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Regulation of CD4-p56<latex>$^{\text{lck}}$</latex>-associated Phosphatidylinositol 3-kinase (PI 3-kinase) and Phosphatidylinositol 4-kinase (PI 4-kinase)

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Phil. Trans. R. Soc. Lond. B 1993 **342**, 35-42
doi: 10.1098/rstb.1993.0132

Regulation of CD4-p56^{lck}-associated phosphatidylinositol 3-kinase (PI 3-kinase) and phosphatidylinositol 4-kinase (PI 4-kinase)

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SUMMARY

CD4 serves as a receptor for MHC class II antigens and as a receptor for the human immunodeficiency virus (HIV-1) viral coat protein gp120. It is coupled to the protein-tyrosine kinase p56^{lck}, an interaction necessary for an optimal response of certain T cells to antigen. Although anti-CD4 crosslinking may increase *lck* activity, the effects of HIV-1 gp120 have been controversial. Activated protein-tyrosine kinases are known to associate with certain intracellular proteins possessing src-homology regions (SH-2 domains) such as phosphatidylinositol 3-kinase (PI 3-kinase). In this paper, we demonstrate that the CD4:p56^{lck} complex associates with significant amounts of phosphatidylinositol (PI) kinase activity. High pressure liquid chromatographic (HPLC) analysis of the reaction products demonstrated the presence of phosphatidylinositol 3-phosphate (PI 3-P) and phosphatidylinositol 4-phosphate (PI 4-P), thus indicating that PI 3 and PI 4 kinases associate with CD4-p56^{lck}. The p85 subunit of PI 3-kinase was also detected in anti-CD4 immunoprecipitates by immunoblotting with anti-p85 antiserum. Significantly, p56^{lck} binding to CD4 appears to be necessary for the detection of lipid kinase activity associated with p56^{lck}. Also, anti-HIV gp120 and anti-CD4 crosslinking induced a 10–15-fold increase in levels of both PI 3- and PI 4-kinase activity in anti-CD4 precipitates. Stimulation of CD4-p56^{lck}-linked PI kinases by crosslinked HIV-1 gp120 may play a role in HIV-1-induced immune defects.

1. INTRODUCTION

CD4 serves as a receptor for MHC class II antigens and as a receptor for the human immunodeficiency virus (HIV-1) viral coat protein gp120 (Reinherz *et al.* 1983; Rudd *et al.* 1989; Littman 1990; Dalgleish *et al.* 1984; Bedinger *et al.* 1988). Both CD4 and CD8 are coupled to the src-related protein-tyrosine kinase p56^{lck} (Rudd *et al.* 1988; Barber *et al.* 1989; Veillette *et al.* 1988). The association is required for an optimal response of certain T cells to antigen (Sleckman *et al.* 1988; Glaichenhaus *et al.* 1991; Abraham *et al.* 1991). CD4-p56^{lck} complex can also physically associate with the TcRζ/CD3 complex (Burgess *et al.* 1991), and as such synergize with the TcRζ/CD3 complex in augmenting T cell proliferation (Emmerich *et al.* 1986; Eichmann *et al.* 1987; Anderson *et al.* 1987). Although anti-CD4 crosslinking may increase lck activity (Veillette *et al.* 1989; Luo & Sefton 1990), the effects of HIV-1 gp120 have been controversial (Horak *et al.* 1990; Juszczak *et al.* 1991; Kaufmann *et al.* 1992). p56^{lck} may be responsible for the tyrosine phosphorylation of substrates induced by CD4 or TcR/CD3 ligation (Veillette *et al.* 1989; June *et al.* 1990; Deans *et al.* 1992). Potential targets include phospholipase Cγ (PLCγ) (Weber *et al.* 1992), MAP-2 kinase (Ettehadieh *et al.* 1992); CD5 (Burgess *et al.* 1992) and p70^{zap} (Chan *et al.* 1992). Identification of downstream targets of the CD4-p56^{lck} complex will be key to unravelling components of the tyrosine phosphorylation in T cell activation.

Phosphatidylinositol 3-kinase (PI 3-kinase) is a potential component of the signalling cascade initiated by CD4-TcRζ/CD3 ligation. PI 3-kinase phosphorylates the D-3 position of the inositol ring of phosphatidylinositol, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5 bisphosphate (Cantley *et al.* 1991; Carpenter & Cantley 1990). It is a heterodimeric protein, comprised of a regulatory subunit (p85) and a catalytic subunit (p110) (Carpenter *et al.* 1990, Escobedo *et al.* 1991; Skolinik *et al.* 1991; Otsu *et al.* 1991; Hiles *et al.* 1992). p85 is comprised of two Src-homology 2 domains (SH2 domains) which mediate binding to phosphotyrosine residues within

Phil. Trans. R. Soc. Lond. B (1993) **342**, 35–42
Printed in Great Britain

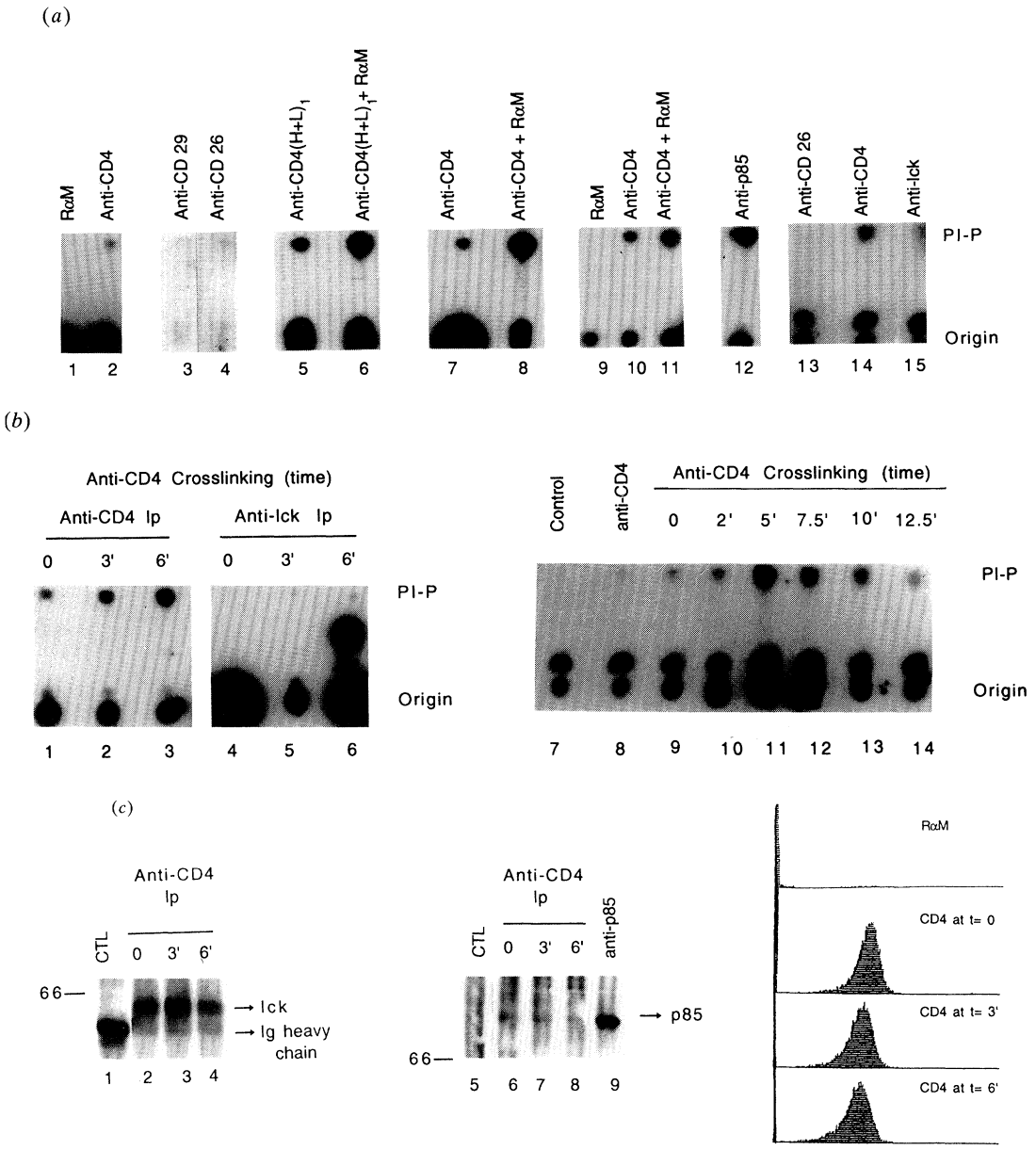

Figure 1. PI kinase activity associated with CD4 is enhanced by antibody induced CD4 crosslinking. TLC analysis of the PI-P formed in anti-CD4 immunoprecipitates is presented. (*a*) Untreated HPB-ALL cells were lysed in a NP40/digitonin lysis buffer (0.5% each) and subjected to immunoprecipitation with rabbit anti-mouse (lane 1), anti-CD4 (lane 2 and 14), anti CD26 (lane 13) and anti-lck (lane 15). Alternatively, cells were pre-treated with anti-CD29 (lane 3), anti-CD26 (lane 4), univalent anti-CD4 $(H+L)_1$ (lane 5), univalent anti-CD4 $(H+L)_1$ plus rabbit anti-mouse (lane 6), bivalent anti-CD4 (lane 7 and 10) or bivalent anti-CD4 plus rabbit anti-mouse (lanes 8 and 11) prior to precipitation, as indicated in the Methods. Addition of anti-CD4 (lanes 7 and 10) for 6 min at 37°C greatly enhanced the levels of precipitable PI kinase activity. Crosslinking of either univalent anti-CD4 (lane 6) or bivalent anti-CD4 (lane 8 and 11) with rabbit anti-mouse increased levels of precipitable activity. Anti-p85 precipitates from cell lysates served as a positive control (lane 12; shorter term exposure of film; see below). (*b*) Left panel: timecourse of effect of rabbit anti-mouse induced anti-CD4 crosslinking at 37°C on precipitable CD4 associated PI kinase activity. Anti-CD4 precipitates (lanes 1–3); anti-$p56^{lck}$ precipitates from CD4 depleted cell lysates (lanes 4–6). Times of incubation: lanes 1 and 4, 0 min; lanes 2 and 5, 3 min; lanes 3 and 6, 6 min. The intermediate spot in lane 6 is a contaminating hot spot unrelated to the experiment. Right panel: kinetic analysis of the regulation of CD4:$p56^{lck}$ associated PI kinase activity. Lane 7 represents immune complexes obtained from HPB-ALL cells treated with anti-CD29 and crosslinked with rabbit anti-mouse (0 min at 37°C). Cells treated with anti-CD4 alone (0 min at 37°C) (lane 8). Anti-CD4/RaM crosslinked samples correspond to: 0 min (lane 9), 2 min (lane 10), 5 min (lane 11), 7.5 min (lane 12), 10 min (lane 13) and 12.5 min (lane 14). (*c*) Left panel: anti-lck immunoblotting of CD4 precipitates during the timecourse of CD4 crosslinking with anti-CD4 and rabbit anti-mouse. Lane 1, rabbit anti-mouse control. Anti-CD4 and rabbit anti-mouse (lanes 2–4). Times of incubation: lane 2, 0 min; lane 3, 3 min; lane 4, 6 min. Middle panel: anti-p85 immunoblotting of CD4 immunoprecipitates during the timecourse of incubation, as in left panel (lanes 5–9). Lane 5, rabbit anti-mouse control. Anti-CD4 plus RaM (lanes 6–8). Times of incubation: lane 6, 0 min; lane 7, 3 min; lane 8, 6 min. Anti-p85 (lane 9). Right panel: flow cytometric analysis of the expression of CD4 receptors after anti-CD4 crosslinking is also shown.

the cytoplasmic tail of receptor tyrosine kinases such as the platelet derived growth factor (PDGF-R) (31–33). p85 also binds to activated pp60src (Fukui & Hanafusa 1989), associated middle T antigen (Talamage *et al.* 1989) and the insulin receptor substrate IRS-1 (Yonezawa *et al.* 1992). By contrast, the p110 subunit is related to the yeast protein *Vps*34 that plays an essential role in protein sorting and transport (Robinson *et al.* 1988; Herman & Emr 1990). The importance of PI 3-kinase has been shown by its requirement in signalling by PDGF-R (Fantl *et al.* 1992).

In this study, we demonstrate that the CD4:p56^{lck} complex associates with significant amounts PI 3- and PI 4-kinase activity. High pressure liquid chromatographic (HPLC) analysis of the reaction products demonstrated the presence of phosphatidylinositol 3-phosphate (PI 3-P) and phosphatidylinositol 4-phosphate (PI 4-P). Both anti-HIV gp120 and anti-CD4 crosslinking induced a 10–15-fold increase in levels of both PI 3- and PI 4-kinase activity in anti-CD4 precipitates. CD4-p56^{lck}–PI 3-kinase binding is likely to constitute a key interaction in HIV-1 gp120 and CD4-TcRζ/CD3 induced T cell function.

2. MATERIALS AND METHODS

(*a*) *Antibodies and immunoprecipitation analysis*

HPB-ALL cells (20×10^6 ml^{-1}) were solubilized in NP-40/Digitonin (0.5% each by volume) in 20 mM Tris-HCL, pH 8.3 containing 150 mM NaCl, 1 mM PMSF (phenyl methylsulphonyl fluoride) and immunoprecipitated with an excess of anti-CD4 antibody (19Thy5D7, 20 μg ml^{-1}), as described (Rudd *et al.* 1988; Barber *et al.* 1989). Alternatively, the same number of cells in cold RPMI containing 2% (by volume) fetal calf serum (FCS) were incubated with an excess of anti-CD4 antibody, or anti-CD26 (1F7, 10 μg ml^{-1}) or anti-CD29 (4B4, 10 μg ml^{-1}) for 30 min at 4°C. Cells were either lysed or further incubated with rabbit anti-mouse antibody (RaM, 6 μg ml^{-1}) for 30 min at the same cell density. After washing twice with ice cold media, cells were resuspended in warm RPMI/2% FCS and incubated for various times at 37°C. Activation through receptor crosslinking was arrested by diluting the cells with ice cold RPMI. Cells were centrifuged, washed twice with ice cold RPMI and solubilized in NP-40/Digitonin lysis buffer, as described (Rudd *et al.* 1988; Barber *et al.* 1989). The cleared lysate was then incubated with Protein A Sepharose beads for 2 h at 4°C under constant rotation. For a positive control, immunoprecipitations from cell lysates were carried out using anti-p85 rabbit antisera raised against the 85 kDa subunit of PI 3-kinase. Immune complexes were washed three times with PBS (1% NP-40), three times with 100 mM Tris, pH 7.5 with 0.5 M LiCl and twice with TNE (10 mM Tris-Hcl, pH 7.5, 150 mM NaCl and 1 mM EGTA). The lipid kinase reaction was carried out on the beads using phosphatidyl inositol and [^{32}Pγ]-ATP (20 μCi), as described (Whitman *et al.* 1985; Auger *et al.* 1989). Lipids were then extracted using chloroform and methanol (1:1) and separated by thin layer chromatography on a silica gel plate precoated with potassium oxalate using a basic system (chloroform, methanol, water, ammonium hydroxide (60: 47, 11.3, 2) (Whitman *et al.* 1985; Auger *et al.* 1989). The plates were then removed dried, wrapped in a plastic wrap and exposed to a X-ray film, overnight at −70°C, except for lane 12 which is a half-hour exposure.

Cell lysates were further depleted of CD4 by sequential precipitation (three times) using anti-CD4 antibody, followed by two preclearing steps using Protein A Sepharose. Depleted lysates were then subjected to precipitation using an anti-lck sera (figure 1*b*, left panel, lanes 4–6). The antisera was raised in rabbits against an amino acid terminal peptide (residues 39–64) coupled to key hole limpet haemocyanin (KLH) (Prasad & Rudd 1992). After transfer to nitrocellulose, membranes were blocked with gelatin (2% by mass) in Tris-buffered saline (10 mM Tris-Hcl, pH 8.0 with 150 mM NaCl) and probed with the anti-lck rabbit antisera (1:1000 dilution) (figure 1*c*, left panel). Immunoblots were visualized using goat anti-rabbit alkaline phosphatase system (Promega). p85 of PI-3 kinase was visualized by immunoblotting with anti-p85 rabbit sera (1:4500) and enhanced by chemiluminescence (ECL) Western blotting (Amersham) (figure 1*c*, middle panel). Aliquots of the cells at the different time points were pelleted, incubated with goat anti-rabbit-FITC (Fisher) and assayed for CD4 surface expression by flow cytometry using an EPICS cell sorter (Coulter Immunology, Florida) (figure 1*c*, right panel).

HPB-ALL cells at a density similar to that described for anti-CD4 crosslinking experiment (figure 1) were exposed to a mixture of anti-CD4 antibody (20 μg ml^{-1}; 19thy5D7, IgG2A) and rat anti-mouse (0.2 μg ml^{-1}, Zymed, California, IgG2a) was added to the cells at 37°C for various times. In contrast to the experiment described in figure 1, cells were shifted to 37°C at the same time as the addition of anti-CD4 and rabbit anti-mouse. In case of control, comparable amounts of anti-CD29 (4B4) and rat anti-mouse were added. Cells were lysed at the indicated times and analysed for CD4-associated PI kinase activity by TLC as described in figure 1. The corresponding PI-P spots were extracted deacylated and subjected to HPLC analysis (Whitman *et al.* 1985; Auger *et al.* 1989).

(*b*) *HIV-1 gp120 crosslinking*

Conditions of HIV gp120 binding were established as previously described (Kaufmann *et al.* 1992). gp120 was radiolabelled with ^{125}I-Bolton Hunter reagent and incubated with 2×10^5 cells for 2 h. Free and bound ligand were separated by centrifugation of cells through silicon oil (specific density: 1.011 g ml^{1}). The bottom of the 300 μl vials (Sarstedt) was cut off for measurement of bound radioligand. Non-specific binding was determined by performing the experiment in the presence of 100 nM soluble CD4. The anti-gp120 sera blocks the binding of soluble gp120, and binds, but does not dissociate CD4 bound gp120.

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Computation of the binding parameters (equilibrium dissociation constant (K_d), etc.) were determined as described (Kaufmann *et al.* 1992). For lipid kinase assays, HPB-ALL cells were harvested and suspended at a density of 20×10^6 cells ml^{-1} in ice cold RPMI (2% by volume FCS) and rotated with recombinant HIV-1 gp120 (ABT, Cambridge) derived from baculovirus Sf9 cell system at 4°C for 2 h (concentration of native pure protein, 1.0×10^{-8} M; K_d, 1.06×10^{-8} M). Based on the measurement of the association kinetics, this procedure results in gp120 binding to 10% of surface CD4 molecules (data not shown). The cells were washed twice with ice cold RPMI (2% FCS) and treated with anti-gp120 rabbit sera (1:100, ABT, Cambridge) for 1 h at 4°C. A 1/100 dilution of rabbit anti-gp120 results in antibody binding to 45–50% of gp120-CD4 complexes on the cell surface (figure 3*a*). The cells were washed and treated with saturating amounts of goat anti-rabbit antibody (1:200, Sigma) for 0.5 h at 4°C. Cells were then incubated at 37°C for the indicated times. Following cell lysis immunoprecipitations were carried out and subjected to a lipid kinase reaction as described (figure 1). The reaction products were deacylated and analysed by HPLC as described.

3. RESULTS

To determine whether the CD4-p56^{lck} complex can associate with PI kinase, anti-CD4 precipitates from the leukemic T cell line HPB-ALL (figure 1*a*) were assayed for the ability to generate monophosphorylated phosphoinositides (PIP) from exogenously added lipids. A 5 min incubation of intact HPB-ALL cells with either univalent $(H+L)_1$ or bivalent anti-CD4 antibodies followed by immunoprecipitation resulted in the detection of significant amounts of PI kinase activity (lanes 5–11). Rabbit anti-mouse (RaM) (lanes 1 and 9), anti-CD29 (4B4) (lane 3) anti-CD26 (1F7) (lanes 4 and 13) failed to co-precipitate enzymic activity, while anti-85 precipitable PI 3-kinase activity served as a positive control (lane 12). Anti-CD4 precipitation from cells that had not been pre-incubated with anti-CD4 sufficed to precipitate PI kinase activity (lanes 2 and 14). Anti-p56^{lck} serum precipitated small levels of activity (lane 15), although consistently less than that precipitated by anti-CD4 (lane 14). Crosslinking of either univalent anti-CD4 (lane 6) or bivalent anti-CD4 (lanes 8, 11) with RaM increased levels of precipitable activity. Similar results were obtained using other T cell lines and peripheral T cells (data not shown).

We next attempted to determine changes in PI kinase activity over the timecourse of anti-CD4 binding. Anti-CD4 crosslinking has previously been reported to increase the level of p56^{lck} phosphorylation within T cells (Veillette *et al.*, 1989; Luo & Sefton 1990). Anti-CD4 crosslinking resulted in a time-dependent increase in precipitable lipid kinase activity (figure 1*b*, lanes 1–3 and 7–14). Further kinetic analysis showed a transient increase in activity, with maximal precipitable kinase activity at about 5 min, followed by a gradual decrease (lanes 9–14); under some conditions, elevated activity was still detected at 15 min. Under the same conditions, surface CD4 underwent a slight decrease in expression (figure 1*c*, right panel). Similarly, co-precipitated lck underwent no detectable change as monitored by anti-lck immunoblotting (figure 1*c*, left panel, lanes 1–4). Anti-p85 PI 3-kinase immunoblotting positively identified co-precipitated p85 associated with CD4-p56^{lck} (figure 1*c*, middle panel, lanes 5–9). The amount of p85 appeared to undergo little change over the timecourse; however, given the low amount of detectable material, a conclusive evaluation of this matter was not possible.

The small amount of activity precipitated by the anti-p56^{lck} serum (figure 1*a*, lane 15) was readily lost by depletion of lysates using anti-CD4 antibody (figure 1*b*, lanes 4–6). These data indicate that PI kinase preferentially binds to the CD4-p56^{lck} complex.

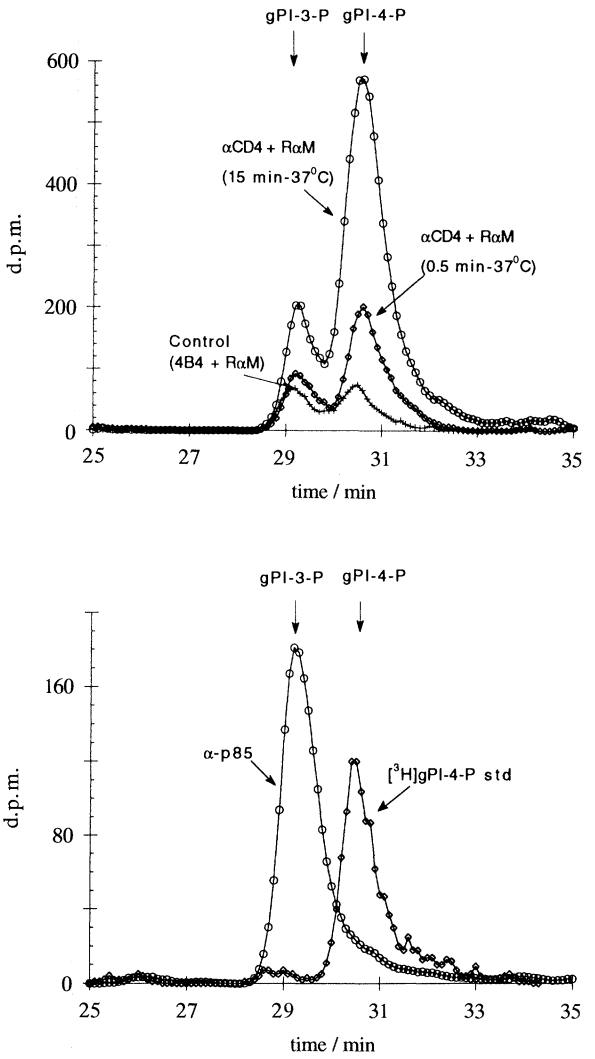

Figure 2. Both PI 3- and PI 4-kinase activities associate with the CD4:p56^{lck} complex and are up-regulated upon anti-CD4 antibody crosslinking. An HPLC analysis of the deacylated lipids. Upper panel: control (crosses); anti-CD4 and RaM, 0.5 min (diamonds) and 15 min (circles). Lower panel: PI-P analysis corresponding to anti-p85 showed only the presence of PI-3P (circles); the position of ^{3}H-gPI-4P standard is indicated (diamonds).

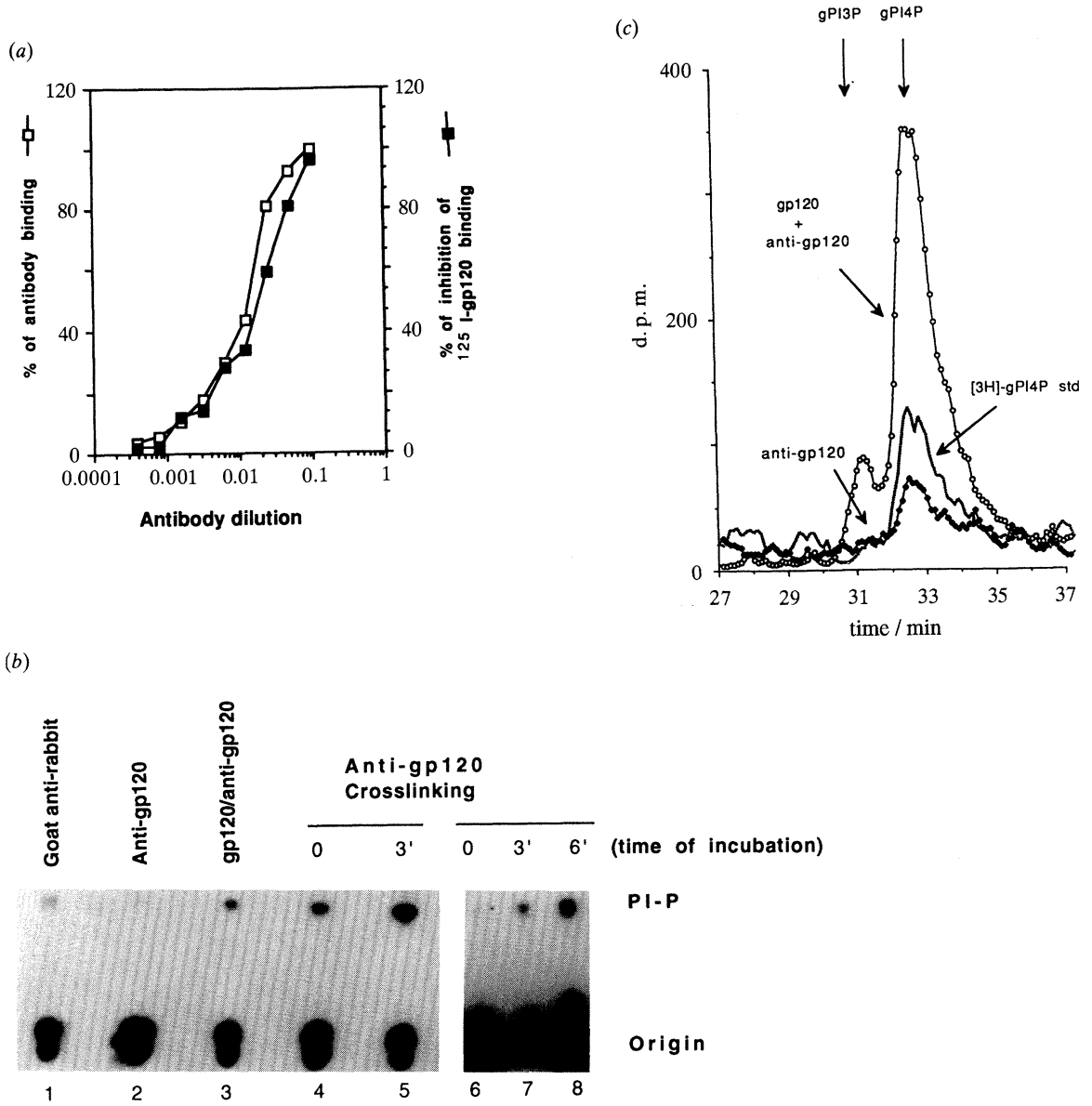

Figure 3. CD4 associated PI kinase activity is also enhanced by HIV-1 anti-gp120-mediated crosslinking of CD4 receptors. (*a*) Binding analysis of HIV-1 gp120 as detected by anti-gp120. Competition analysis of soluble HIV-gp120 by anti-gp120 antisera. (*b*) TLC analysis of CD4-precipitable PI kinase activity after gp120 mediated crosslinking. Cells were treated for various periods of time with the following antibodies: goat anti-rabbit (lane 1); rabbit anti-gp120 (lane 2); HIV-1 gp120 and rabbit anti-gp120 (lane 3); HIV-1 gp120, rabbit anti-gp120 and goat anti-rabbit (lanes 4–8). Time of incubation: 0 min (lanes 4 and 6); 3 min (lanes 5 and 7); 6 min (lane 8). Lanes 6–8 are from a separate experiment. (*c*) HPLC analysis of the deacylated reaction products. PI-P produced by immunocomplexes from cells treated with anti-gp120 alone (filled circles), or HIV-1 gp120, rabbit anti-gp120 plus goat anti-rabbit (open circles) were deacylated and analysed by HPLC; [^{3}H]-γPI 4P standard is indicated (dots).

HPLC analysis of the deacylated products of the anti-CD4 associated PI kinase confirmed the presence of PI 3-P and, surprisingly, significant amounts of PI 4-P (figure 2, upper panel). The levels of the two phospholipids varied slightly from experiment to experiment; however, PI 4-P formation exceeded PI 3-P by some 2- to 5-fold. Anti-p85 precipitates showed exclusive labelling of the PI 3-P product (figure 2, lower panel). Crosslinking of CD4-p56^{lck} complexes resulted in time-dependent increase in both PI 3- and PI 4-kinase activities (figure 2, upper panel). Both kinases showed a two- to threefold increase from 0.5 to 15 mm of crosslinking (see legend figure 2).

Of potential importance was the finding that the crosslinking of CD4-p56^{lck} by HIV gp120 also increased associated PI kinase activity. Purity of HIV-1 gp120 from Baculovirus Sf9 cells (>80%) and percent native protein (15.7%) were assessed as previously described (Kaufmann *et al.* 1992). Displacement studies showed gp120 binding to a single binding site with $K_d = 1.06 \times 10^{-8}$ M (Kaufmann *et al.* 1992). Studies were conducted using concentrations of gp120 designed to bind to a relatively low percent (approximately 10%) of CD4 surface receptors. This was followed by exposure of cells to a 1/100 dilution of rabbit anti-gp120 designed to bind 50% of gp120-

CD4 complexes (figure 3*a*). The combined exposure to gp120 and rabbit anti-gp120 precipitated moderate levels of PI kinase activity (figure 3*b*, lane 3). Further crosslinking of the immune complexes with a saturating concentration of goat anti-rabbit serum (1/200 dilution) demonstrated that HIV-1 gp120 aggregation induced a time-dependent increase in precipitable PI kinase activity (figure 3*b*, lanes 4–8). As controls, neither goat anti-rabbit, nor rabbit anti-gp120 alone precipitated activity (lanes 1 and 2). HPLC analysis of the products revealed the presence of increased levels of PI 3-P and PI 4-P compared with controls (figure 3*c*). Although previous studies have raised controversy about the effects of HIV-1 gp120-mediated CD4 crosslinking of $p56^{lck}$ activity (Horak *et al.* 1990; Juszczak *et al.* 1991; Kaufmann *et al.* 1992), our data from four experiments indicated that the crosslinking of HIV-1 gp120 has a marked modulatory effect on the activity of PI 3- and PI 4-kinases associated with the receptor.

4. DISCUSSION

CD4-$p56^{lck}$ associates and synergizes with the TcRζ/CD3 complex in the optimal response of T cells to antigen (Rudd 1992). A key issue has been to uncover the downstream events mediated by ligation of the CD4 complex. T cell activation has been reported to generate PI(3,4)P_2 and PI(3,4,5)P_3, thereby implicating PI 3- and PI 4-kinases in the activation cascade (Ward *et al.* 1991). In this study, we demonstrate that the CD4:$p56^{lck}$ complex associates with significant amounts PI 3- and PI 4-kinase activity, an interaction modulated by anti-HIV gp120 and anti-CD4 crosslinking. Confirmation of the interaction was further made by the detection of p85 by immunoblotting. A previous report by Thompson and co-workers (1992) detected CD4-$p56^{lck}$ associated PI 3-kinase activity; however, others failed to detect activity associated with $p56^{lck}$ (Augustine *et al.* 1991). The basis of this discrepancy may be related to the fact that the PI 3-kinase was detected primarily when present in a complex with CD4. Anti-lck precipitates derived from cell lysates depletion of CD4 failed to show detectable PI 3-kinase activity (Thompson *et al.* 1992). Our studies have also shown that PI 3-kinase binds to the SH3 domain of lck (data not shown). Therefore, it appears that the association of $p56^{lck}$ with the CD4 cytoplasmic tail may play a role in facilitating the ability of the SH3 domains to interact with PI 3-kinase. Whether this would involve the unfolding of $p56^{lck}$ from a restrained C-terminal-SH2 binding remains to be determined.

Surprisingly, unlike previous reports (Augustine *et al.* 1991; Thompson *et al.* 1992), PI 4-kinase was also found associated with CD4-$p56^{lck}$ at high levels and was regulated by receptor ligation (figures 1 and 2). Although structurally less well characterized, it is a key component in the classical PI pathway, replenishing PI 4,5-P_2 for PI turnover and PIP_3 production. PI 4-kinase associates with the epidermal growth factor receptor (EGF-R) independent of receptor activation and with a region distinct from the kinase domain (Cochet *et al.* 1991). If PI 3-kinase associates with internalized vesicles (Kelly *et al.* 1992), recruitment of PI 4-kinase onto the same vesicles may be necessary for the sustained production of PI 3,4-P_2 and/or PI 3,4,5-P_3. PI 4-kinase failed to bind the SH2/SH3 domains, and may instead bind to other regions of the kinase, or the CD4 receptor.

The CD4-$p56^{lck}$–PI 3/PI 4-kinase interaction may influence the pathogenesis of HIV-1 infectivity. Infection is characterized by the impaired function and loss of $CD4^+$ T cells, an event possibly mediated by apoptosis or syncytia (Fauci 1988; Weinhold *et al.* 1989; Groux *et al.* 1992). The yeast homolog of the p110 subunit of PI 3-kinase, *Vps* 34 kinase regulates membrane sorting and morphogenesis (Robinson *et al.* 1988; Herman & Emr 1990; Hiles *et al.* 1992). In contrast to CD4-$p56^{lck}$ activity (Horak *et al.* 1990; Kaufmann *et al.* 1992) crosslinking by HIV-1 gp120 of a small percent of CD4 molecules on the cell surface (about 5%) was sufficient to allow the detection of a marked increase in associated PI 3- and PI 4-kinase activity. By altering lipid metabolism, these enzymes may facilitate viral entry and inappropriately activate pathways involved in syncytium formation, or be detrimental to T cell signaling. Early activation events have been reported to render T cells susceptible to HIV-1-induced syncytia (Mohagheghpour *et al.* 1992). Similarly, CD4-$p56^{lck}$ has been reported to inhibit T cell growth (Haughn *et al.* 1992).

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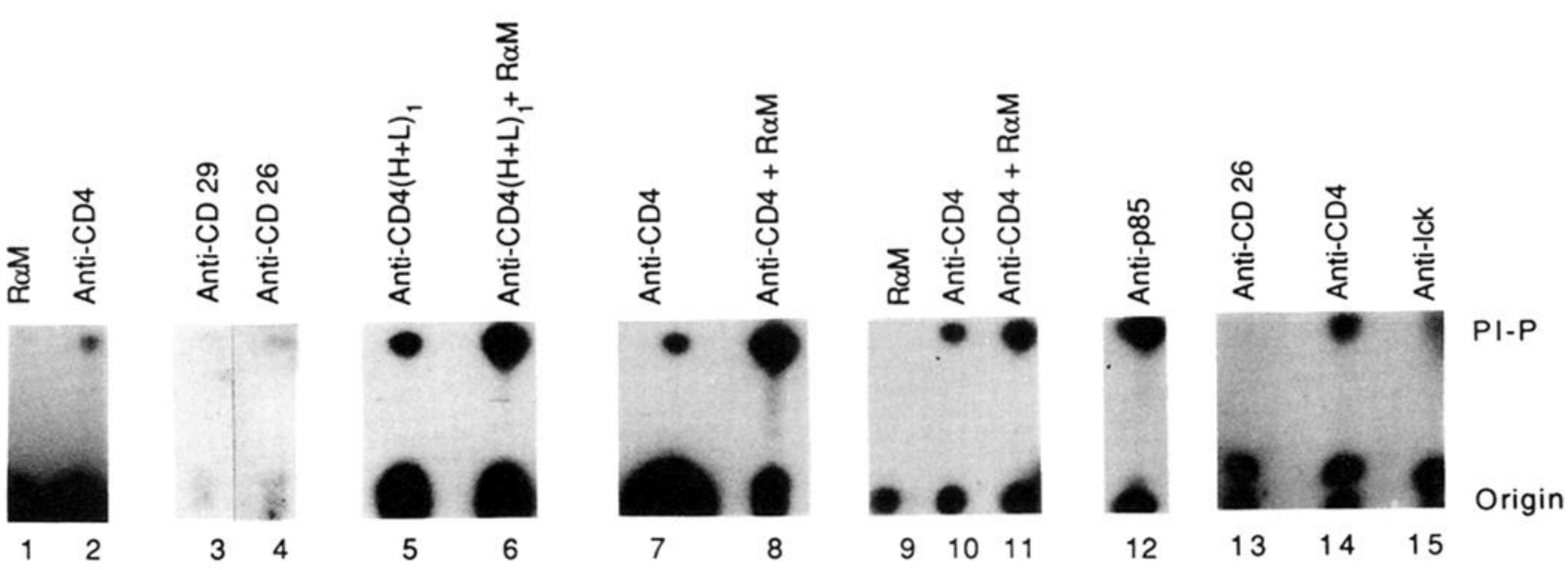
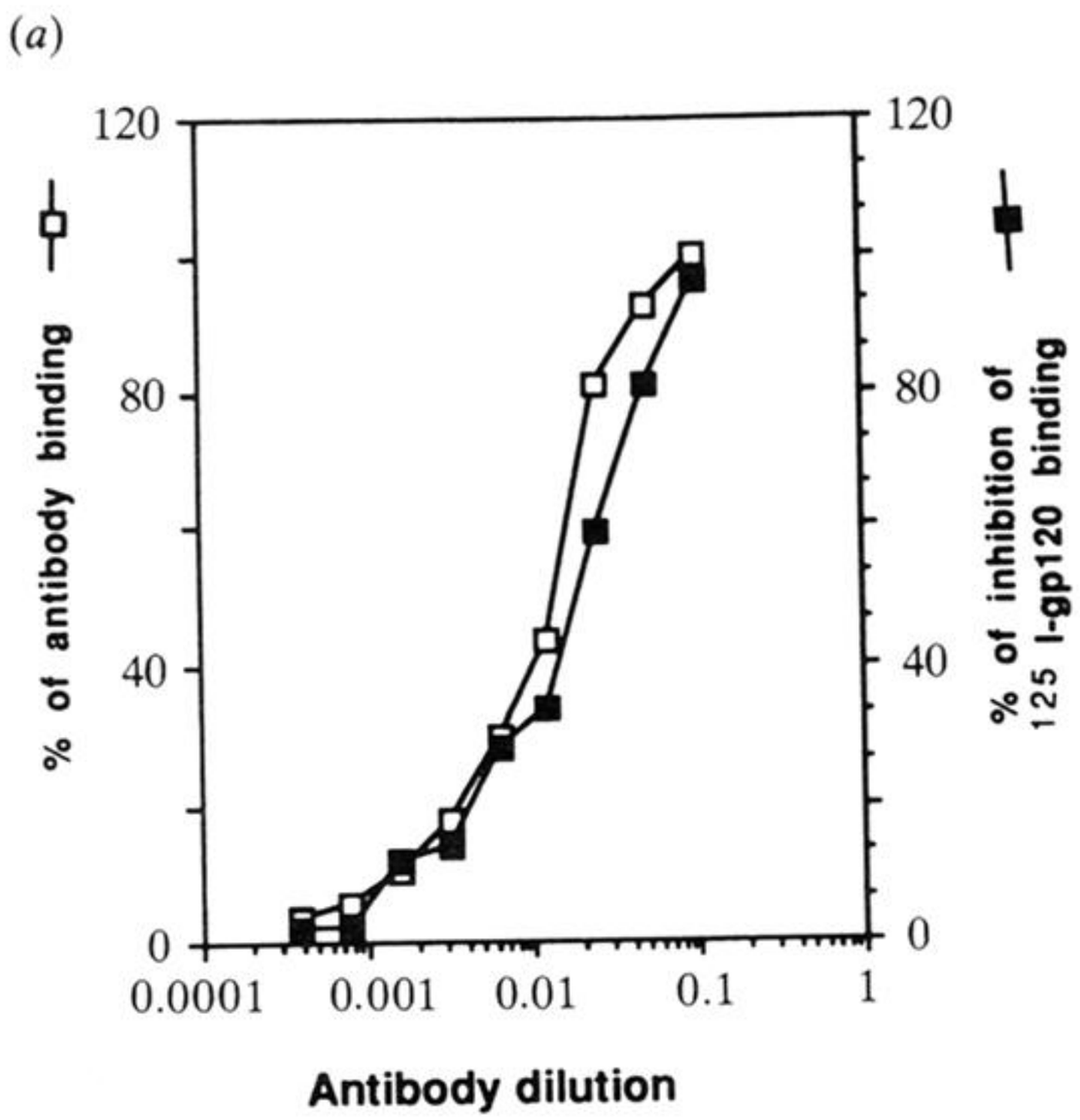
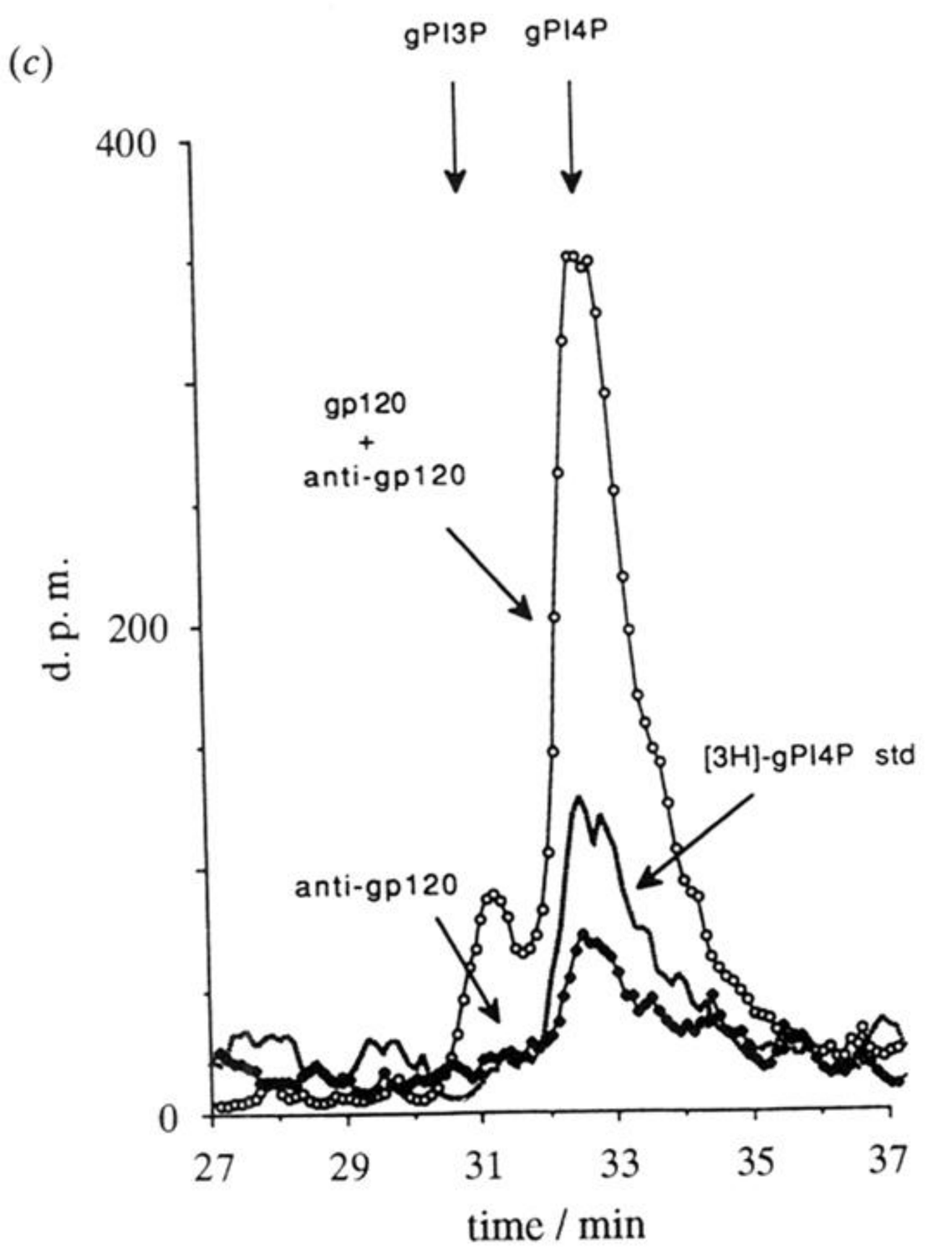


Figure 1. PI kinase activity associated with CD4 is enhanced by antibody induced CD4 crosslinking. TLC analysis of the PI-P formed in anti-CD4 immunoprecipitates is presented. (*a*) Untreated HPB-ALL cells were lysed in a NP40/digitonin lysis buffer (0.5% each) and subjected to immunoprecipitation with rabbit anti-mouse (lane 1), anti-CD4 (lane 2 and 14), anti CD26 (lane 13) and anti-lck (lane 15). Alternatively, cells were pre-treated with anti-CD29 (lane 3), anti-CD26 (lane 4), univalent anti-CD4 $(H+L)_1$ (lane 5), univalent anti-CD4 $(H+L)_1$ plus rabbit anti-mouse (lane 6), bivalent anti-CD4 (lane 7 and 10) or bivalent anti-CD4 plus rabbit anti-mouse (lanes 8 and 11) prior to precipitation, as indicated in the Methods. Addition of anti-CD4 (lanes 7 and 10) for 6 min at 37°C greatly enhanced the levels of precipitable PI kinase activity. Crosslinking of either univalent anti-CD4 (lane 6) or bivalent anti-CD4 (lane 8 and 11) with rabbit anti-mouse increased levels of precipitable activity. Anti-p85 precipitates from cell lysates served as a positive control (lane 12; shorter term exposure of film; see below). (*b*) Left panel: timecourse of effect of rabbit anti-mouse induced anti-CD4 crosslinking at 37°C on precipitable CD4 associated PI kinase activity. Anti-CD4 precipitates (lanes 1–3); anti-$p56^{lck}$ precipitates from CD4 depleted cell lysates (lanes 4–6). Times of incubation: lanes 1 and 4, 0 min; lanes 2 and 5, 3 min; lanes 3 and 6, 6 min. The intermediate spot in lane 6 is a contaminating hot spot unrelated to the experiment. Right panel: kinetic analysis of the regulation of CD4:$p56^{lck}$ associated PI kinase activity. Lane 7 represents immune complexes obtained from HPB-ALL cells treated with anti-CD29 and crosslinked with rabbit anti-mouse (0 min at 37°C). Cells treated with anti-CD4 alone (0 min at 37°C) (lane 8). Anti-CD4/RaM crosslinked samples correspond to: 0 min (lane 9), 2 min (lane 10), 5 min (lane 11), 7.5 min (lane 12), 10 min (lane 13) and 12.5 min (lane 14). (*c*) Left panel: anti-lck immunoblotting of CD4 precipitates during the timecourse of CD4 crosslinking with anti-CD4 and rabbit anti-mouse. Lane 1, rabbit anti-mouse control. Anti-CD4 and rabbit anti-mouse (lanes 2–4). Times of incubation: lane 2, 3 min; lane 3, 3 min; lane 4, 6 min. Middle panel: anti-p85 immunoblotting of CD4 immunoprecipitates during the timecourse of incubation, as in left panel (lanes 5–9). Lane 5, rabbit anti-mouse control. Anti-CD4 plus RaM (lanes 6–8). Times of incubation: lane 6, 0 min; lane 7, 3 min; lane 8, 6 min. Anti-p85 (lane 9). Right panel: flow cytometric analysis of the expression of CD4 receptors after anti-CD4 crosslinking is also shown.





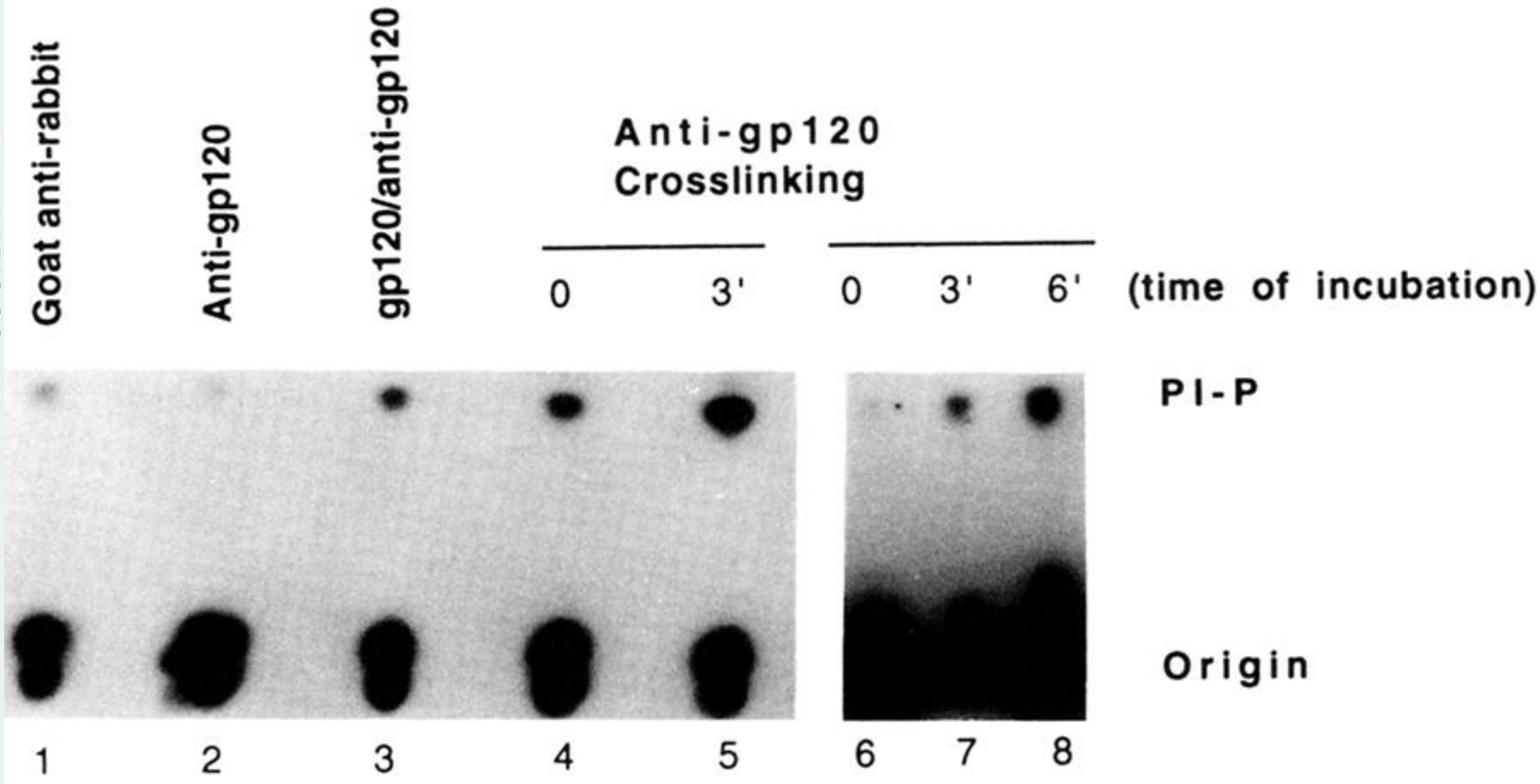


Figure 3. CD4 associated PI kinase activity is also enhanced by HIV-1 anti-gp120-mediated crosslinking of CD4 receptors. (*a*) Binding analysis of HIV-1 gp120 as detected by anti-gp120. Competition analysis of soluble HIV-gp120 by anti-gp120 antisera. (*b*) TLC analysis of CD4-precipitable PI kinase activity after gp120 mediated crosslinking. Cells were treated for various periods of time with the following antibodies: goat anti-rabbit (lane 1); rabbit anti-gp120 (lane 2); HIV-1 gp120 and rabbit anti-gp120 (lane 3); HIV-1 gp120, rabbit anti-gp120 and goat anti-rabbit (lanes 4–8). Time of incubation: 0 min (lanes 4 and 6); 3 min (lanes 5 and 7); 6 min (lane 8). Lanes 6–8 are from a separate experiment. (*c*) HPLC analysis of the deacylated reaction products. PI-P produced by immunocomplexes from cells treated with anti-gp120 alone (filled circles), or HIV-1 gp120, rabbit anti-gp120 plus goat anti-rabbit (open circles) were deacylated and analysed by HPLC; [^{3}H]-γPI 4P standard is indicated (dots).