Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7566–7570, August 1992
Immunology

Disruption of the CD4–p56^{lck} complex is required for rapid internalization of CD4

(T-cell activation/receptor internalization/protein-tyrosine kinase)

BARRY P. SLECKMAN*, JAEKYOON SHIN†, VIVIEN E. IGRAS*, TASSIE L. COLLINS*, JACK L. STROMINGER†, AND STEVEN J. BURAKOFF*

*Divisions of Pediatric Oncology and †Tumor Virology, Dana–Farber Cancer Institute, Departments of Pediatrics and Pathology, Harvard Medical School, Boston, MA 02115

Contributed by Jack L. Strominger, May 19, 1992

ABSTRACT **CD4 is a cell surface glycoprotein expressed by a subset of T lymphocytes and functions to enhance T-cell activation. CD4 is noncovalently associated via the cytoplasmic domain with the protein-tyrosine kinase p56^{lck}, a member of the src protein-tyrosine kinase family. Upon activation of protein kinase C by phorbol ester, CD4 is phosphorylated on cytoplasmic serine residues and internalized from the cell surface, and disruption of the CD4–p56^{lck} complex occurs. The exact relationship between these events is likely to be functionally significant, as cytoplasmic-domain serine phosphorylation and internalization have been shown to regulate the function of receptors that possess intrinsic protein-tyrosine kinase activity. Here we demonstrate that p56^{lck} slows the rate of phorbol 12-myristate 13-acetate-induced internalization of CD4 in a manner that depends on a physical association between p56^{lck} and CD4. This decreased rate is due at least in part to a requirement for disruption of the CD4–p56^{lck} complex prior to internalization of CD4. Furthermore, disruption of the CD4–p56^{lck} complex appears to depend on the integrity of the cytoplasmic-domain serine at position 408, probably due to a requirement for phosphorylation.**

The CD4 molecule is a cell surface glycoprotein expressed by a subset of T lymphocytes with T-cell receptors that recognize antigen in association with major histocompatibility complex (MHC) class II molecules. Upon binding MHC class II molecules on the surface of an antigen-presenting cell, CD4 functions to enhance T-cell activation (1, 2). The CD4 molecule is noncovalently associated with the protein-tyrosine kinase p56^{lck}, which is a member of the src family of protein-tyrosine kinases (3, 4). This association occurs via the cytoplasmic domain of CD4 and involves cysteine motifs within both the cytoplasmic domain of CD4 and the N terminus of p56^{lck} (5–8). Signal transduction via p56^{lck} is likely to be an important mechanism by which CD4 enhances T-cell activation, as mutant forms of CD4 unable to associate with p56^{lck} are also unable to augment activation (9, 10).

During T-cell activation CD4 is phosphorylated on cytoplasmic serine residues and internalized (11–14). These events are most likely due to activation of protein kinase C (PKC), as activation of PKC by phorbol esters such as phorbol 12-myristate 13-acetate (PMA) results in serine phosphorylation and internalization of CD4 (11–13, 15, 16). The structural requirements of the cytoplasmic domain of CD4 necessary for PMA-induced internalization have been analyzed in detail. The membrane-proximal region of the cytoplasmic domain of CD4 from amino acids 397–414 is homologous to a membrane-proximal region of the epidermal growth factor (EGF) receptor and is predicted to form an α-helical structure (17). Disruption of this region by amino acid substitutions that do not affect phosphorylation abolishes PKC-induced internalization of CD4 (17). In addition, amino acid substitutions at Ser^{408} abolish internalization, suggesting that phosphorylation of this residue is an important step in internalization (16).

Here we demonstrate that PMA-induced internalization of CD4 requires disruption of the CD4–p56^{lck} complex. Disruption of the complex is required prior to rapid internalization of CD4 and depends on the integrity of Ser^{408}, probably due to a requirement for phosphorylation of this residue.

MATERIALS AND METHODS

Expression of CD8, CD4, and CD4 Mutants and p56^{lck} in BW5147 and HeLa Cells. The CD4SA1 mutant contains an alanine substitution at Ser^{408} of CD4 (16). The CD4LD413 mutant contains a leucine substitution at Asp^{413} of CD4 (17). The CD4CS420, CD4CS422, and CD4CS430 mutants contain serine substitutions at Cys^{420}, Cys^{422}, and Cys^{430} of CD4, respectively.

CD4, CD8, CD4SA1, CD4CS420, CD4CS422, and CD4CS430 cDNAs were individually subcloned into the pMNC plasmid with subsequent construction of a retroviral producer line by use of the DAMP packaging line (18). BW5147 murine thymoma cells were infected with the defective retroviruses from the resulting producer lines and then selected in medium with G418 (1). G418-resistant cell lines were screened for surface expression of CD8 or CD4 by indirect immunofluorescence as described below. CD4LD413 was subcloned into the pCDM8 plasmid (pCDM8CD4LD413) (16). BW5147 was coelectroporated with pCDM8CD4LD413 and pMoNEO, a plasmid containing the G418-resistance gene driven by the long terminal repeat of Moloney murine leukemia virus. G418-resistant cell lines were screened for expression of CD4LD413 as described below. Representative cell lines were chosen for analysis.

Stable CD4-expressing HeLa cell lines were produced by transient expression of the pCDM8CD4 plasmid followed by several rounds of cell sorting for CD4 expression. The p56^{lck} cDNA (19) was subcloned into the pCDM8 plasmid and transiently expressed in HeLa cell lines as described (16).

Analysis of Cell Surface Expression. Cells were incubated in the presence or absence of PMA (Sigma) as described in the text. CD4 and CD8 surface expression by BW5147 was determined by incubation with the anti-CD4 monoclonal antibody (mAb) anti-Leu3a or the anti-CD8 mAb OKT8, followed by incubation with fluorescein-conjugated rat anti-mouse immunoglobulin antiserum (18). CD4 expression by HeLa cell lines was determined by a one-step incubation with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; EGF, epidermal growth factor.

phycoerythrin conjugated anti-Leu3a (Becton Dickinson). Immunofluorescence analysis was carried out on a Becton Dickinson FACScan analyzer. For quantitative analysis the mean fluorescence was determined using the CONSORT 30 program (Hewlett–Packard). The mean fluorescence at time zero less the background fluorescence was set at 100%. Percent surface expression at different times of incubation with PMA was calculated using the mean fluorescence at the time point less the background fluorescence divided by the mean fluorescence at time zero less the background fluorescence.

Protein Analysis. A total of 2×10^7 BW5147 or 5×10^6 HeLa cells were incubated at 37°C in 20 ml of RPMI 1640 (BW5147) or 10 ml of Dulbecco's modified Eagle's medium (HeLa), each supplemented with 10% fetal bovine serum in the presence or absence of PMA as described in the text. Cells were washed twice with phosphate-buffered saline (PBS) and lysed for 20 min at 4°C in a buffer containing 0.5% Triton X-100, 150 mM NaCl, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride, and 10 μg of leupeptin and aprotinin per ml. The resulting lysate was then clarified by centrifugation at 17,000 × *g* for 10 min, and 1.8 μg of the anti-CD4 mAb anti-Leu3a or 50 μl of a cell culture containing the anti-CD8 mAb OKT8 was added and incubated at 4°C for 60 min. Protein A-Sepharose (35 μl) was added and incubated at 4°C for 60 min. The protein A-Sepharose was washed once with PBS, twice with a solution containing 500 mM LiCl/50 mM Tris at pH 8.0, and once with water. SDS/10% PAGE was performed under reducing conditions (18). Proteins were transferred to nitrocellulose with a Hoeffer dry blot apparatus at 150 mA for 1 hr. The nitrocellulose was then blocked for 1 hr at 37°C with 150 mM NaCl/10 mM Tris, pH 7.4 (TBS), containing 2% gelatin (Bio-Rad) and subsequently incubated with a solution containing a 1:200 dilution of a rabbit anti-p56^{lck} antiserum (a gift from Kurt Amrein, Hoffman–LaRoche) in TBS/0.5% Tween 20 (Bio-Rad)/0.5% gelatin for 1 hr at 37°C. The nitrocellulose was washed three times for 15 min at 37°C with TBS/0.5% Tween 20/0.5% gelatin and then incubated with a 1:10,000 dilution of goat anti-rabbit antiserum conjugated to alkaline phosphatase (Promega) in TBS/0.5% Tween 20/0.5% gelatin for 1 hr at 37°C. The nitrocellulose was then washed three times for 15 min at room temperature in TBS/0.5% Tween 20 and developed with 10 ml of a solution containing 3.3 mg of nitroblue tetrazolium and 1.7 mg of 5-bromo-4-chloro-3-indolyl phosphate in 100 mM NaCl/5 mM $MgCl_2$ (Promega).

RESULTS

Disruption of the CD4–p56^{lck} Complex But Not the CD8–p56^{lck} Complex Occurs Upon Incubation with PMA. The protein-tyrosine kinase p56^{lck} has been shown to associate with the CD4 and CD8 T-cell surface molecules (3, 4). CD4 and CD8 are rapidly phosphorylated on cytoplasmic serine residues during T-cell activation or activation of the PKC pathway by phorbol esters (11–13, 15, 16, 20). However, CD4 is rapidly internalized from the surface of the cell whereas CD8 surface expression is not affected (11–13, 15, 16, 20). Furthermore, phorbol esters induce a disruption of the CD4–p56^{lck} but not the CD8–p56^{lck} complex (21).

The human CD4 and CD8 molecules were expressed in the murine thymoma BW5147 as described. Upon incubation with PMA, the CD4, but not the CD8, molecule was rapidly internalized from the surface of BW5147 cells as determined by indirect immunofluorescence (Fig. 1*A*, compare *b* and *d*). There was no appreciable staining of the parent BW5147 cell line with either anti-CD4 (Fig. 1*Aa*) or anti-CD8 (Fig. 1*Ac*) mAb.

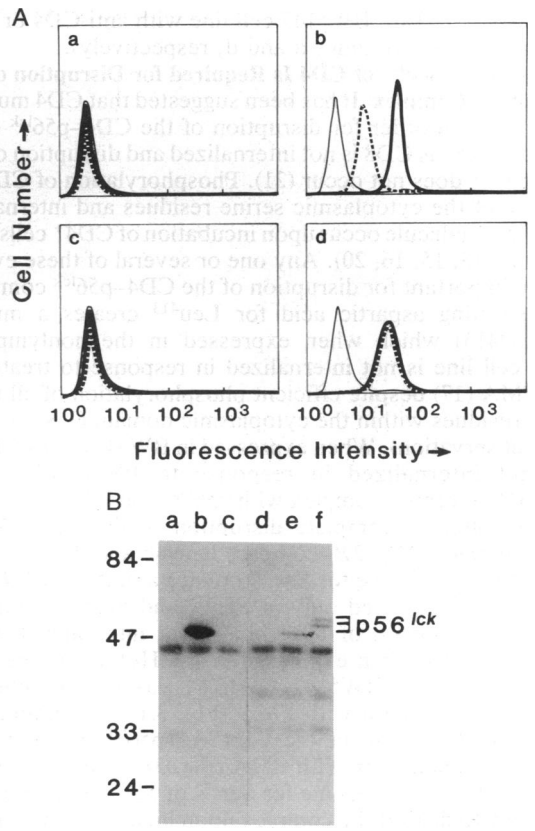

FIG. 1. (*A*) CD4 but not CD8 is internalized in response to PMA when expressed in BW5147. CD4$^-$/CD8$^-$ (*a* and *c*), CD4$^+$ (*b*) or CD8$^+$ (*d*) BW5147 cells were incubated in the presence (---) or absence (—, —) of PMA (100 ng/ml) for 60 min at 37°C. Cells were incubated with the anti-CD4 mAb anti-Leu3a (*a* and *b*; ---, —) or the anti-CD8 mAb OKT8 (*c* and *d*; ---, —) followed by fluorescein-conjugated goat anti-mouse immunoglobulin or with fluorescein-conjugated goat anti-mouse immunoglobulin alone (—). The data are from a representative experiment of three experiments that were carried out. (*B*) PMA induces disruption of the CD4–p56^{lck} complex but not the CD8–p56^{lck} complex. CD4$^-$/CD8$^-$ (lanes a and d), CD4$^+$ (lanes b and c), or CD8$^+$ (lanes e and f) BW5147 cells were incubated in the presence (lanes c and f) or absence (lanes a, b, d, and e) of PMA (100 ng/ml) for 60 min at 37°C. Immunoprecipitations were carried out with the anti-CD4 mAb anti-Leu3a (lanes a–c) or the anti-CD8 mAb OKT8 (lanes d–f). The presence of p56^{lck} in immunoprecipitates was determined by immunoblot analysis. The p56^{lck} molecule is indicated. Bio-Rad prestained low molecular weight markers were used (bovine serum albumin, 84 kDa; chicken ovalbumin, 47 kDa; bovine carbonic anhydrase, 33 kDa; soybean trypsin inhibitor, 24 kDa). The data are from a representative experiment of two that were carried out.

In the absence of PMA, the anti-CD4 mAb immunoprecipitated the p56^{lck} molecule complexed with CD4 (Fig. 1*B*, lane b), and the anti-CD8 mAb immunoprecipitated the p56^{lck} molecule complexed with CD8 (lane e). There was a marked difference in the amount of p56^{lck} associated with CD8 as compared to that associated with CD4 (compare lanes b and e). This difference has been previously reported and may be due to a lower affinity of CD8, as compared to CD4, for p56^{lck} (6). Incubation of the CD4$^+$ and CD8$^+$ cell lines with PMA resulted in disruption of the CD4–p56^{lck} complex (Fig. 1*B*, compare lanes b and c) but not the CD8–p56^{lck} complex (compare lanes e and f). The higher molecular weight forms of p56^{lck} associated with CD8 upon incubation with PMA have been described and are due primarily to serine phosphorylation of p56^{lck} (ref. 22; J.S., unpublished observation). The p56^{lck} molecule was not immunoprecipitated from the

parent CD4⁻/CD8⁻ BW5147 cell line with anti-CD4 or anti-CD8 mAb (Fig. 1*B*, lanes a and d, respectively).

Integrity of Ser^{408} of CD4 Is Required for Disruption of the CD4–p56^{lck} Complex. It has been suggested that CD4 must be internalized in order for disruption of the CD4–p56^{lck} complex to occur, as CD8 is not internalized and disruption of the CD8–p56^{lck} does not occur (21). Phosphorylation of CD4 on all three of the cytoplasmic serine residues and internalization of the molecule occur upon incubation of CD4$^+$ cells with PMA (11–13, 15, 16, 20). Any one or several of these events may be important for disruption of the CD4–p56^{lck} complex.

Substituting aspartic acid for Leu^{413} creates a mutant (CD4LD413) which when expressed in the nonlymphoid HeLa cell line is not internalized in response to treatment with PMA (17) despite efficient phosphorylation of all three serine residues within the cytoplasmic domain (J.S., unpublished observation). When expressed in BW5147, CD4LD413 was not internalized in response to PMA (Fig. 2*A*). CD4LD413 forms a complex with p56^{lck}, and incubation with PMA resulted in complete disruption of the CD4LD413–p56^{lck} complex (Fig. 2*B*, compare lanes c and d).

Substituting alanine for Ser^{408} creates a mutant (CD4SA1) that is phosphorylated only at Ser^{415} and Ser^{431} within the cytoplasmic domain of CD4 but is not internalized in response to PMA when expressed in the HeLa cell line (16). When expressed in BW5147, CD4SA1 was also not internalized upon incubation with PMA (Fig. 3*A*). In addition, no significant disruption of the CD4SA1–p56^{lck} complex was noted upon incubation with PMA (Fig. 3*B*, compare lanes c and d). Substituting alanine for Ser^{415} or Ser^{431} did not affect the ability to disrupt the complex upon incubation with PMA (data not shown).

These data demonstrate that internalization of CD4 is not required for disruption of the CD4–p56^{lck} complex, as the CD4LD413 mutant is not internalized in response to PMA yet disruption of the CD4LD413–p56^{lck} complex occurs (Fig. 2*B*). Further, the integrity of the Ser^{408} appears to be critical for disruption of CD4–p56^{lck}, as disruption of the CD4SA1–p56^{lck} complex was not observed (Fig. 3*B*). The integrity of Ser^{408} may be critical due to a requirement for phosphorylation.

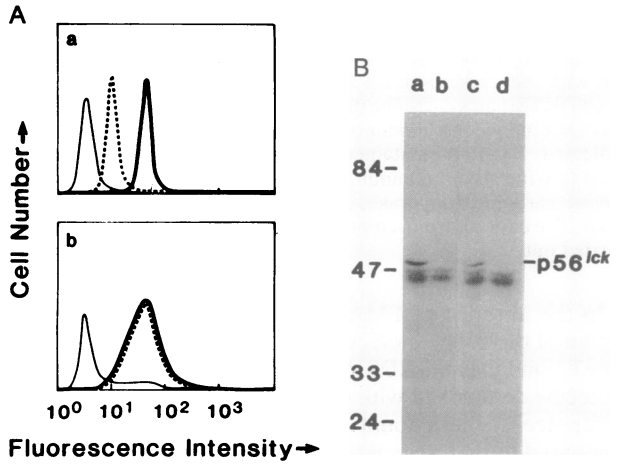

FIG. 2. (*A*) CD4LD413 is not internalized in response to incubation with PMA. Internalization of CD4 (*a*) and CD4LD413 (*b*) expressed in BW5147 in response to PMA was assessed as described in the legend to Fig. 1. The data are from a representative experiment of three that were carried out. (*B*) PMA induces disruption of the CD4LD413–p56^{lck} complex. CD4$^+$ (lanes a and b) and CD4LD413$^+$ (lanes c and d) BW5147 cell lines were incubated in the presence (lanes b and d) or absence (lanes a and c) of PMA. Immunoprecipitation and p56^{lck} immunoblot analysis were as described in the legend to Fig. 1. The data are from a representative experiment of two that were carried out.

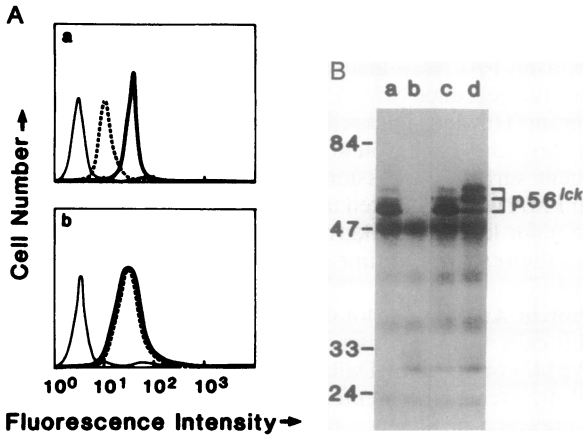

FIG. 3. (*A*) CD4SA1 is not internalized in response to incubation with PMA. Internalization of CD4 (*a*) and CD4SA1 (*b*) expressed in BW5147 in response to PMA was assessed as described in the legend to Fig. 1. The data are from a representative experiment of three that were carried out. (*B*) PMA does not induce disruption of the CD4SA1–p56^{lck} complex. CD4$^+$ (lanes a and b) and CD4SA1$^+$ (lanes c and d) BW5147 cell lines were incubated in the presence (lanes b and d) or absence (lanes a and c) of PMA. Immunoprecipitation and anti-p56^{lck} immunoblot analysis were as described in the legend to Fig. 1. The data are from a representative experiment of three that were carried out.

Disruption of the CD4–p56^{lck} Complex Is Required for Rapid Internalization of the CD4 Receptor. Substituting serine for Cys^{420} (CD4CS420) or Cys^{422} (CD4CS422) abolishes CD4–p56^{lck} complex formation (6, 7). When these mutants and a mutant in which serine was substituted for Cys^{430} (CD4CS430) were expressed in BW5147, CD4 and CD4CS430 but not CD4CS420 or CD4CS422 formed complexes with p56^{lck} (Fig. 4*A*). Analysis of the kinetics of internalization of these mutants in response to PMA revealed that the rate of internalization of the CD4CS420 and CD4CS422 molecules, which fail to associate with p56^{lck}, was significantly faster than the rate of internalization of CD4 or CD4CS430, which do associate with p56^{lck} (Fig. 4*B*). Maximal internalization was observed at 120 min and was similar for all mutants (data not shown). The difference in kinetics appears to be due to the association between CD4 and p56^{lck}, as CD4 and CD4CS420 were internalized at approximately the same rate when expressed in the p56^{lck}-negative HeLa cell line (data not shown).

To directly assess the effect of p56^{lck} on internalization of CD4, p56^{lck} was transiently expressed in a HeLa cell line that stably expressed CD4. Optimal expression of p56^{lck} was observed at 48 hr after transient transfection of HeLa cells with a plasmid containing the p56^{lck} cDNA (Fig. 5*A*, lane c). Immunoprecipitation with anti-CD4 mAb demonstrated that p56^{lck} forms a complex with CD4 in HeLa and that this complex is not disrupted upon incubation with PMA (Fig. 5*A*, compare lanes g and h). Expression of p56^{lck} in CD4$^+$ HeLa cells significantly decreased the kinetics of internalization of CD4 in response to PMA (Fig. 5*B*). The maximal level of CD4 internalization was achieved at 120 min and was significantly lower in CD4$^+$ HeLa cells that transiently expressed p56^{lck} (Fig. 5*B*).

DISCUSSION

During T-cell activation or PMA-induced activation of PKC, CD4 is phosphorylated on cytoplasmic serine residues and internalization and disruption of the CD4–p56^{lck} complex occurs (11–16, 21). Structural analysis of the cytoplasmic domain of CD4 has revealed that it can be divided into at least two distinct regions. The membrane-distal domain contains a

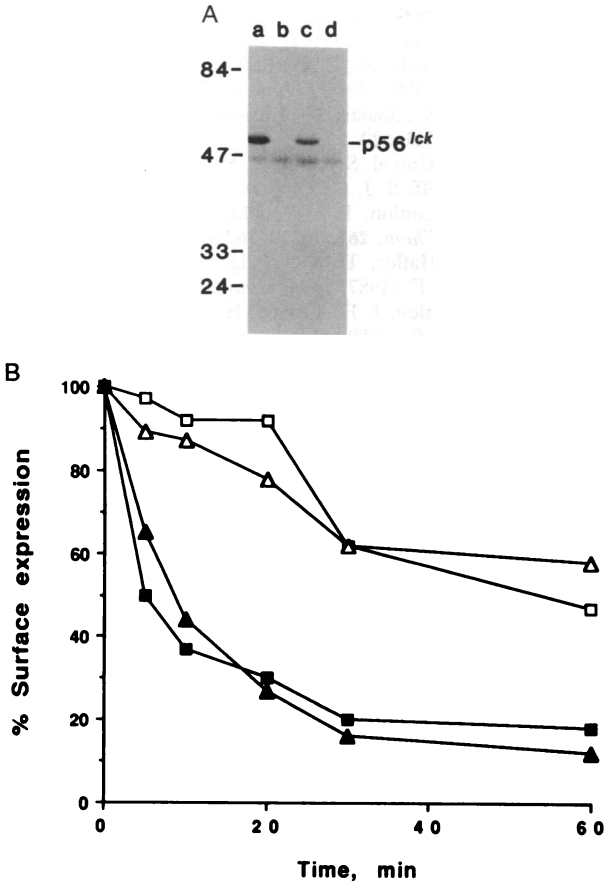

FIG. 4. (*A*) p56^{lck} forms a complex with CD4 and CD4CS430 but not with CD4CS420 or CD4CS422 when expressed in BW5147. Immunoprecipitations were carried out with the anti-CD4 mAb anti-Leu3a, on 20 × 10^6 BW5147 cells that expressed CD4 (lane a), CD4CS420 (lane b), CD4CS430 (lane c), or CD4CS422 (lane d), followed by anti-p56^{lck} immunoblot analysis. The data are from a representative experiment of three that were carried out. (*B*) When expressed in BW5147 cells, CD4 and CD4CS430, which associate with p56^{lck}, are internalized at a slower rate in response to PMA when compared to CD4CS420 and CD4CS422, which do not associate with p56^{lck}. BW5147 cell lines expressing CD4 (□), CD4CS430 (△), CD4CS420 (▲), or CD4CS422 (■) were incubated with PMA (10 ng/ml) for 0, 5, 10, 20, 30, and 60 min. Cells were stained with the anti-CD4 mAb anti-Leu3a as described in the legend to Fig. 1. Quantitative analysis of cell surface expression was carried out as described. The data are from a representative experiment of two that were carried out.

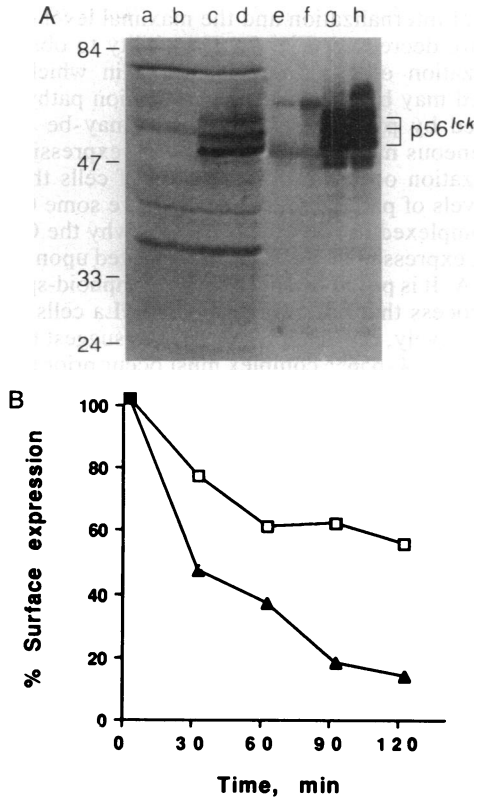

FIG. 5. (*A*) The CD4–p56^{lck} complex expressed in HeLa is not disrupted by incubation with PMA. CD4$^+$ HeLa cells (5 × 10^6) that expressed p56^{lck} (lanes c, d, g, and h) or did not express p56^{lck} (lanes a, b, e, and f) were incubated for 2 hr in the presence (lanes b, d, f, and h) or absence (lanes a, c, e, and g) of PMA (5 ng/ml). p56^{lck} present in whole cell lysates (lanes a–d) and in anti-CD4 immunoprecipitates (lanes e–h) was analyzed as described in the legend to Fig. 1. The data shown are representative of two experiments carried out. (*B*) Expression of p56^{lck} in HeLa decreases the rate of internalization of CD4 in response to PMA. CD4$^+$ HeLa cells (5 × 10^6) that did (□) or did not (▲) express p56^{lck} were incubated with PMA (5 ng/ml) for 0, 30, 60, 90, and 120 min. Surface expression of CD4 was determined as described. The data shown are from a representative experiment of two that were carried out.

critical cysteine motif necessary for noncovalent association with p56^{lck} (5–8). The membrane-proximal domain is homologous to a membrane-proximal region of the EGF receptor and is predicted to form an α-helical structure, the integrity of which appears to be critical for PKC-induced internalization of CD4 (17). In addition, the membrane-proximal domain contains Ser^{408}, the integrity of which is required for internalization of CD4 probably due to a requirement for phosphorylation (16).

Hormone receptors such as the insulin and EGF receptors possess distinct regions of the cytoplasmic domain that encode internalization and intrinsic protein-tyrosine kinase activity (refs. 23 and 24; reviewed in ref. 25). Activation of the tyrosine kinase domain of both the insulin and EGF receptors occurs upon binding of their respective ligands (reviewed in ref. 26). Activation of the kinase domain appears to be critical for internalization and/or intracellular sorting of these receptors (refs. 23, 24, and 27–32; reviewed in ref. 25). It has been suggested that kinase activation of these receptors results in a conformational change in the cytoplasmic domain exposing regions necessary for internalization (23, 33).

Here we demonstrate that incubation with phorbol ester results in disruption of the CD4–p56^{lck} complex and internalization of CD4 (Fig. 1). Internalization of CD4 is not required for disruption of the complex, as the CD4LD413 molecule is not internalized although complete disruption of the CD4LD413–p56^{lck} complex was observed (Fig. 2). This process depends on the integrity of Ser^{408}, as disruption of the CD4SA1–p56^{lck} complex was not observed (Fig. 3). These data suggest that phosphorylation of Ser^{408}, which is intact in CD4LD413, may lead to disruption of the CD4–p56^{lck} complex. However, the possibility of a PKC-mediated event independent of phosphorylation but requiring the integrity of Ser^{408} cannot be eliminated.

When expressed in BW5147 cells, CD4 and CD4CS430, which do associate with p56^{lck}, were internalized at a slower rate than CD4CS420 and CD4CS422, which do not associate with p56^{lck} (Fig. 4). The maximal levels of internalization of all of these molecules when expressed in BW5147 were equivalent (data not shown). The slower kinetics of internalization of the CD4 and CD4CS430 molecules is most likely due to a requirement for disruption of the CD4–p56^{lck} complex prior to internalization, as disruption of the CD4–p56^{lck} complex does not occur in HeLa cells and thus both the

kinetics of internalization and the maximal level of internalization are decreased (Fig. 5). The ability to observe some internalization of CD4 in HeLa cells in which $p56^{lck}$ is expressed may be due to an internalization pathway that is unaffected by $p56^{lck}$. Alternatively, it may be due to the heterogeneous nature of $p56^{lck}$ transient expression, so that internalization occurs in a population of cells that express lower levels of $p56^{lck}$ and, therefore, have some CD4 which is not complexed to $p56^{lck}$. It is unclear why the CD4–$p56^{lck}$ complex expressed in HeLa is not disrupted upon incubation with PMA. It is possible that there is a lymphoid-specific step in the process that does not occur in HeLa cells.

Cumulatively, the data presented here suggest that disruption of the CD4–$p56^{lck}$ complex must occur prior to the rapid internalization of the CD4 molecule observed upon incubation with PMA. Further, the integrity of Ser^{408} is critical for disruption of the complex, probably due to a requirement for phosphorylation. The requirement for disruption of the CD4–$p56^{lck}$ complex prior to internalization of CD4 may be analogous to the conformational change that occurs upon activation of the kinase domain of receptors with intrinsic tyrosine kinase activity, thus exposing the membrane-proximal region of CD4 and allowing for internalization. Endocytosis of CD4 in resting lymphocytes occurs at a slow rate, whereas in nonlymphoid cells, which do not express $p56^{lck}$, the rate is much faster (34). In resting lymphocytes, endocytosis of CD4 does not occur via coated pits, whereas in nonlymphoid cells, endocytosis via coated pits is observed (34). Further, it has recently been shown that expression of $p56^{lck}$ in nonlymphoid cells slows the rate of endocytosis of CD4 and results in the exclusion of CD4 from coated pits (35). The disruption of the CD4–$p56^{lck}$ complex that occurs upon activation may be necessary to allow CD4 to occupy a position within coated pits resulting in rapid internalization.

We are indebted to Dr. Kurt Amrein for generously providing us with the $p56^{lck}$ rabbit antiserum. In addition, we thank Dr. Sheldon Ratnofsky for providing us with the CD8 DAMP retroviral producer line and Dr. David Diamond for providing us with the CD4SA1 DAMP retroviral producer line. This work was supported by a grant from Hoffman–LaRoche and by grants from the National Institutes of Health (AI17258 to S.J.B.; AI15669 and CA47554 to J.L.S.).

1. Sleckman, B. P., Peterson, A., Jones, W. A., Foran, J. A., Greenstein, J. L., Seed, B. & Burakoff, S. J. (1987) *Nature (London)* **328,** 351–353.
2. Doyle, C. & Strominger, J. L. (1987) *Nature (London)* **330,** 256–259.
3. Rudd, C. E., Trevillyan, J. M., Dasgupta, J. D., Wong, L. L. & Schlossman, S. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5190–5194.
4. Veillette, A., Bookman, M. A., Horak, E. M. & Bolen, J. M. (1988) *Cell* **55,** 301–308.
5. Shaw, A. S., Amrein, K. E., Hammond, C., Stern, D. F., Sefton, B. M. & Rose, J. K. (1989) *Cell* **59,** 627–636.
6. Turner, J. M., Brodsky, M. H., Irving, B. A., Levin, S. D., Perlmutter, R. A. & Littman, D. R. (1990) *Cell* **60,** 756–765.
7. Shaw, A. S., Chalupny, J., Whitney, A., Hammond, C., Amrein, K. E., Kavathas, P., Sefton, B. M. & Rose, J. K. (1990) *Mol. Cell. Biol.* **10,** 1853–1862.
8. Veillette, A., Sleckman, B. P., Ratnofsky, S., Bolen, J. B. & Burakoff, S. J. (1990) *Eur. J. Immunol.* **20,** 1397–1400.
9. Glaichenhaus, N., Shastri, N., Littman, D. R. & Turner, J. M. (1991) *Cell* **64,** 511–520.
10. Collins, T. L., Uniyal, S., Shin, J., Strominger, J. L., Mittler, R. S. & Burakoff, S. J. (1992) *J. Immunol.* **148,** 2159–2162.
11. Acres, R. B., Conlon, P. J., Mochizuki, D. Y. & Gallis, B. (1986) *J. Biol. Chem.* **261,** 16210–16214.
12. Blue, M.-L., Hafler, D. A., Craig, K. A., Levine, H. & Schlossman, S. F. (1987) *J. Immunol.* **139,** 3949–3954.
13. Blue, M.-L., Daley, J. F., Levine, H., Branton, K. R., Jr., & Schlossman, S. F. (1989) *J. Immunol.* **142,** 374–380.
14. Weyand, C. M., Goronzy, J. & Fathman, C. G. (1987) *J. Immunol.* **138,** 1351–1354.
15. Sleckman, B. P., Bigby, M., Greenstein, J. L., Burakoff, S. J. & Sy, M. S. (1989) *J. Immunol.* **142,** 1457–1462.
16. Shin, J., Doyle, C., Yang, Z., Kappes, D. & Strominger, J. L. (1990) *EMBO J.* **9,** 425–434.
17. Shin, J., Dunbrack, R. L., Jr., Lee, S. & Strominger, J. L. (1991) *J. Biol. Chem.* **266,** 10658–10665.
18. Sleckman, B. P., Peterson, A., Foran, J. A., Gorga, J. C., Kara, C. J., Strominger, J. L., Burakoff, S. J. & Greenstein, J. L. (1988) *J. Immunol.* **141,** 49–54.
19. Voronova, A. F. & Sefton, B. M. (1986) *Nature (London)* **219,** 682–685.
20. Acres, R. B., Conlon, P. J., Mochizuki, D. Y. & Gallis, B. (1987) *J. Immunol.* **139,** 2268–2274.
21. Hurley, T. R., Luo, K. & Sefton, B. M. (1989) *Science* **245,** 407–409.
22. Marth, J. D., Lewis, D. B., Cooke, M. P., Mellins, E. D., Gearn, M. E., Samelson, L. E., Wilson, C. B., Miller, A. D. & Perlmutter, R. M. (1989) *J. Immunol.* **142,** 2430–2437.
23. Chen, W. S., Lazar, C. S., Lund, K. A., Welsh, J. B., Chang, C.-P., Walton, G. M., Der, C. J., Wiley, H. S., Gill, G. N. & Rosenfeld, M. G. (1989) *Cell* **59,** 33–43.
24. Backer, J. M., Kahn, C. R., Cahill, D. A., Ullrich, A. & White, M. F. (1990) *J. Biol. Chem.* **265,** 16450–16454.
25. Ullrich, A. & Schlessinger, J. (1990) *Cell* **61,** 203–212.
26. Backer, J. M. & King, G. L. (1991) *Biochem. Pharmacol.* **41,** 1267–1277.
27. Hari, J. & Roth, R. A. (1987) *J. Biol. Chem.* **262,** 15341–15344.
28. McClain, D. A., Maegawa, H., Lee, J., Dull, T. J., Ullrich, A. & Olefsky, J. M. (1987) *J. Biol. Chem.* **262,** 14663–14671.
29. Russell, D. S., Gherzi, R., Johnson, E. L., Chou, C.-K. & Rosen, O. M. (1987) *J. Biol. Chem.* **262,** 11833–11840.
30. Felder, S., Miller, K., Moehren, G., Ullrich, A., Schlessinger, J. & Hopkins, C. R. (1990) *Cell* **61,** 623–634.
31. Chen, W. S., Lazar, C. S., Poenie, M., Tsien, R. Y., Gill, G. N. & Rosenfeld, M. G. (1987) *Nature (London)* **328,** 820–823.
32. Honegger, A. M., Dull, T. J., Felder, S., Van Obberghen, E., Bellot, F., Szapary, D., Schmidt, A., Ullrich, A. & Schlessinger, J. (1987) *Cell* **51,** 199–209.
33. Perlman, R., Bottaro, D. P., White, M. F. & Kahn, C. R. (1989) *J. Biol. Chem.* **264,** 8946–8950.
34. Pelchen-Matthews, A., Armes, J. E., Griffiths, G. & Marsh, M. (1991) *J. Exp. Med.* **173,** 575–587.
35. Pelchen-Matthews, A., Boulet, I., Littman, D. R., Fagard, R. & Marsh, M. (1992) *J. Cell. Biol.* **117,** 279–290.