Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 6676–6680, July 1993
Immunology

Human immunodeficiency virus proteins induce the inhibitory cAMP/protein kinase A pathway in normal lymphocytes

(signal transduction/cell activation/proliferation/anergy)

BO HOFMANN*, PARUNAG NISHANIAN, THANG NGUYEN, PRAPHAPHONE INSIXIENGMAY, AND JOHN L. FAHEY

Center for Interdisciplinary Research in Immunology and Disease, The Johnson Comprehensive Cancer Center, and Multi Center AIDS Cohort Study (MACS), School of Medicine, University of California, Los Angeles, CA 90024-1747

Communicated by Paul D. Boyer, January 7, 1993

ABSTRACT **Proliferation of normal T lymphocytes is impaired by human immunodeficiency virus (HIV) proteins. In this paper, we demonstrate important parts of this mechanism. Initially, HIV-induced impairment of proliferation was shown to be an active process involving induction of protein tyrosine kinases in both CD4 and CD8 T cells. Furthermore, the impairment of cell proliferation was demonstrated to be linked to induction of the inhibitory protein kinase A (PKA) pathway by HIV proteins. This induction of PKA was accompanied by an increase in intracellular cAMP, which is necessary for the activation of PKA. Finally, increases in cAMP/PKA activity were shown to induce biochemical changes that impaired proliferation when cells were stimulated with phytohemagglutinin. This was demonstrated by showing that (*i*) agents, other than HIV proteins, that increase cAMP/PKA activity (cholera toxoid and 8-bromo-cAMP) also decreased T-lymphocyte proliferation; (*ii*) exposure of lymphocytes to HIV or cholera toxoid led to decreased membrane activity of the proliferation promoter protein kinase C upon stimulation; and (*iii*) agents that reduced cAMP generation neutralized the effect of HIV proteins and restored lymphocyte proliferation. These studies show that the HIV-induced augmentation of cAMP/PKA activity may be a key part of the mechanism responsible for all or part of the HIV-induced anergy of T lymphocytes.**

Infection with human immunodeficiency virus (HIV) leads to a decline in CD4 T-cell numbers and causes an impaired cellular immune function leading to development of AIDS (1). Factors other than the virus infection itself seem to be involved in the suppression of the immune system, because the number of HIV-infected cells is low in most stages of infection (2, 3). Further, the decrease in CD4 T cells alone cannot account for the suppression of the immune system, because a functional T-cell defect is detectable early in HIV infection, at a time when the CD4 T-cell numbers are within the normal range (4, 5).

We (6, 7) and others (8, 9) have previously reported an inhibitory effect of various preparations of inactivated HIV or HIV lysates in nanogram quantities on lymphocyte proliferation *in vitro* that is similar to the effect reported for animal retroviruses (10). HIV impaired proliferation of both CD4 and CD8 T cells, indicating that the suppression is not connected to the CD4 receptor (7). Further, we showed that HIV impaired the very early steps of activation, because exposure to HIV shortly after cell stimulation failed to impair proliferation (7). Apart from impairment of cell proliferation, exposure to HIV decreased the metabolism of inositol phospholipids, but without acting on the cells' ability to increase intracellular free Ca^{2+} (7). However, exposure to HIV *in nanogram quantities* did not by itself induce cell proliferation, increase intracellular Ca^{2+}, or induce metabolism of inositol phospholipids (7), indicating that the effect of HIV does not directly involve the T-cell CD3 receptor. The HIV glycoprotein gp120 has been described to have a mitogenic effect leading to inositolphospholipid metabolism and cell proliferation (11). However, this mitogenic effect of gp120 is obtained only with *microgram quantities* of gp120, which is an unphysiological amount.

Lymphocyte activation leading to cell proliferation involves signaling through the biochemical intracellular pathways. The signaling is regulated through activation of various protein tyrosine and serine threonine kinases. These kinases activate enzymes and receptors by phosphorylation and thereby regulate the activation process (12). The best-characterized kinases are the inhibitory protein kinase A (PKA) and the growth-promoting protein kinase C (PKC). Protein tyrosine kinases (PTKs) are less well characterized, and various types are present.

In this paper we have explored the effect of HIV proteins on these protein kinases. We show that the impairment of lymphocyte proliferation induced by HIV is an active process involving induction of tyrosine and serine/threonine protein kinases. Further, we link the HIV-induced activation of protein kinases of the cAMP-dependent PKA pathway to the impairment of proliferation of normal peripheral blood mononuclear cells (PBMC).

MATERIALS AND METHODS

Cell Cultures. PBMC from healthy blood donors were isolated by Lymphoprep (Nyegaard, Oslo) density gradient centrifugation. Proliferation assays were performed as described (6, 7). Cell cultures were prepared in Eppendorf tubes and contained 5–10 × 10^6 cells per ml for the phosphotyrosine experiments and 2–5 × 10^6 cells per ml for the kinase experiments and cAMP determinations. Optimal concentrations of the following were used: phytohemagglutinin (PHA)-P (Wellcome), 10 μg/ml; phorbol 12-myristate 13-acetate (PMA), 200 ng/ml; cholera toxoid, 0.2–10 μg/ml; and 8-bromo-cAMP (Br-cAMP), 0.2–40 μg/ml (the last three all from Sigma). HIV proteins were added to final dilutions of 108–360 pg of p24 antigen per ml. 2',5'-Dideoxyadenosine (ddAdo) was used in a concentration of 10 ng/ml according to an initial titration showing that this was sufficient to block cAMP generation without having an effect on normal PHA-induced cell proliferation. T cells were prepared as described

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: HIV, human immunodeficiency virus; PTK, protein tyrosine kinase; PKC, protein kinase C; PKA, protein kinase A; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; Br-cAMP, 8-bromoadenosine 3',5'-cyclic monophosphate; PBMC, peripheral blood mononuclear cells; ddAdo, 2',5'-dideoxyadenosine.

*To whom reprint requests should be sent at present address: Department of Infectious Diseases 144, Hvidovre Hospital, Municipality of Copenhagen University, Kettegaard Alle 30, DK-2650 Hvidovre, Denmark.

earlier (7). T-cell subsets were purified by depleting T cells of either CD4 or CD8 T cells as described earlier (5, 7). Phosphate-buffered saline (PBS), RPMI medium 1640, and Hepes for T-cell purification were all specially endotoxin-free preparations (Sigma). β-Propiolactone-inactivated HIV was prepared as described (13) and quantitated by measuring the amount of HIV p24 by ELISA [DuPont/NEN (14)].

Phosphotyrosine Determinations. PBMC were lysed with lysing buffer (1% Triton X-100/5 mM Tris·HCl, pH 7.0/5 mM EDTA/10 mM sodium vanadate/100 μg of phenylmethylsulfonyl fluoride per ml (Sigma) at 100 μl per 12 × 10^6 cells and sonicated (50 W) three times for 10 sec on ice. The cell lysates were electrophoresed on an SDS/4–20% polyacrylamide gradient gel and electroblotted to a nitrocellulose membrane (Bio-Rad). The membrane was incubated with a monoclonal antibody to phosphotyrosine (Sigma) for 3 hr and probed with an ^{125}I-labeled goat antibody to mouse immunoglobulin (ICN) for 2 hr. An x-ray film (Kodak) was exposed to the membrane for 1–3 days. The bands were identified according to the film and cut from the membrane, and radioactivity was measured in a γ counter.

PKC, PKA, and cAMP Measurements. For the PKC and PKA assays, the reagents were obtained as kits from GIBCO and the manufacturer's protocol was followed. *PKC*: Cell membrane fractions were obtained by ultracentrifugation (100,000 × *g* for 1 hr at 4°C). Membrane fractions were then sonicated in the presence of 0.05% Triton X-100. Specific PKC activity was determined as the difference between phosphorylation of a PKC-specific substrate without and with a specific PKC inhibitor present. The specific PKC substrate was a synthetic peptide from myelin basic protein (amino acids 4–14) with an acetylated N-terminal glutamine, and the specific inhibitor was a peptide (amino acids 19–36) from the same protein which binds to the "pseudosubstrate" region of the regulatory domain (15, 16). *PKA*: Cells were sonicated, and the specific PKA activity in the cell lysates was determined as the difference between phosphorylation of a PKA-specific substrate without and with a specific PKA inhibitor present. The specific PKA substrate was the synthetic peptide Leu-Arg-Arg-Ala-Ser-Leu-Gly, called "kemptide," and the specific inhibitor was a peptide called "PKI," which has an alanine to serine replacement in the consensus sequence Xaa-Arg-Arg-Xaa-Ser-Xaa (Xaa = any amino acid) and binds to the pseudosubstrate region of the regulatory domain (17, 18). The reaction mixtures containing enzyme, [^{32}P]-ATP, substrate, and either inhibitor or extra buffer were kept at 37°C for 5 min and then spotted on cellulose paper. Unbound [^{32}P]ATP was removed by washing, and the radioactivity on the paper discs was measured in a β counter after addition of scintillation fluid. The amounts of lysate over which the assay was linear were determined in initial experiments. All experiments were repeated two to five times. *cAMP*: This assay is a commercial scintillation proximity RIA (Amersham) and the manufacturer's protocol was followed.

RESULTS

HIV Induces Sustained Phosphorylation of Protein Tyrosine Residues in Normal PBMC. Addition of HIV proteins or PHA to normal PBMC for 1 hr led to the generation of phosphorylated protein tyrosine residues (Fig. 1). Detection of phosphotyrosine in protein bands from about 10 to 200 kDa indicates activity of various PTKs. Three bands, about 45, 55, and 80 kDa, were particularly strong. For quantitating activity, we focused on these bands although other bands reflected the activation as well. Because the activity of PTKs is low in resting T lymphocytes (19, 20), demonstration of an activation of PTKs shows that HIV proteins actively induce signals in normal PBMC.

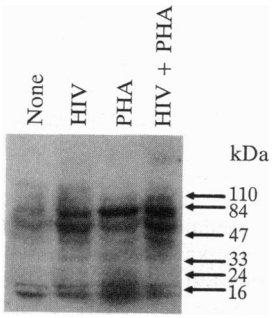

FIG. 1. Induction of sustained protein tyrosine phosphorylation in normal PBMC by HIV proteins or PHA. The autoradiography of a Western blot shows phosphotyrosine generation when HIV proteins or PHA is added to normal PBMC for 1 hr. For the HIV + PHA combination, cells were treated with HIV for 10 min before 1-hr PHA stimulation. Quantitation of the 45-, 55-, and 80-kDa bands together gave nonstimulated, 931 cpm; +HIV, 1974 cpm; +PHA, 2472 cpm, and HIV + PHA, 2464 cpm after subtraction of background.

PTK Activity Induced by HIV Can Be Measured as Early as 5 min After Stimulation. Normal PBMC were stimulated with HIV proteins or PHA and were tested for generation of phosphotyrosine after 0, 2, 5, and 10 min. Phosphotyrosine bands, measured on a Western blot, were increased over background after 5 min of HIV stimulation (Fig. 2). It was also found that exposure of normal PBMC to HIV for 10 min before PHA stimulation did not prevent subsequent induction of tyrosine phosphorylation by PHA (Fig. 2).

HIV Induces Phosphorylation of Tyrosine in Both CD4 and CD8 T Cells. CD4 and CD8 T cells were purified by first E-rosetting T cells [sheep erythrocytes (E) bind to the CD2 receptors but do not lead to signaling]. Subsequently, these E^+ lymphocytes were depleted of either CD4 or CD8 T cells to a purity of CD4 T cells of 89% (±2%) with remaining CD8

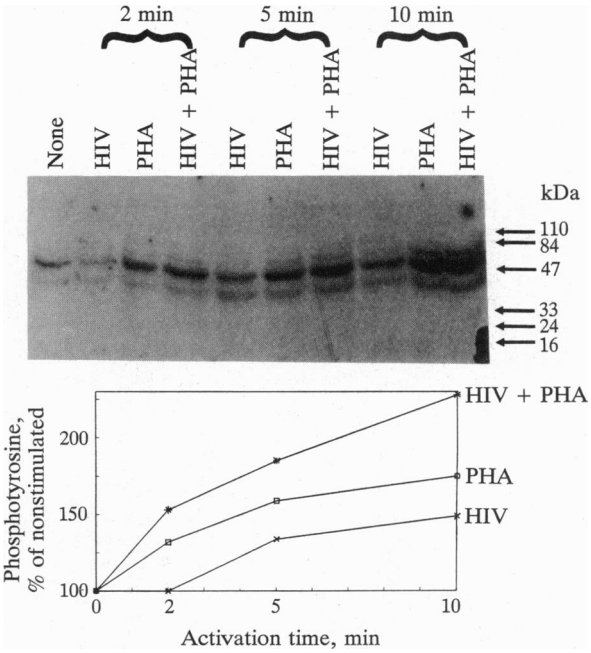

FIG. 2. HIV-induced protein tyrosine phosphorylation is detectable within 5 min. (*Upper*) HIV proteins or PHA was added to normal PBMC in a time-course experiment. For the HIV + PHA combination, cells were treated with HIV for 2, 5, or 10 min before 1-hr PHA stimulation. (*Lower*) Quantitation of the combined 45-, 55-, and 80-kDa bands shows that HIV as well as PHA induces phosphotyrosine progressively over the first 10 min of activation, and addition of PHA to cells previously exposed to HIV proteins for 10 min does not reduce PHA-induced PTK activity (line labeled HIV + PHA).

of 3% (±1%) and CD8 T cells of 76% (±3%) with remaining CD4 of 19% (±2%). HIV proteins induced phosphorylation of tyrosine residues in both CD4- and CD8-enriched T cells in 10 min. In three experiments, exposure to HIV increased the intensity of the 45-, 55-, and 80-kDa bands to 206% ± 90% (mean ± SEM) for CD4 T cells and 181% ± 20% for CD8 T cells. Because the activations obtained in CD4 and CD8 T cells are similar, it is unlikely that the activation of CD8 T cells is due to the remaining CD4 T cells in the preparation. The experiment shows that activation of PTKs by HIV proteins is not mediated via the CD4 receptor. The finding is consistent with earlier published data showing that HIV proteins impair the proliferation of both CD4 and CD8 T cells (7).

HIV Induces an Increase of PKA Activity. Addition of HIV proteins or PHA to normal PBMC induced PKA activity in time-course experiments, reaching a maximum after 5 min of activation (Fig. 3 *Upper*). A 5-min activation was chosen for further studies. The induction of PKA activity by HIV was confirmed in four of four experiments ($P < 0.05$) (Fig. 3 *Lower*). Exposure of PBMC to HIV for 10 min prior to stimulation did not decrease subsequent induction of PKA activity by PHA. Although 70% of PBMC are T cells, only T cells respond to PHA. The experiment was repeated on separated T cells. During separation, particular care was taken to avoid endotoxin-containing media, which may cause fluctuating preactivation. In three experiments, PKA activity increased from 2.5 ± 1.0 (mean ± SEM) to 10.0 ± 1.6 pmol of ^{32}P incorporated per million T cells after HIV stimulation ($P < 0.05$) and to 7.8 ± 2.2 pmol of ^{32}P incorporated per million T cells after activation with PHA, demonstrating that results obtained with PBMC and T cells are similar. These experiments show that HIV activates the inhibitory PKA pathway in T lymphocytes, which has an inhibitory effect on cell processes leading to proliferation (21).

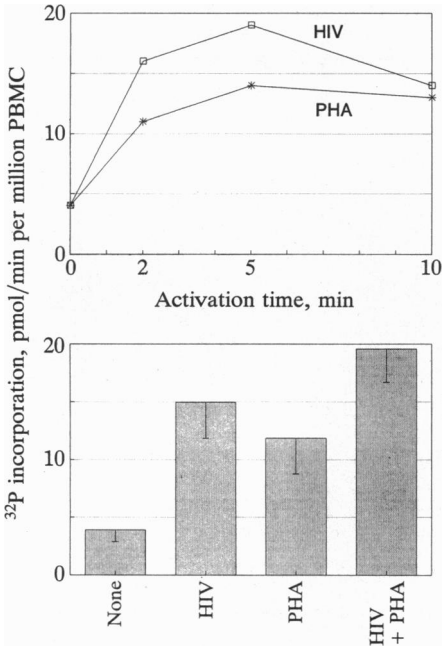

FIG. 3. HIV proteins induce an increase of cAMP-dependent PKA activity in normal PBMC. (*Upper*) Addition of HIV proteins or PHA to normal PBMC induced an increase in PKA activity in a time-course experiment, reaching a maximum after 5 min of activation. The 5-min time was chosen for further studies. (*Lower*) Induction of PKA activity by HIV and PHA was confirmed in four of four experiments ($P < 0.05$). Exposure of PBMC to HIV proteins for 10 min prior to stimulation did not alter subsequent induction of PKA activity by PHA. Each column represents mean and SEM of four experiments.

HIV Induces an Increase in Intracellular cAMP Concentration. Addition of HIV proteins as well as PHA to normal PBMC induced an immediate increase in intracellular cAMP as measured in cell lysates. In three time-course experiments, the intracellular cAMP increased and reached a maximum after 2–5 min (data not shown). More donor variability was observed than for PKA activity. The induction of cAMP by HIV was shown in five of five experiments ($P < 0.01$) (Fig. 4). Again, carefully separated T cells were also tested, and they showed similar results. In three experiments cAMP increased after addition of HIV or PHA from 0.39 ± 0.09 (mean ± SEM) to 0.61 ± 0.11 and 1.09 ± 0.36 pmol per million T cells ($P < 0.05$), demonstrating that results obtained with PBMC and T cells are similar. PKA is active only in the presence of cAMP. The generation of cAMP in T cells in response to HIV, therefore, confirms the above results showing activation of the inhibitory cAMP/PKA pathway.

HIV-Induced Increase in PKA Activity Is Similar to That Induced by Cholera Toxoid. In one of two experiments showing similar results, PKA activity induced by HIV was 19 pmol of ^{32}P incorporated per min per 10^6 PBMC and 16 pmol/min per 10^6 PBMC induced by cholera toxoid. The activity in nonstimulated cells was 6 pmol/min per 10^6 PBMC. The HIV-induced increase in cAMP/PKA activity is, therefore, of the same magnitude as cholera toxoid-induced increase.

Reagents That Increase Intracellular cAMP Impair Proliferation of Normal PBMC. Both Br-cAMP, a stable form of cAMP, and cholera toxoid, a well-established stimulator of cAMP accumulation (22), impaired PHA-induced cell proliferation as measured by [^{3}H]thymidine uptake (Table 1). The experiment confirmed that cAMP impairs PHA-induced proliferation of T lymphocytes and confirms results of others (22).

Blocking of Intracellular cAMP Generation by Chemical Means Reverses the Effect of HIV on T-Lymphocyte Proliferation. Intracellular cAMP can be decreased by blocking adenylate cyclase, the enzyme responsible for cAMP generation, with the adenosine analogue ddAdo. If HIV impairs T-lymphocyte proliferation mainly by increasing intracellular cAMP, it should be possible to restore proliferation by decreasing intracellular cAMP. To demonstrate this, HIV proteins were added to PBMC for 1 hr and then washed away. As expected, the proliferative response to PHA in these cells was reduced (Fig. 5). However, when the HIV-treated cells were subsequently treated with ddAdo for 10 min and then washed, the proliferative response to PHA was mostly restored (Fig. 5). This effect of ddAdo was seen in five of five experiments ($P < 0.05$). ddAdo alone had only a minor and variable effect on

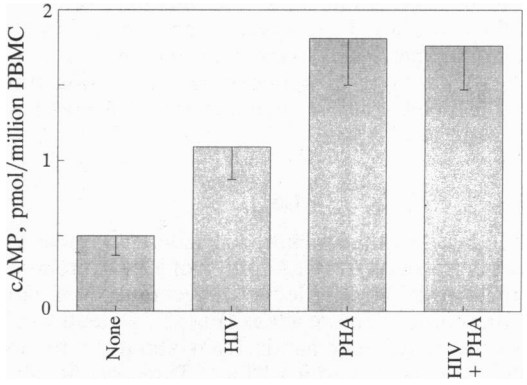

FIG. 4. HIV proteins induce an increase in intracellular cAMP. Addition of PHA or HIV to normal PBMC induced in five of five experiments ($P < 0.01$) an increased intracellular level of cAMP. Treatment of PBMC with HIV protein for 10 min before PHA stimulation did not change the subsequent induction of cAMP (HIV + PHA).

Table 1. Comparison of impairment of PHA-induced cell proliferation induced by various agents that increase intracellular cAMP

Addition to PHA*	Conc., μg/ml	[^{3}H]Thymidine incorporation,† cpm × 10^{-3}	Residual activity, %‡
None	—	254.2 ± 9.0	100
HIV	§	122.0 ± 3.8	48
Cholera toxoid	1.25	84.6 ± 9.7	33
	0.63	120.5 ± 9.2	47
	0.33	168.7 ± 21.9	66
Br-cAMP	400	41.6 ± 2.1	16
	200	96.3 ± 14.4	38
	100	168.7 ± 21.9	66

HIV, cholera toxoid, and Br-cAMP each decreased PHA-induced thymidine uptake significantly ($P < 0.01$ comparing triplicate determinations). HIV, cholera toxoid, and Br-cAMP alone did not stimulate [^{3}H]thymidine uptake above background.
*PHA concentration was 10 μg/ml in all cases.
†Entries are mean ± SEM of triplicate determinations.
‡Percentages are (cpm in presence of PHA and addition/cpm in PHA alone) × 100.
§HIV was 360 pg of HIV p24 per ml.

PBMC (the response to PHA increased in two, decreased in two, and did not change in one experiment).

HIV Induces Lymphocyte Membrane PKC Activity. An important step in early cell activation is the translocation of PKC from cytoplasm to the plasma membrane. Addition of the specific PKC activator PMA to normal PBMC induced a lasting PKC activity in the cell membrane fraction within the first 2 min of activation (Fig. 6 *Upper*). Addition of HIV proteins or PHA to normal PBMC also induced membrane PKC activity within 2–5 min of activation. This initial membrane PKC activity decreased to about baseline after 10 min (Fig. 6 *Upper*). This increase/decrease in membrane PKC activity is similar to what has been reported for activation with other mitogens (23). A 5-min incubation was used for subsequent experiments. Again, carefully separated T cells were also tested and showed similar results ($P < 0.01$). In two experiments PKC activity increased after addition of HIV for 5 min from 0.1 ± 0.0 (mean ± SEM) to 5.0 ± 0.7 pmol per 10^6 PBMC.

HIV Impairs Subsequent PHA-Induced Membrane Translocation of PKC. Normal PBMC were exposed to HIV proteins for 10 min prior to PHA activation (5 min). The PHA-induced membrane PKC activity obtained at 5 min after exposure to HIV proteins was clearly lower than with PHA induction alone (four of four experiments) ($P < 0.05$) (Fig. 6 *Lower*). When the cells were stimulated with the direct PKC activator PMA, pretreatment with HIV had less and more variable effect (decreased in three of four experiments) (Fig. 6 *Lower*).

HIV-Induced Impairment of Membrane PKC Activity Is Similar to That Induced by Cholera Toxoid. In three experiments, membrane PKC activity induced by PHA was reduced from 6.6 ± 2.0 (mean ± SEM) to 3.1 ± 0.9 pmol/min per 10^6 PBMC by 10-min exposure to HIV or to 4.1 ± 1.3 pmol/min per 10^6 PBMC when cells were exposed to cholera toxoid (values decreased in three of three experiments but statistical significance was not reached due to variability between experiments). The level in nonstimulated cells was 1.3 ± 0.2 pmol/min per 10^6 PBMC. The HIV-induced signals, therefore, reduce subsequent induction of membrane PKC activity to the same degree as the well-characterized PKA/cAMP inducer cholera toxoid (24).

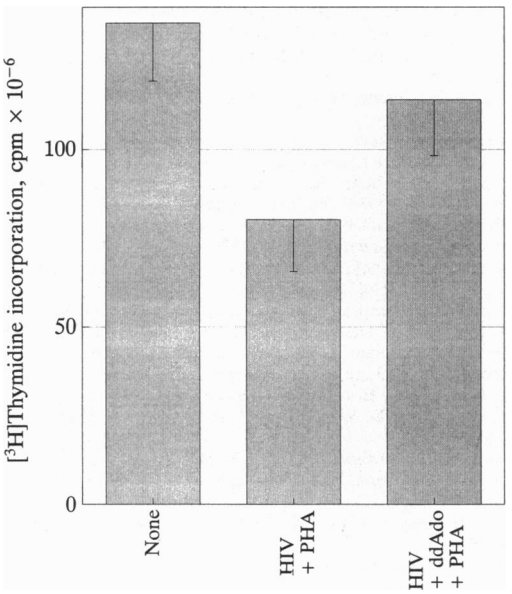

FIG. 5. Chemically blocking intracellular cAMP generation (adenylate cyclase) reverses the effect of HIV on T-lymphocyte proliferation. Exposure of normal PBMC to HIV proteins for 60 min followed by washing impaired cell proliferation in response to PHA (middle column). When ddAdo (an inhibitor of adenylate cyclase) was added subsequently for 10 min (followed by washing) to these cells after HIV exposure the effect of HIV proteins was mostly reversed (right column) in five of five experiments ($P < 0.01$). ddAdo alone, in a concentration of 10 ng/ml as used in this experiment, had only a minor and variable effect on PBMC (the response to PHA increased in two experiments, decreased in two experiments, and did not change in one experiment).

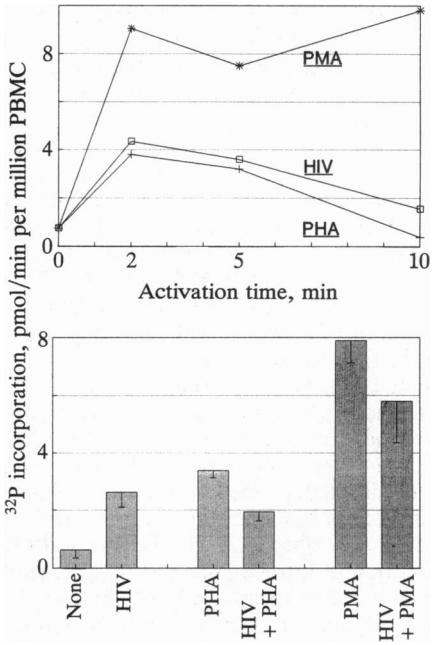

FIG. 6. HIV proteins induce lymphocyte membrane PKC activity and impair subsequent PHA-induced membrane PKC activity. (*Upper*) Addition of PMA to normal PBMC induced a lasting PKC activity, whereas addition of HIV proteins or PHA induced an increase in membrane PKC activity reaching a maximum after 2–5 min and then decreasing. A 5-min incubation was chosen for further studies. (*Lower*) Induction of PKC activity by HIV, PHA, and PMA was confirmed in four of four experiments ($P < 0.01$). Exposure of PBMC to HIV for 10 min prior to stimulation with PHA (HIV + PHA) clearly decreased the ability of PHA to induce membrane PKC activity in four of four experiments ($P < 0.05$). When cells were stimulated with PMA, pretreatment with HIV had less and more variable effect (decreased in three of four experiments, not significant). Each column represents mean and SEM of four experiments.

DISCUSSION

In this paper the impairment of T-lymphocyte proliferation by HIV proteins is shown to be an active process mediated by the same biochemical processes that are involved in normal lymphoid regulation. In particular, we demonstrate the involvement of the inhibitory cAMP/PKA pathway in the proliferative impairment induced by HIV proteins.

That HIV proteins induce signals in T cells was shown by the lasting activation of tyrosine kinases in both CD4 and CD8 T cells immediately after exposure to HIV proteins. The finding that HIV proteins induce phosphorylation of proteins of various sizes is consistent with activation of many PTKs and is similar to what is seen after stimulation with PHA. More than seven human lymphoid PTKs have been described, and both inhibitory and activating properties have been attributed to them (20).

The signals that HIV proteins induce in T lymphocytes lead to induction of two functionally well-characterized serine/threonine kinases with opposite functions. PKC activation promotes cell proliferation (20, 25–30), whereas PKA, in the presence of cAMP, leads to inhibition of proliferation (26, 27). HIV proteins were shown not only to induce PKA in normal PBMC and T cells but also to induce increased cytoplasmic cAMP, which is necessary for PKA activity. cAMP is generated by the membrane-bound enzyme adenylate cyclase (30, 31). cAMP/PKA is also induced by PHA, since it is part of the network of activating/inhibiting processes following normal lymphocyte activation (27). PKC is normally present in the cytoplasm and translocates to the plasma membrane after activation with 1,2-diacylglycerol, although other molecules such as arachidonic acid are also able to activate PKC (32). HIV proteins induce PKC activity although they do not induce phospholipid metabolism (7). A similar activation pattern has been reported for interleukin 2 (IL-2) in mitogenic concentrations. Like HIV, IL-2 activates PKC without inducing inositolphospholipid metabolism or elevating intracellular calcium concentration (33, 34).

HIV proteins do not induce cell proliferation. As described above, both PHA and HIV proteins induce cell activation that leads to induction of protein kinases. HIV-induced cell activation differs from PHA-induced cell activation in that PHA induces inositolphospholipid metabolism and increases intracellular Ca^{2+}, whereas HIV does not (7). The difference may explain why HIV exposure does not lead to cell proliferation. Induction of anergy is well known as a part of normal dynamic immune activation (35).

HIV-induced changes are responsible for T lymphocytes becoming refractory to subsequent reactivation. In particular, an increased intracellular level of cAMP induced by HIV may contribute to the decreased proliferation when cells are subsequently stimulated. Decreased cell proliferation in response to increases in intracellular cAMP and cAMP/PKA activity is well described (27, 31). In this and other studies (24, 36) induction of intracellular cAMP with cholera toxoid or addition of a stable form of cAMP (Br-cAMP) to normal PBMC efficiently impaired proliferation in response to PHA. The data are also consistent with a report showing that cAMP blocks progression but not initiation of T-cell proliferation (31). Strong evidence for the involvement of cAMP/PKA activity in HIV-induced impairment of T-lymphocyte proliferation is that addition of ddAdo, an inhibitor of adenylate cyclase, reversed the effect of HIV proteins and restored cell proliferation. Increases of cAMP have also been shown in cell lines after 4 days of infection with HIV (37).

It is difficult to prove that the *in vivo* and *in vitro* defects are the same. However, a study of intracellular cAMP showed a 4-fold greater mean level in HIV-seropositive subjects without AIDS than in controls (38). The finding of increased cAMP in HIV-infected subjects supports the view that the cellular impairment found *in vivo* has metabolic derangements similar to those shown here *in vitro* and is induced by HIV proteins.

This work was supported by National Institutes of Health Grants AI23606 and AI72631. B.H. was supported by Grant AIDS 12-8445 from the Danish Medical Council.

1. Schroff, R. W., Gottlieb, M. S., Prince, H. E., Chai, L. L. & Fahey, J. L. *Clin. Immunol. Immunopathol.* **27**, 300–314.
2. Harper, M. E., Marselle, L. M., Gallo, R. C. & Wong-Staal, F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 772–776.
3. Schnittman, S. M., Greenhouse, J. J., Psallidopoulos, M. C., Davey, V., Lane, H. C. & Facui, A. S. (1990) *Ann. Int. Med.* **113**, 438–443.
4. Hofmann, B., Lindhardt, B. O., Gerstoft, J., Petersen, C. S., Platz, P., Ryder, L. P., Odum, N., Dickmeiss, E., Nielsen, P. B., Ullman, S. & Svejgaard, A. (1987) *Br. Med. J.* **295**, 293–296.
5. Hofmann, B., Jakobsen, K., Odum, N., Dickmeiss, E., Platz, P., Ryder, L., Petersen, C., Mathisen, L., Bygbjerg, I., Faber, V. & Svejgaard, A. (1989) *J. Immunol.* **142**, 1874–1880.
6. Hofmann, B., Langhoff, E., Lindhardt, B. O., Odum, N., Hyldig-Nielsen, J. J., Ryder, L. P., Platz, P., Jakobsen, B. K., Bentzen, K., Jacobsen, N., Lerche, B., Schafer-Nielsen, C., Dickmeiss, E., Ulrich, K. & Svejgaard, A. (1989) *Cell. Immunol.* **121**, 336–348.
7. Hofmann, B., Nishanian, P., Baldwin, R. L., Insixiengmay, P., Nell, A. & Fahey, J. L. (1990) *J. Immunol.* **145**, 3699–3705.
8. Pahwa, S., Pahwa, R., Saxinger, C., Gallo, R. C. & Good, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8198–8202.
9. Henderson, L. A., Qureshi, N. M., Rasheed, S. & Garry, R. (1988) *Clin. Immunol. Immunopathol.* **48**, 174–186.
10. Orosz, C. G., Zinn, N. E., Olsen, R. G. & Mathes, L. E. (1985) *J. Immunol.* **134**, 3396–3403.
11. Kornfeld, H., Cruikshank, W. W., Pyle, S. W., Berman, J. S. & Center, D. M. (1988) *Nature (London)* **335**, 445–448.
12. Flier, J. S. & Underhill, L. H. (1989) *N. Engl. J. Med.* **321**, 1383–1391.
13. Nishanian, P., Ojo-Amaize, E., Uittenbogaart, C. H. & Giorgi, J. V. (1987) *J. Immunol. Methods* **107**, 261–271.
14. McDougal, J. S., Cort, S. P., Kennedy, M. S., Cabridilla, C. D., Feorino, P. M., Francis, D. P., Hicks, D., Kalyanaraman, V. S. & Martin, L. S. (1985) *J. Immunol. Methods* **76**, 171–183.
15. House, C. & Kemp, B. E. (1987) *Science* **238**, 1726–1728.
16. Yasuda, I., Kishimoto, A., Tanaka, S., Tominaga, M., Sakurai, A. & Nishizuka, Y. (1990) *Biochem. Biophys. Res. Commun.* **166**, 1220–1227.
17. Maller, J. L., Kemp, B. E. & Krebs, E. G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 248–251.
18. Kemp, B. E., Cheng, H. C. & Walsh, D. A. (1988) *Methods Enzymol.* **159**, 173–183.
19. Veillette, A., Bookman, M. A., Horak, E. M., Samelson, L. E. & Bolen, J. B. (1989) *Nature (London)* **338**, 257–259.
20. Mustelin, T. & Altman, A. (1989) *Immunol. Today* **10**, 189–192.
21. Kammer, G. M. (1988) *Immunol. Today* **9**, 222–229.
22. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y. & Shearer, G. M. (1974) *Science* **184**, 19–28.
23. Do-Kyun, K., Lancki, D. W., Hui, F. H. & Fitch, F. W. (1989) *J. Immunol.* **142**, 616–622.
24. Nel, A. E., Vandenplas, M., Wooten, M. M., Cooper, R., Vandenplas, S., Rheeder, A. & Daniels, J. (1988) *Biochem. J.* **256**, 383–390.
25. Weiss, A. & Imboden, J. B. (1987) *Adv. Immunol.* **41**, 1–38.
26. Ledbetter, J. A., June, C. H., Martin, P. J., Spooner, C. E., Hansen, J. A. & Meier, K. E. (1986) *J. Immunol.* **136**, 3945–3952.
27. Kim, D., Lancki, D. W., Hui, F. H. & Fitch, F. W. (1989) *J. Immunol.* **142**, 616–622.
28. Droge, W. (1986) *Immunol. Today* **7**, 340–343.
29. Takayama, H., Trenn, G. & Sitkovsky, M. V. (1988) *J. Biol. Chem.* **263**, 2330–2336.
30. Bastin, B., Payet, M. D. & Dupuis, G. (1990) *Cell. Immunol.* **128**, 385–389.
31. Lingk, D. S., Chan, M. A. & Gelfand, E. W. (1990) *J. Immunol.* **145**, 449–455.
32. McPhail, L. D., Clayton, C. C. & Snydermann, R. (1984) *Science* **224**, 622–625.
33. Mills, G. B., Girald, P., Grinstein, S. & Gelfand, E. W. (1988) *Cell* **55**, 91–100.
34. Friedrich, B. & Gullberg, M. (1988) *Eur. J. Immunol.* **18**, 486–492.
35. Jenkins, M. K. (1992) *Immunol. Today* **13**, 69–73.
36. Sitkovsky, M. V., Trenn, G. & Takayama, H. (1988) *Ann N.Y. Acad. Sci.* **532**, 350–358.
37. Nokta, M. & Pollard, R. (1991) *Virology* **181**, 211–217.
38. Hofmann, B., Nishanian, P., Nguyen, T., Liu, M. & Fahey, J. L. (1993) *AIDS* **7**, in press.