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# Impaired CD45-Associated Tyrosine Phosphatase Activity during HIV-1 Infection: Implications for CD3 and CD4 Receptor Signalling

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**Proper function of the protein tyrosine phosphatase CD45 is required for the positive regulation of the activity of *src* tyrosine kinases p56$^{lck}$ and p59$^{fyn}$ which participate in T-cell receptor and CD4 receptor signalling. In this study, the effect of HIV-1 infection on the function of CD45-associated tyrosine phosphatase activity in the H9 T-cell line has been investigated with respect to CD3 and CD4 ligation. A significant reduction in CD45-associated phosphatase activity was observed following CD3 + CD4 ligation in virally infected cells, whereas CD45 activity was not compromised following CD3 receptor ligation. Dysfunctional CD45 activity in infected cells was not attributable to reduced receptor surface expression induced by HIV-1, since CD4, CD3 and CD45 expression levels were found to be intact. Defective CD45 activity correlated with inhibted downstream signalling events as evidenced by reduced CD4-associated tyrosine kinase activity and inhibition of PLC-γ1. Impaired CD45 function is likely to play a critical role in the inhibition of CD3/CD4 signalling thereby contributing to HIV-1 pathogenesis.** 

Human immunodeficiency virus-1 (HIV-1) infection results in quantitative and qualitative defects in the function of CD4 positive T lymphocytes (1). Evidence suggests that perturbation in signal transduction events which can be caused by the virus and/or viral proteins play a role in cellular dysfunction (2, 3). The mechanisms and nature by which HIV-1 interferes with intracellular signalling are ill-defined. In this regard, CD4/p56$^{lck}$ signalling events have been demonstrated to be affected during HIV-1 infection (4, 5). Distinct protein tyrosine phosphorylation (6) and other T-cell signal activation events such as stimulation of p56$^{lck}$ activity (7), the inositol phosphate and $Ca^{2+}$ signal pathway (8) were found to be modified by gp120 and by whole virus. Work from our laboratory and a report (4) demonstrated that HIV-1 infection mediated dissociation of p56$^{lck}$ from the CD4 receptor thereby impairing CD4-mediated co-stimulation events of the T-cell receptor (TcR)/CD3 complex. CD4 molecules act as co-receptors that amplify TcR/CD3-induced signal transduction by a mechanism that requires the interaction of CD4 with the *src*-related protein tyrosine kinase (PTK) p56$^{lck}$ (9). The earliest biochemical events that follow stimulation of T-cells through the TcR/CD3 and CD4 complex is the activation of the PTKs of the *src* family such as p56$^{lck}$, p59$^{fyn}$ followed by the subsequent activation of ZAP-70, PLC-γ1, and the p21$^{ras}$ signal transduction pathways (9), eventually leading to T-cell effector function. Proximal signalling events in T-cells are regulated by the activity of the tyrosine phosphatase CD45 (10). In particular, CD45 regulates positively the activity of the *src* kinases p56$^{lck}$ and p59$^{fyn}$ by dephosphorylation of their respective negative-regulatory, C-terminal tyrosine residues (10–13). Given its critical role in the regulation of the activation state of p56$^{lck}$ and p59$^{fyn}$ we addressed the question whether CD45-associated tyrosine phosphatase activity was affected in T-cells which were productively infected with HIV-1. In addition, downstream targets of CD45 signalling such as CD4-associated PTK and PLC-γ1 function were assessed in control and infected cells. It was demonstrated in this study that the functional state of CD45 was compromised during HIV-1 infection after CD4 and CD3 + CD4 ligation. CD45 dysfunction directly correlated with aborted

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TcR/CD4 signalling, as evidenced by inhibited PLC-γ1 and CD4-associated tyrosine kinase activity.

## MATERIALS AND METHODS

*Cell culture and HIV infection of H9 cells.* H9 cells ($4 \times 10^6$/ml) were incubated with the cell free HIV-1 RF isolate (kindly provided by the Medical Research Council, UK) for 2 h at 37°C. The infectious dose varied between 2000–5000 pg/ml of p24, as was measured by p24 ELISA (14). Following this incubation period, unbound virus was removed by repeated washing with phosphate buffered saline (PBS) and both virus infected and uninfected cells were maintained in RPMI-1640 medium supplemented with 10% FCS (TCS, Oxford, UK), 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin (all from Sigma, Poole, UK) for 7 days. Supernatants were subsequently analysed for the expression of HIV-p24 antigen by ELISA.

*Staining of intracellular HIV-1 p24 antigen and immunofluorescence analysis.* The proportion of HIV-1 infected cells in culture was assessed using the anti-p24 monoclonal antibody KC57 (Coulter, Luton, UK) in a direct immunofluorescence assay as previously described (5). Control and virally exposed H9 cells were fixed, permeabilised and cells were incubated with anti-p24 antibody or FITC-labelled control antibody. Cells were washed thrice and resuspended in Facs-Flow before FACScan analysis (Becton Dickinson, Oxford, UK). For surface expression of CD3, CD4 and CD45 receptors, H9 cells were incubated with FITC-conjugated anti-CD4 antibody (Becton Dickinson, Oxford, UK), anti-CD3 (Dako, High Wycombe, UK) or anti-CD45 antibody (Serotech, Oxford, UK), washed twice and fixed with 2% paraformaldehyde overnight at 4°C before FACS analysis.

*Stimulation and lysis of H9 cells.* H9 cells were allowed to rest by incubation in serum-free RPMI-1640 medium for 2 h at 37°C. Cells were washed twice in a 0.9% NaCl solution and resuspended at $5 \times 10^7$. The cells were treated with a mixture of anti-CD4 (IOT 4B) and anti-CD3 (UCHT-1, Immunotech, Marseilles, France) monoclonal antibodies (both at 10 μg/ml) and then cross-linked with rabbit anti-mouse immunoglobulin (RAM) at 5 μg/ml at 37°C for 1 min. Stimulation was terminated by the addition of ice-cold 0.9% NaCl and centrifugation at 800g for 5 min. Cells were washed twice in ice-cold 0.9% NaCl and lysed for 45 min on ice in buffer containing 0.1% Nonidet-P-40, 20 mM Tris-HCl, pH 8, 150 mM NaCl, with freshly added protease inhibitors benzamidine, aprotinin, leupeptin (all at 10 μg/ml) and 1 mM PMSF, for the generation of CD45 immunoprecipitates. Alternatively for CD4 immunoprecipitations, cells were lysed in RIPA buffer (1% v/v Nonidet-P-40, 50 mM Tris-HCl, pH 8, 150 mM NaCl, 0.25% w/v sodium deoxycholate, 1 mM EDTA, 1 mM EGTA, 1 mM $Na_3VO_4$, with freshly added protease inhibitors.

*Immunoprecipitation of the CD4 and CD45 receptors.* Lysates were centrifuged at 14000g for 10 min at 4°C and post-nuclear supernatants were subjected 3 times to gel filtration using Sephadex G-25 columns according to the manufacturer's instructions (Promega, Southampton, UK). Lysates were centrifuged and supernatants were then incubated with anti-CD45 or anti-CD4 antibody for 2 h which was followed by the addition of protein A-Sepharose. Aliquots were washed 3 times with ice-cold lysis buffer and CD4 and CD45 immunoprecipitations were used for *in vitro* phosphatase and kinase assays, respectively.

*In vitro protein tyrosine phosphatase (PTP) assay of CD45 immunoprecipitates.* CD45 immunoprecipitates were resuspended in phosphatase assay buffer containing 100 mM sodium acetate pH 6.0, 1 mM EDTA, 1 mg/ml BSA and 0.1% mercaptoethanol and subjected to a non-radioactive based PTP microplate assay for 30 min at 30°C (Promega, Madison USA). The chemically synthesised phosphopeptide-1 END(pY)INASL at 100 μM served as the substrate for the reaction. The reactions were terminated and free phosphates were detected by the addition of 100 μl of a molybdate dye additive/ molybdate dye solution mix which was then followed by incubation for 30 min at room temperature. The samples were quantitated by measuring the absorbance at 630 nm using an ELISA microplate reader (Dynatech, Guernsey, UK).

*ELISA based protein tyrosine kinase assay.* CD4 immunoprecipitates were resuspended in kinase assay buffer (25 mM Hepes, pH 7.4, 20 mM $MnCl_2$, 1 mM $CaCl_2$, 1% w/v bovine serum albumin (BSA), 1 mM DTT, 5 mM $MnCl_2$, 2mM ATP, 100 μM $Na_3VO_4$) and were prepared for an ELISA based tyrosine kinase assay utilising synthetic human gastrin-17 (EGPWLEEEEEAYGWMDF-amide) as the substrate. The reactions were quantitated by measuring the absorbance at 450 nm.

Background readings (mean O.D. of negative controls + 2 S.D.) was subtracted from the O.D. values of the test samples to give the corrected O.D. Stimulatory index (S.I.) obtained from CD4 and CD45 activities was then calculated as follows:

$$\text{S.I.} = \frac{\text{Mean (corrected O.D. of stimulated sample)}}{\text{Mean (corrected O.D. of unstimulated sample)}}$$

*Immunoblotting of cell lysates.* For immunoblotting, aliquots of CD45 immunoprecipitates which were prepared as described above were resuspended in 4 × SDS sample buffer and subjected to SDS/ PAGE gel electrophoresis under reducing conditions as described by Laemmli (15). Gels were electrophoretically transferred on to polyvinylidene difluoride (PVDF) membranes (Sartorius, Epsom, UK) and blocked with 5% BSA in TBS buffer (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.4). Membranes were probed with anti-CD45 monoclonal antibody (UBI, Lake Placid, USA) for 1h, washed and incubated with alkaline phosphatase (AP)-conjugated secondary anti-mouse antibody (Sigma, Poole, UK). Immunoblots were washed extensively in TBS buffer and proteins visualised by the addition of the AP specific substrate 5-bromo-4-chloro-3 indolyl phosphate/nitro blue tetrazolium (Sigma, Poole, UK) according to the manufacturer's instructions.

*Myo-inositol labelling of H9 cells and HPLC analysis of inositol polyphosphates.* H9 cells ($1 \times 10^6$/ml) which were co-cultured for 5 to 6 days in the presence of HIV-1 RF were resuspended in complete DMEM medium with 5 μCi/ml of $^3$H myo-inositol (NEN Du-Pont, Stevenage, UK) and were incubated at 37°C for a further 48 h. The cells were washed twice and allowed to rest in serum-free DMEM medium for 2 h and then incubated with 10 mM of LiCl for 30 min in order to inhibit inositol monophosphate (InsP) phosphatase and inositol 1,4 bisphosphate (Ins,4-$P_2$)/inositol 1,3,4 trisphosphate (Insufficient,3,4-$P_3$) 1-phosphatase. Samples of $10^7$ cells were then stimulated with a combination of anti-CD3 and CD4 antibodies as described above for 30 min in the presence of 10 mM LiCl. Samples were washed 5 times in ice-cold PBS containing 10 mM LiCl and pellets were resuspended in 200 μl of PBS/ 10 mM LiCl. Cell lysis was achieved by the addition of 10% ice-cold trichloroacetic acid for 10 minutes on ice. Cell debris was removed by centrifugation and supernatants were neutralised to pH 7 by the addition of 1 M KOH. The aqueous phase was run on HPLC using a 200 μl sample loop and a strong anion exchange column. The water-soluble inositol polyphosphates were then eluted employing a linear gradient from HPLC-grade water to 1 M ammonium phosphate (pH 3.5). The eluate was then analysed by an in-line β-counter (Canberra Packard, Pangbourne, UK). Eluted inositol polyphosphates were identified by the use of Ins1-P, Ins4-P, Ins1,4-$P_2$, Ins1,3,4-$P_3$, Ins1,4,5-$P_3$, Ins1,3,4,5-$P_4$, Ins-$OHP_5$ standards (Du-Pont, Stevenage, UK).

*Statistical analysis.* Statistical analysis using the Mann-Whitney U test was performed using the Instat software package.

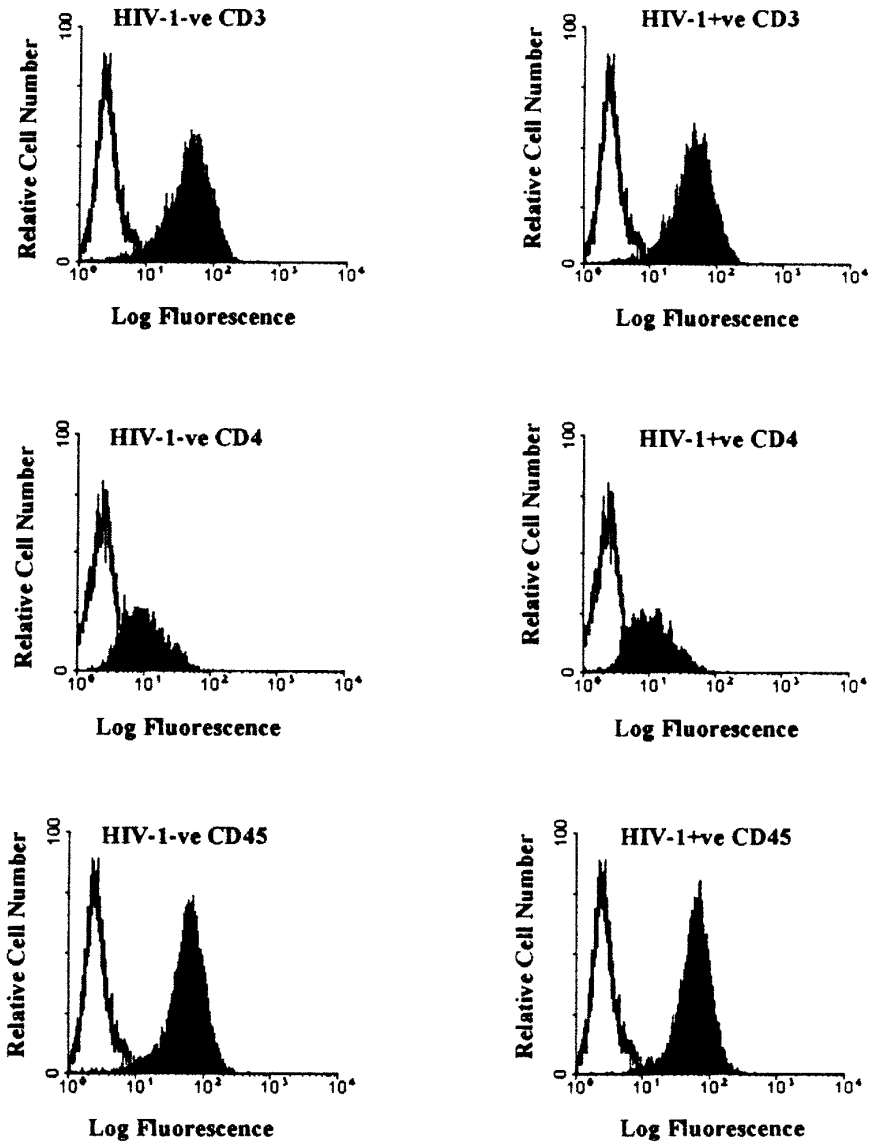


**FIG. 1.** Flow cytometric analysis of CD3, CD4 and CD45 surface expression levels in uninfected and HIV-1 RF infected H9 cells after 1 week in culture. H9 cells were incubated with FITC-labelled anti-CD3, CD4 and/or CD45 antibodies, washed, and analysed by flow cytometry. Results are shown from a representative of three experiments.

## RESULTS

*Effect of early HIV-1 infection on cell viability and cell surface receptor expression.* In this study we used the laboratory HIV-1 RF isolate as a laboratory model for HIV-1 pathogenesis. Extracellular levels of p24 antigen were found to range between ~4000 pg/ml to 7000 pg/ml and the percentage of infected H9 cells ranged between 55% to 61% 7 days post-infection. Following this culture period, H9 cells showed no significant loss of viability in this system as was measured by trypan blue exclusion. To address the question whether alterations in the receptor surface expression of CD3, CD4 and CD45 occurred following culture of H9 cells with HIV-1 RF, we stained the cells with anti-CD3, CD4 and CD45 antibody for FACSscan analysis. Data from a representative experiment are shown in Figure 1 which demonstrates that the level of CD3, CD4 and CD45 receptor expression on these cells was not significantly affected by HIV-1 infection and after 1 week culture. Thus, because no changes in cell viability and receptor expression between uninfected and infected cells were detected, they served as a cellular model for receptor signalling.

*Reduced CD45-associated protein tyrosine phosphatase activity in HIV-1 infected cells.* We went on to compare the enzymatic activation state of CD45 in con-

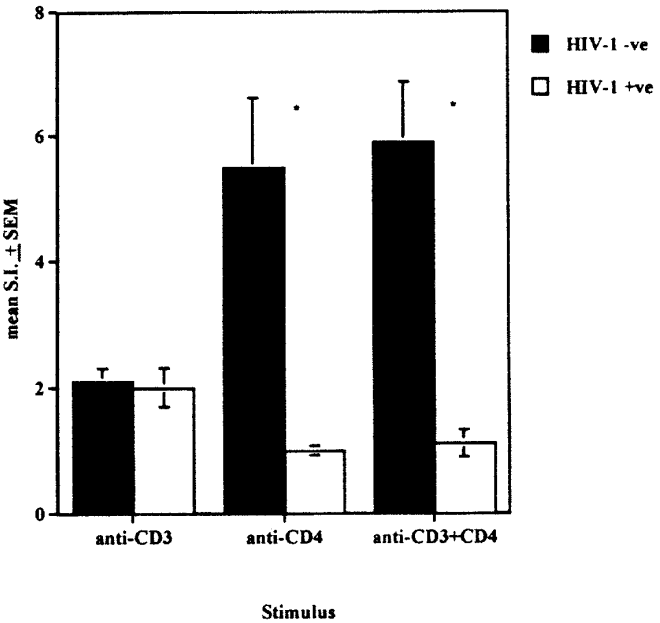


**FIG. 2.** Effect of CD3 and CD4 receptor cross-linking on induction of CD45-associated protein tyrosine phosphatase activity in uninfected and HIV-1 RF infected H9 cells. Quiescent H9 cells were stimulated as indicated, lysed in modified RIPA buffer and CD45 was immunoprecipitated. CD45 immunoprecipitates were then subjected to *in vitro* protein tyrosine phosphatase assay. Results are presented as the mean S.I. ± SEM from three independent experiments (* = $P < 0.05$).

trol and in HIV-1 infected lysates following CD3 and/or CD4 ligation. Immune-complex phosphatase activity tests were performed using an *in vitro* phosphatase assay in the presence of an exogenous substrate. In uninfected H9 cells anti-CD3 ligation resulted in a moderate induction of CD45 activity (~2 fold) as compared to the unstimulated controls (cells exposed to cross-linking RAM only; (Figure 2)). A similar increase in CD45 activity following exposure to anti-CD3 was observed in virally infected cells. Exposure to anti-CD4 alone mediated a rapid increase in CD45-associated activity (5.5 fold). By contrast, following CD4 cross-linking of virally infected cells, only a slight increase in activity was observed. This difference in reduced activity was found to be significant compared to the uninfected controls ($p < 0.05$ by the Mann-Whitney U test). In uninfected cells, simultaneous CD3 and CD4 receptor stimulation resulted in induction of CD45 phosphatase activity (5.9 fold) which was similar to exposure to anti-CD4 alone, whereas in HIV-1 infected cells a significant down-regulation of activity (mean S.I. = 1.1) could be observed compared to uninfected cells ($p < 0.05$ by the Mann-Whitney U test). Thus, in HIV-1 infected cells, CD45-associated protein tyrosine phosphatase was significantly down-modulated upon CD4 and CD3 + CD4 ligation. In order to exclude the possibility of a post-activation restoration and/or of a modified kinetic pattern of CD45 phosphatase activity in infected cells, we performed time course experiments on CD45 immunoprecipitates following CD3 + CD4 stimulation. As is depicted in Figure 3, CD45-associated phosphatase activity originating from control lysates was rapidly induced and reached a peak after 1 min and declined thereafter. By contrast in HIV-1 infected lysates, stimulation induced CD45 activity was down-regulated throughout the time course. However, a slight increase in activity was observed between 1 to 5 min post-activation (~1.8 fold), but it never approached values as high as in control cells (Figure 3). All activities could be almost completely inhibited by incubation with the selective tyrosine phosphatase inhibitor sodium orthovanadate, indicating a high degree of specificity of the phosphatase assay system (data not shown). In parallel experiments, we subjected CD45 immunoprecipitates to western-blot analysis to confirm that the phosphatase activity which mediated the tyrosine dephosphorylation of the exogenous substrate represented indeed CD45. In Figure 4, a representative immunoblot is shown of CD45 immunoprecipitates originating from infected and uninfected lysates, demonstrating that similar amounts of CD45 have been subjected to analyses.

*Reduced CD4-associated tyrosine kinase activity during HIV-1 infection.* It was further investigated whether the observed downregulation of CD45 activity following CD3 + CD4 ligation had implications on the activity state of CD4-associated tyrosine kinases. Control cells were pre-treated with the tyrosine phosphatase inhibitor sodium orthovanadate, stimulated with anti-CD3 + CD4 antibody and CD4 immunoprecipita-

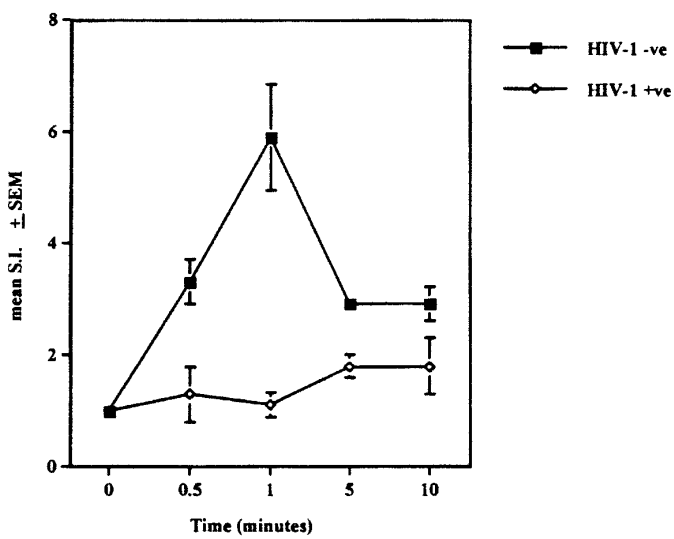


**FIG. 3.** Time course experiment of CD45 associated protein tyrosine phosphatase activity of uninfected and HIV-1 RF infected H9 cells following anti-CD3 + CD4 antibody stimulation. CD45 immunoprecipitations were subjected to an *in vitro* tyrosine phosphatase assay. Individual values represent the mean S.I. ± SEM from three experiments.

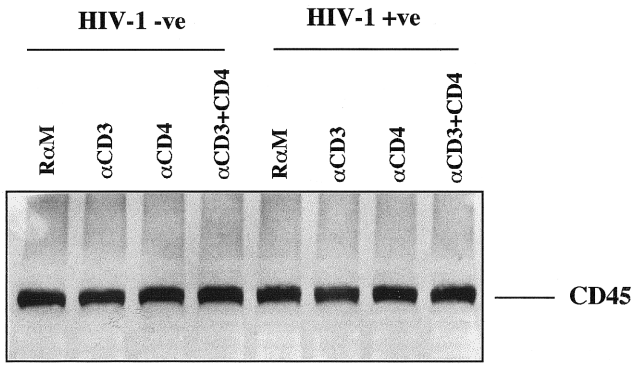


**FIG. 4.** Western blot analysis of CD45 immunoprecipitates originating from uninfected and HIV-1 RF infected H9 cells. Cells were stimulated as indicated, lysed and CD45 immunoprecipitates were subjected to 12% SDS PAGE. Membranes were probed with anti-CD45 antibody followed by the addition of AP-conjugated secondary antibody. Bands were revealed by the addition of the AP specific substrate BCIP/NBT. Blot is from a representative experiment, similar results were obtained in three different experiments.

tions were subjected to tyrosine kinase assays. It was established in preliminary experiments that inhibitor treatment of H9 cells inhibited CD45-associated phosphatase activity (data not shown). Figure 5a demonstrates that in inhibitor treated and CD3 + CD4 ligated cells the inducibility of tyrosine kinase(s) associated with the CD4 receptor is also profoundly inhibited, indicating the requirement of tyrosine phosphatase activity for induction of CD4-associated tyrosine kinases. It was further analysed whether HIV-1 infected cells which displayed inhibited CD45 activity following CD3 + CD4 ligation also exhibited reduced CD4-tyrosine kinase activity. Similar to inhibitor treated cells, virus exposed cells also showed markedly reduced tyrosine kinase activity which could be co-immunoprecipitated with the CD4 receptor compared to the uninfected controls (Figure 5b). Thus, there was a correlation between inhibition of CD45 phosphatase and tyrosine kinase activity during HIV-1 infection.

*Inhibition of PLC-γ1 function in HIV-1 RF exposed H9 cells.* It was further examined whether defective CD45 signalling events had an adverse effect on the activity state of PLC-γ1, a downstream target for TcR/CD4 signalling. Exposure of control and infected cells to anti-CD3 antibody alone resulted in hydrolysis of membrane PI 4,5-$P_2$, albeit a modified inositol polyphosphate profile was generated in infected cells (Figure 6b). PLC-γ1-mediated lipid hydrolysis was almost undetectable in infected cells, whereas the isomer 2-OH $IP_5$ predominated in control lysates (Figure 6c). The most pronounced difference in the production of isomers between infected and control cells was observed following CD3 + CD4 ligation. Compared to control cells HIV-1 exposed cells displayed a severely inhibited activation state of PLC-γ1, as was evidenced by completely abolished production of any inositol polyphosphates (Figure 6d). Thus, impaired PLC-γ1 function correlated with dysfunctional CD45/CD4 signalling during HIV-1 infection.

## DISCUSSION

T cell dysfunction appears early during the course of HIV-1 infection and results in the down-modulation of immune function which is reflected by the loss of responsiveness to recall antigen (16) before the loss of CD4 T-cells. The biochemical mechanisms by which

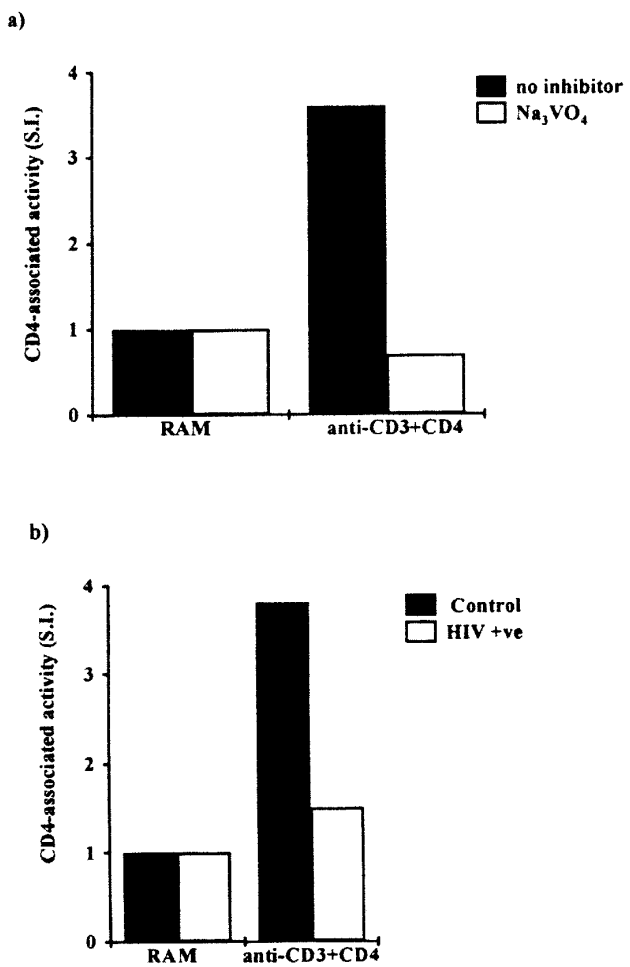


**FIG. 5.** Reduced CD4-associated tyrosine kinase activity following treatment with the tyrosine phosphatase inhibitor sodium orthovanadate and in HIV-1 infected cell lysates. H9 cells were pre-incubated with 5 mM sodium orthovanadate ($Na_3VO_4$) for 1 hour and were stimulated with anti-CD3 + CD4 antibody for 1 minute (a). Alternatively, infected cells were allowed to rest and subjected to the same stimulation regime (b). Cells were lysed in RIPA buffer containing 1 mM $Na_3VO_4$, CD4 was immunoprecipitated and then subjected to *in vitro* protein tyrosine kinase assay (a). Alternatively, infected cells were lysed in RIPA buffer, CD4 was immunoprecipitated and was subjected to an ELISA-based tyrosine kinase assay (b).

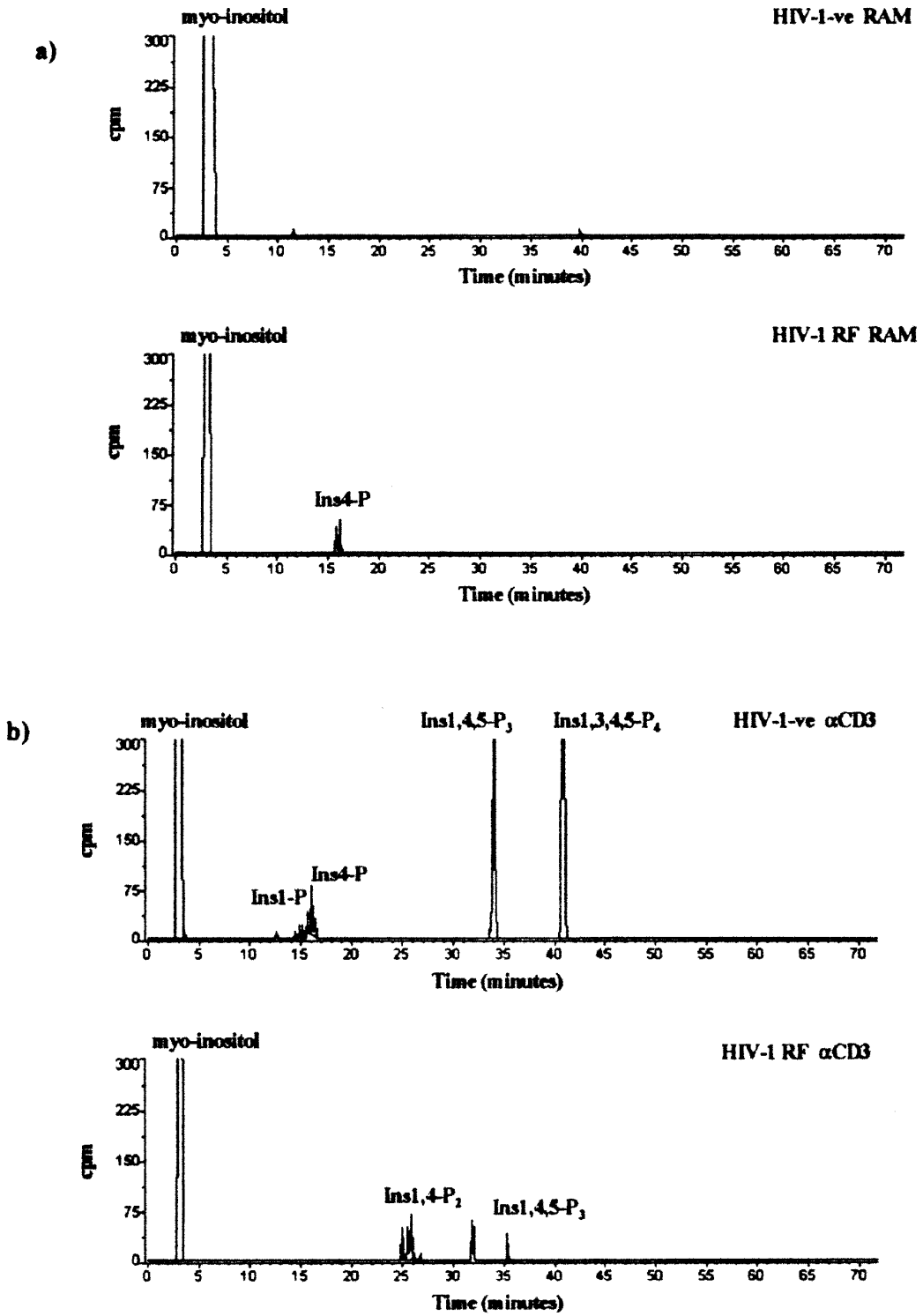


**FIG. 6.** HPLC profiles of productively infected and uninfected cell lysates following stimulation. Uninfected and HIV-1 RF infected H9 cells were loaded with $^{3}H$ myo-inositol and stimulated with RAM (a), anti-CD3 (b), CD4 (c) and anti-CD3 + CD4 (d) antibody in the presence of 10 mM LiCl for 30 minutes. Cells were washed, the water-soluble inositol phosphates were extracted and samples were subjected to HPLC. The eluates were detected by an in-line $\beta$ scintillation counter and peaks were identified by inositol phosphate standards.

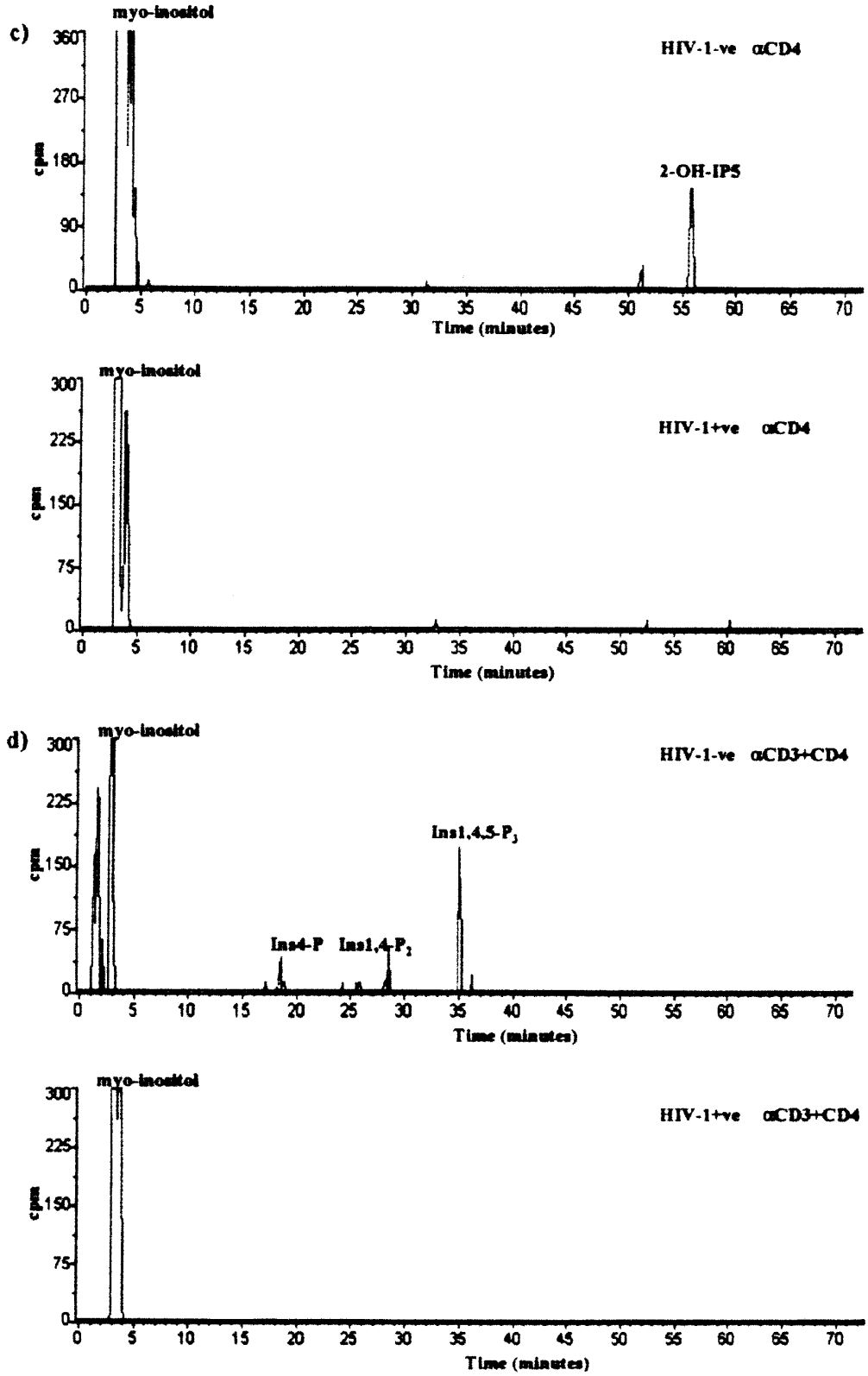


**FIG. 6**—*Continued*

HIV-1 down-regulates cellular function are currently ill-defined, but a growing body of evidence points to impairment of signalling events induced by the virus (3, 5), potentially resulting in apoptosis (17). Protein tyrosine phosphorylation participates in early T-cell signalling and is crucial for positive regulation of distal signal transduction (18). Since CD45 phosphatase regulates $p56^{lck}$ and $p59^{fyn}$ activity, we investigated the effect of virus infection of H9 T-cells on CD45-associated tyrosine phosphatase activity in context of TcR/CD3-CD4 activation. There have been conflicting results on the effect of direct ligation of the CD45 molecule by anti-CD45 antibody on lymphocyte activation, depending on the specific antibody used, whether the antibody is cross-linked with other surface molecules, or on the experimental protocol. Thus, anti-CD45 antibodies have been shown to inhibit T-cell activation when cross-linked with anti-CD3 (19–21), or to inhibit T-cell stimulation directly (22, 23). We were able to induce CD45 activity in uninfected cells by exposure to anti-CD4 alone or in combination with anti-CD3 antibody. This increase in CD45 activity without direct ligation probably occurred *via* stimulation-induced aggregation of these receptors thereby positioning CD45 in close proximity to the CD3/CD4 receptors and leading to its activation. Support for this hypothesis comes from a study which has been shown that CD45 co-immunoprecipitates with the TcR and CD4, suggesting that it is physically and functionally associated with these molecules (9, 24). Alternatively, it is possible that CD4 stimulation either alone or in combination with CD3 might have generated an "inside out" signal resulting in elevated CD45-associated tyrosine phosphatase activity. In this regard it would be intriguing to determine whether PTKs would be critically involved in the regulation of CD45 activity. Attempts were made to assess the tyrosine phosphorylation state of CD45 following CD3 + CD4 ligation in the presence of the tyrosine phosphatase inhibitors sodium orthovanadate and phenylarsine oxide. Preliminary results suggest no differences in the tyrosine phosphorylation state of CD45 in infected and control cells. Currently, we are examining whether serine phosphorylation of CD45 is affected by HIV-1 infection. We report here that CD45 activity was compromised in productively infected cells, which was more prominent following CD4-induced stimulation. Since we did not find any HIV-1 mediated down-regulation in the expression levels of CD3, CD4 and CD45 surface molecules, these results suggest that functional impairment of CD45 was attributable to defective signalling rather than modified expression of these molecules. Compromised CD45 function is expected to have an inhibitory effect on the activation of the TcR-CD4 signalling pathway *via* $p56^{lck}$ and/or $p59^{fyn}$. Evidence was provided that CD3 + CD4-mediated activation events were inhibited in the cells under study. Inhibition of CD45 correlated with reduced CD4-associated protein tyrosine kinase activity and with the subsequent abrogation of PLC-$\gamma$1-mediated inositol polyphosphate metabolism in infected cells. The increase in CD3-driven PLC-$\gamma$1 activity in infected cells might be a result of activation of TcR/CD3 complexed $p59^{fyn}$ and/or $p56^{lck}$ or other kinases. This stimulation regime also resulted in comparable CD45 activity, indicating that CD3 signalling was not as severely affected by HIV infection and that CD45 controls preferably the CD4 signal pathway. Although not determined in this study, it is most likely that the reduced tyrosine kinase activity in CD4 immunoprecipitation represented $p56^{lck}$. Reduced CD4-associated kinase activity was also reported in other studies which demonstrated $p56^{lck}$ dissociation from the CD4 receptor in HIV-1 infected PBLs (5) and in H9 cells (4), further underscoring that those virally-induced signalling defects do not constitute a cell-line specific phenomenon. It is possible that the observed dysfunctional CD45 and CD4-associated kinase activity reflect epiphenomena. However, the parallel occurrence of these defects following the same antibody treatment strongly suggests that the observed CD45 and CD4-signalling abnormalities are closely related to each other, rather than constitute independent events. Either of these two defects are likely to impair T-cell signalling and function. It is therefore important to establish whether CD45 not only regulates $p56^{lck}$ and $p59^{fyn}$ activity, but also critically influences the association state of $p56^{lck}$ with the CD4 receptor. Alternatively, it is possible that the *src*-kinases regulate CD45 activity. HIV-1 p24 antigen levels were relatively high at the time of the experiments which suggests that direct viral cytopathic mechanisms were responsible for the observed dysfunctional signal events. Thus, it is feasible that the observed high titre of HIV-1 or its proteins induces CD4/$p56^{lck}$ modifications which may in turn affect CD45 function. In particular, gp120 or soluble factors may prevent the interaction between CD45 and CD4/CD3 receptors, thereby preventing the formation of a functional and intact signalling matrix. However, the reduced CD4-associated kinase activity which was observed after sodium orthovanadate treatment places CD45 as the upstream regulator of CD4/$p56^{lck}$.

In summary, we have demonstrated that during HIV-1 infection the activity state of CD45 was profoundly impaired which also correlated with reduced tyrosine kinase signalling and PLC-$\gamma$1 function. Hence, defective CD3 and CD4 signalling is likely to adversely affect T-cell effector function during HIV-1 pathogenesis, such as induction of apoptosis, impaired proliferative response to antigen and clonal anergy.

## ACKNOWLEDGMENT

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