plus RAR IgG. Exposure: 12 h. (B) Phosphoamino acid analyses. Phosphoamino acid analyses were performed on metabolically labeled CD4-bound Lck proteins as described before (14). Panel 1, Untreated control; panel 2, MAb GK1.5 plus RAR IgG for 1 min. The positions of ninhydrin-stained phosphoamino acid standards are indicated. Y, Tyrosine; T, threonine; S, serine. Exposure, 3 days.

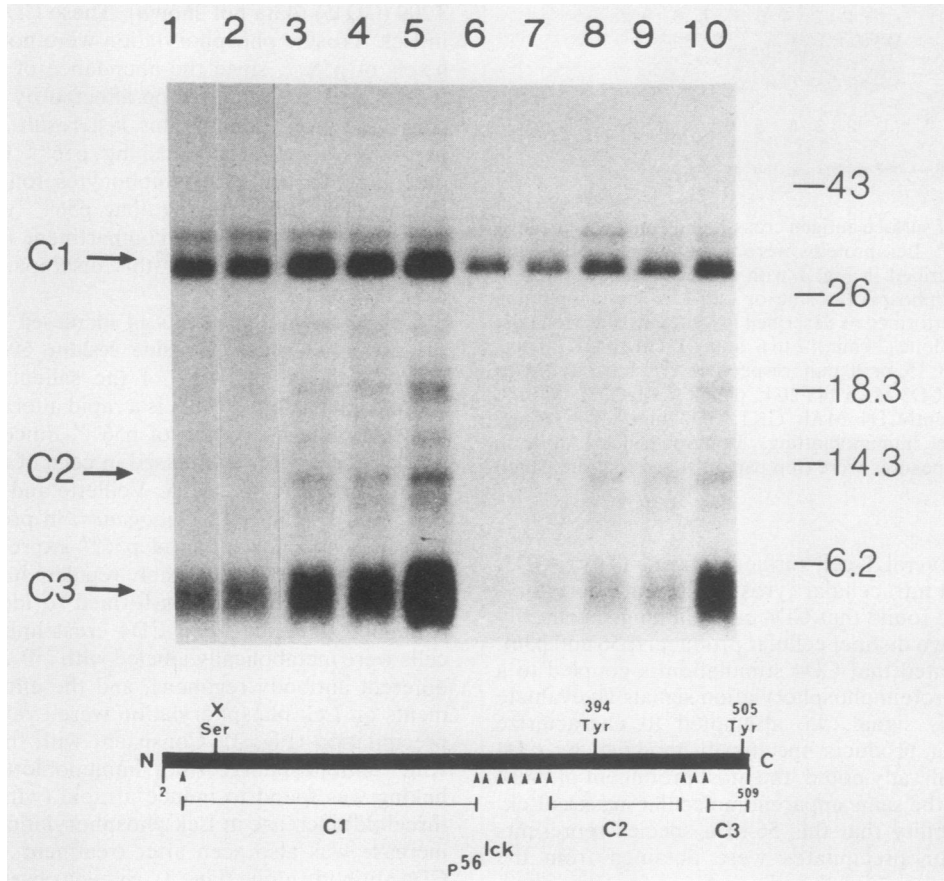

FIG. 4. Cyanogen bromide cleavage. Cyanogen bromide cleavage of phospholabeled Lck and CD4-bound Lck was performed as described before (14). Lanes: 1 to 5, total Lck; 6 to 10, CD4-bound Lck; 1 and 6, untreated controls; 2 and 7, RAR IgG alone; 3 and 8, MAb GK1.5 alone; 4 and 9, MAb GK1.5 (with RAR IgG added to lysate); 5 and 10, MAb GK1.5 plus RAR IgG. Cross-linking was performed for 1 min. A schematic diagram of the predicted cyanogen bromide cleavage sites (arrowheads) is shown below (14). C1 corresponds to the phosphoserine containing the amino-terminal portion of the molecule, while C2 and C3 contain the major site of in vitro (Y-394) and in vivo (Y-505) tyrosine phosphorylation, respectively. The specific serine residue(s) phosphorylated within the C1 fragment has not been identified and is therefore denoted as X.

tion of small increases in Lck-associated tyrosine kinase activity under the same treatment conditions (17).

CD4 (MAb GK1.5) immunoprecipitates obtained from these lysates (lanes 6 to 10) also contained a 56-kDa phosphoprotein demonstrating enhanced phosphorylation in response to CD4 cross-linking (lane 10). Additional studies identified this phosphoprotein as $p56^{lck}$ (see below; data not shown). Acid hydrolysis of CD4-bound $p56^{lck}$ (Fig. 3B) confirmed that, while these molecules contained low levels of phosphotyrosine in untreated resting T cells (approximately 15% of their phosphoamino acid content being phosphotyrosine) (Fig. 3B, panel 1), significantly higher levels of phosphotyrosine were detected after CD4 cross-linking (approximately 40% of phosphoamino acids being phosphotyrosine) (Fig. 3B, panel 2).

To evaluate which tyrosine residue(s) was involved in these phosphorylation changes, mapping studies with cyanogen bromide fragmentation were performed (Fig. 4). These experiments revealed that cross-linking of CD4 (lane 5) resulted in a marked increase in occupancy of the carboxy-terminal C3 fragment of $p56^{lck}$ (which contains Y-505), with smaller increases affecting the amino-terminal C1 fragment and Y-394-containing C2 fragment. Similar results were obtained upon examination of CD4-bound $p56^{lck}$ (lane 10). Interestingly, this population of Lck molecules demonstrated rather low relative levels of carboxy-terminal phosphorylation prior to CD4 cross-linking. The localization of the CD4-induced phosphorylation changes to Y-505 and Y-394 was also confirmed by tryptic peptide mapping studies (data not shown).

DISCUSSION

It has previously been reported that the CD4 T-cell surface antigen is physically associated with the internal membrane tyrosine protein kinase $p56^{lck}$ (12, 16) and that the enzymatic activity of $p56^{lck}$ is significantly enhanced upon antibody-mediated cross-linking of CD4 (17). These findings have provided preliminary evidence that CD4 can transduce an intracellular signal involving alterations of Lck and possibly resulting in changes in tyrosine protein phosphorylation. Using antiphosphotyrosine immunoblotting assays, we have now demonstrated that CD4 cross-linking stimulates the tyrosine phosphorylation of a number of cellular proteins. Although changes in tyrosine protein phosphorylation can also be observed after cross-linking of other T-cell surface antigens (such as CD3 and Thy1), the distinct ability of CD4 to regulate the phosphorylation of at least two cellular products (p56 and p36) implies that CD4 indeed transduces a specific tyrosine protein phosphorylation signal.

While we do not know the identity of the 36-kDa "substrate" (it does not react with an anti-calpactin I antibody; our unpublished data), our data demonstrate that the major CD4-specific tyrosine phosphorylation substrate is $p56^{lck}$. Coupled with the observation that these molecules become rapidly activated upon CD4 cross-linking, this finding adds to the evidence of important functional interactions between these two membrane-associated proteins. Although it remains to be demonstrated that $p56^{lck}$ (either directly or indirectly) mediates these CD4-induced changes in tyrosine protein phosphorylation, the specificity and rapidity of the interactions between these two products imply that at least part of the function of CD4 in signal transduction is mediated through $p56^{lck}$. Interestingly, the delayed onset of certain CD4-induced changes in tyrosine phosphorylation (such as those involving the 64- to 72-kDa products) raises the possibility that other tyrosine phosphorylation regulatory pathways may also be implicated in CD4-related signalling events.

How can $p56^{lck}$ mediate these CD4-induced changes in tyrosine protein phosphorylation? The findings that monovalent Fab fragments of anti-CD4 antibodies are incapable of stimulating the CD4-associated tyrosine kinase activity and do not induce changes in tyrosine protein phosphorylation indicate that cross-linking (and probably dimerization or oligomerization) of cell surface CD4 is likely to be required for the initiation of the tyrosine protein phosphorylation signal. By analogy with growth factor receptors with intrinsic tyrosine kinase function (such as the epidermal growth factor receptor) (for a review, see reference 8), one can propose that the cross-linking and dimerization of surface CD4 result in allosteric changes of Lck which stimulate its catalytic activity. In the context of facilitated protein-protein interactions, this enzymatic activation could result in phosphorylation of cellular substrates (including $p56^{lck}$ itself) on tyrosine residues. Alternatively, it is conceivable that CD4 cross-linking and the putative CD4-Lck dimerization primarily facilitate the tyrosine phosphorylation of $p56^{lck}$ (possibly in intermolecular autophosphorylation reactions) and that some of the resulting changes in phosphorylation are responsible for the increase Lck enzymatic activity.

Consistent with the evidence of enzymatic activation of $p56^{lck}$ detected in immune complex kinase reactions, CD4 cross-linking was found to result in an increased in vivo occupancy of the major site of in vitro phosphorylation of Lck (Y-394). This change is characteristic of activated *src*-related tyrosine kinases (for a review, see references 1 and 11 and Veillette and Bolen, in press). While this finding further supports the view that CD4 mediates an intracellular signal involving the activation of $p56^{lck}$, we have also observed that CD4 cross-linking results in a marked increase in Y-505 phosphorylation. This last finding is surprising from several points of view. First, it implies that Y-505 is hypophosphorylated in untreated resting T cells. Indeed, we have found that the CD4-bound $p56^{lck}$ demonstrates a low extent of Y-505 phosphorylation prior to CD4 cross-linking. While one would predict from studies of the equivalent residue of $pp60^{c\text{-}src}$ (for a review, see Veillette and Bolen, in press) that these Lck molecules may be enzymatically activated, it appears that the "specific" enzymatic activity of CD4-bound Lck molecules is either similar or even lower than that of non-CD4-bound $p56^{lck}$ (17; A. Veillette, unpublished). While it remains possible that the activity of CD4-bound Lck is regulated by transient Y-505 phosphorylation (not efficiently detected under the conditions used in our assays), these data raise the alternative possibility that, prior to antibody-mediated cross-linking of surface CD4, the enzymatic activity of CD4-bound Lck molecules may be regulated by mechanisms other than phosphorylation. One such manner could be through regulatory physical interactions between Lck and CD4.

Second, cross-linking of CD4 results in a marked and rapid (within 1 min) increase in phosphorylation of Y-505. Therefore, enzymatic activation of $p56^{lck}$ by antibody-mediated cross-linking of CD4 is associated with the increased phosphorylation of $p56^{lck}$ at a site where phosphorylation is thought to be inhibitory. This apparent paradox may be explained by one of the following possibilities. (i) CD4 cross-linking results in a rapid activation of $p56^{lck}$, which is followed by rapid inhibition mediated through Y-505 phosphorylation. The increase in Lck enzymatic activity measured in our immune complex kinase reactions may therefore represent Lck molecules that have not yet acquired Y-505 phosphorylation. (ii) Although the occupancy of Y-505 downregulates the Lck-associated tyrosine kinase activity in most conditions (such as for the non-CD4-bound Lck or when $p56^{lck}$ is expressed in fibroblasts), another alteration(s) of $p56^{lck}$ (possibly changes in conformation) induced by CD4 cross-linking may act as the dominant positive regulatory event(s). (iii) As is the case for certain growth factor receptors with intrinsic tyrosine kinase function (for example, the epidermal growth factor receptor [4]), the ligand-induced carboxy-terminal tyrosine phosphorylation of Lck may in fact promote the activation of the enzyme by facilitating the interaction of its active site with exogenous substrates.

Finally, the mechanism by which Y-505 becomes phosphorylated upon CD4 cross-linking remains unclear. Since the increased Y-505 occupancy occurs in parallel with a rapid increase in Lck tyrosine kinase activity, one can propose that the CD4-induced Y-505 phosphorylation is the result of a facilitated in vivo autophosphorylation reaction (possibly intermolecular). Alternatively, it may indicate that CD4 cross-linking renders Y-505 inaccessible to phosphatase-mediated dephosphorylation. It also remains possible that the CD4-induced Lck tyrosine phosphorylation is the result of the action of another cellular tyrosine kinase. While the mechanism and function of Y-505 phosphorylation of $p56^{lck}$ in CD4-mediated signalling events remain unclear, future experiments are likely to improve our understanding of these observations.

In summary, the data presented in this report demonstrate that antibody-mediated cross-linking of the CD4 receptor of T lymphocytes results in rapid and specific intracellular tyrosine protein phosphorylation signal. This signal appears to require dimerization (or oligomerization) of surface CD4, since no change in tyrosine phosphorylation was observed following treatment with monovalent Fab fragments of anti-CD4 antibodies. Importantly, the major substrate for the CD4-triggered signal transducer(s) is the internal membrane tyrosine kinase $p56^{lck}$, which becomes phosphorylated on its carboxy-terminal tyrosine residue 505 and, to a lesser extent, tyrosine residue 394 within 1 min of CD4 cross-linking. Coupled with the observation that CD4 cross-linking induces a rapid enzymatic activation of $p56^{lck}$ (17), the data presented in this report provide further evidence that CD4 can transduce a specific intracellular signal that is likely to be mediated through $p56^{lck}$ and that results in the increased phosphorylation of a number of cellular products on tyrosine residues.

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