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Human Immunodeficiency Virus Induction of Corticotropin in Lymphoid Cells*

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ABSTRACT

Disruption of the linkage among the immune, nervous, and endocrine systems may contribute to the pathology and symptoms of acquired immunodeficiency syndrome (AIDS). We investigated the role of human immunodeficiency virus (HIV) in altering these linkages via induction of corticotropin (ACTH) by lymphocytes. Cultured T lymphocytes (H9 cell line) were infected with HIV-1, after which ACTH production was measured and characterized at various time intervals by immunofluorescence and Western blotting. We report a coordinate expression of ACTH and p24 HIV core protein in H9 cells. Also, the kinetics of HIV-induced ACTH production by H9 T lymphoma cells are demonstrated using three different strains of HIV as well as UV-inactivated HIV. ACTH production corresponded with the appearance of p24 antigen and was maximal 35 days after infection. UV-inactivated HIV and the viral envelope protein, gp120, were also able to induce ACTH production in these cells, indicating that viral replication was not required for the ACTH induction. The HIV-induced ACTH was synthesized *de novo* and had the size and biological activity of pituitary ACTH. Inhibition of ACTH in HIV-infected lymphocyte cultures by anti-ACTH antiserum enhanced viral p24 expression. The significance of lymphocyte ACTH in AIDS is not clear, but these results suggest that it may restrict HIV replication and possibly infection. (*J Clin Endocrinol Metab* **83:** 4373–4381, 1998)

HUMAN immunodeficiency virus (HIV) infection and the subsequent acquired immunodeficiency syndrome (AIDS) have profound systemic effects on the body. The infection is limited to certain cell types, and therefore, the mechanism(s) by which these widespread effects occur is probably indirect. The immune, nervous, and endocrine systems appear to be the most affected (1–5). Coincidentally, a growing body of evidence demonstrates that these three systems are interconnected in a regulatory fashion (6), and a novel immunoendocrinological hypothesis for AIDS that links these processes to HIV pathogenesis has been proposed (2). HIV infection may disrupt this connection. There is much evidence showing that the types and levels of cytokines (7) as well as hormones (1, 8–10) change with the progression of HIV infection. One of the current hypotheses suggests that a switch in T helper lymphocyte type 1 to T helper lymphocyte type 2 (Th2) cytokines may be a major factor in progression of the disease. As many hormones have cytokine-like activity (6), hormonal changes in the neuro-immune axis may result in or contribute to cytokine dysregulation.

Endocrine dysfunction is not typically thought of as a cardinal clinical feature of AIDS, probably in part because there is a wide spectrum of abnormalities (1–5). Some clinical manifestations of AIDS similar to adrenal insufficiency occur and the function of the pituitary and adrenal glands in HIV patients has been the focus of numerous studies (3–5, 10–21). Overall, the range of findings, using the 1-h provocative ACTH test include normal basal and stimulated cortisol responses in most of HIV patients and low stimulated cortisol levels in about 10–20% of cases (13, 15). Elevated ACTH is observed especially in the early phase of infection (5) and also in instances of adrenal insufficiency with a lack of dexamethasone suppression of cortisol (19). Some advanced cases have been found with the anomalous feature of elevated cortisol and not ACTH (10).

The sum of these findings is that multiple mechanisms appear to be involved. The first are examples of overt destruction of the adrenal gland by secondary infections or processes. Secondly and more typically, the presence of clinical adrenal insufficiency despite a lack of tissue destruction sufficient to cause adrenal hypofunction suggests damage to tissues that mediate the adrenal gland response. The hypothalamus, pituitary, and possibly lymphoid tissues are likely candidates for mediating the changes in adrenal gland activity described in HIV infections due to their responsiveness to cytokines. The other potential mechanism in regard to immune activation is the production of neuroendocrine hormones by lymphoid cells. We found that HIV induced lymphocytes produce POMC, releasing ACTH- and MSH-like molecules (22). This has been confirmed by Barcellini *et al.*, who detected the β-endorphin product of POMC (23). *In vivo* support of these findings comes from a study showing elevated ACTH and MSH levels in patients at late stages of AIDS. The ACTH and MSH levels were inversely proportional, which supports our study showing that ACTH could be processed by a neutral endopeptidase-like enzyme to an immunoreactive MSH (8, 24).

ACTH is one of the best characterized of the neuroendocrine hormones involved in immune regulation (25). It was

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originally identified as a stress-related peptide processed by proteolytic cleavage from POMC that is released by the pituitary gland and induces corticosteroid hormone release. ACTH has direct actions on lymphocytes, and most of its actions are Th2 cytokine-like, such as the inhibition of interferon-γ (26). Lymphocytes not only possess specific receptors for ACTH, but also synthesize ACTH, which is identical to its pituitary prototype (25, 27–31). Previously, we reported on HIV-induced production of ACTH by a T lymphocyte cell line (22). The primary aim of this study was to examine the kinetics and mechanisms of HIV-induced ACTH production by H9 T lymphoma cells and peripheral blood lymphocytes (PBLs). Also, the bioactivity of the immunoreactive ACTH was examined for effects on adrenal steroidogenesis and HIV replication.

Materials and Methods

Virus isolates

HIV-1 isolates, MCK-1, SK-1, and 213, were gifts from Dr. Miles Cloyd, University of Texas Medical Branch (Galveston, TX). The specific biological properties, propagation, and titration of these isolates have been described previously (32). All HIV preparations were harvests of HIV-infected H9 cell cultures that had gone through one freeze-thaw cycle to release the intracellular HIV and were sterilized by filtration (HIV-1 virus stock titer, 0.5–2.0 $\times$ 10^6 TCID/mL).

Cells

H9 cells ($CD4^+$, T cell lymphoma, American Type Culture Collection HTB 176) were cultured in RPMI 1640 supplemented with 10% newborn bovine serum (NBS) or in serum-free medium (NBS was substituted with an insulin, transferrin, and sodium selenite medium supplement; Sigma Chemical Co., St. Louis, MO). Penicillin, streptomycin, lincomycin (200 μg/mL), and spectinomycin (20 μg/mL) were routinely used in the H9 cultures. Human PBLs were purified by Ficoll-Hypaque density centrifugation (33) from buffy coats obtained from the University of Texas Medical Branch Blood Bank. PBLs were suspended at 2 $\times$ 10^6 cells/mL in RPMI 1640 supplemented with 10% NBS and interleukin-2 (20 U/mL). The culture conditions were similar to the H9 cell culture conditions. In experiments in which PBLs were used to grow HIV, cultures were initially treated with phytohemagglutinin (100 U/mL; Sigma Chemical Co.) for 3–4 days before infection to stimulate T lymphocyte blastogenesis. AtT20 and Y-1 cells were grown in F-10 medium supplemented with 2.5% FBS and 15% horse serum as previously described (34).

Reagents

Monoclonal mouse antibody (M26) reactive with the HIV p24 capsid protein was provided by Dr. Miles Cloyd and was used undiluted for the indirect immunofluorescence (IF) assays. The affinity-purified polyclonal sheep anti-gp120 IgG (Accurate Chemical & Scientific Corp., Westbury, NY) was used at 1 μg/mL. Polyclonal rabbit anti-ACTH-(1–24) Ig (ICN Immunochemicals, Lisle, IL) was used for immunofluorescent staining, Western blotting, and ACTH neutralization studies.

UV inactivation of HIV

Suspensions of HIV-1 were UV-inactivated by decanting 10 mL of the viral stock into a sterile petri dish under a laminar flow hood and exposing the open dish to 4000 erg/cm^2 UV light for 20 min at a distance of 30 cm. A sample of 100 μL was taken from this suspension and titered for the presence of infectious HIV as described previously (32).

Immunoassays

The IF assay was performed as previously described (35). Briefly, approximately 5 $\times$ 10^4 cells were acetone or ethanol fixed onto glass slides. After rehydration, they were treated with polyclonal rabbit anti-ACTH-(1–24) at a dilution of 1:100 in a humid chamber for 1 h at room temperature. Control samples received normal rabbit serum (1:500). After washing, fluorescein isothiocyanate- or phycoerythrin-labeled goat antirabbit IgG antiserum was applied, and samples were scored, using epifluorescent microscopy, as the percentage of positive cells per field. For detection of HIV p24 antigen, the same overall methodology was used; however, a monoclonal anti-p24 mouse antibody was applied followed by the addition of fluorescein isothiocyanate-labeled goat antimouse IgG antiserum. The inter- and intraassay variabilities in this procedure were approximately 15% and 25%, respectively.

Samples were assayed for ACTH using a commercial RIA kit (Incstar Corp., Irvine, CA) as previously described (22). The procedure for the Western blot method was also previously described (36). Briefly, cell lysates were prepared by low speed centrifugation of culture harvests and treating the pellets to three cycles of freeze-thawing after decanting their supernatant fluid. Ten microliters of the lysates were run on a 15% discontinuous SDS-PAGE (150 V for 1.5 h). The protein bands were then electrophoretically transferred onto a nitrocellulose membrane (100 V for 1.5 h). Samples were treated with rabbit anti-ACTH-(1–24) (1:500), washed, and treated with an alkaline phosphatase-conjugated goat antirabbit (1:3000) antiserum, then washed and exposed to substrate. Typically, AtT20 pituitary cell lysate (diluted 1:100) was run as a positive control, and normal rabbit serum was used as a negative serum control.

SDS-PAGE

Lysates of experimental and control cells were reduced by boiling in SDS sample buffer (2% SDS and 5% 2-mercaptoethanol) for 5 min and electrophoresed on a 15% reducing polyacrylamide gel using a Mini-Protein II slab cell (Bio-Rad Laboratories, Hercules, CA). After electrophoresis, the lanes containing radiolabeled samples were sliced into 0.3-cm fractions. Prestained reference markers, run simultaneously in each gel, were used to derive a standard curve, which was used to determine the mol wt of the proteins in the unknown samples.

ACTH bioassay

ACTH bioactivity was determined as previously described in an assay in which mouse Y-1 adrenal cells round up morphologically in response to ACTH (34). Briefly, 5 $\times$ 10^4 mouse Y-1 adrenal cells were seeded and grown to confluence in a 96-well microtiter plate. These cells were then treated with ACTH or experimental samples. Specificity was determined in duplicate assays, one with antiserum to ACTH and the other with normal rabbit serum. After 2 h, the cells are monitored for morphological changes (rounding), and the end point dilution was compared to the ACTH standard dilution series to calculate the units of activity.

Results

Kinetics of HIV-induced ACTH production by H9 lymphocytes

To begin characterizing the relationship between ACTH induction and HIV infection, the kinetics of HIV-induced ACTH production were examined in H9 lymphocytes. Three strains of HIV with varying cytopathogenic properties (32) were used to infect H9 cells at an approximate multiplicity of infection (MOI) of 0.1. Figure 1 shows that the SK-1 strain (the most cytopathogenic strain used) was the most rapid inducer of ACTH in H9 cells. Fifteen days after the infection, about 50% of the HIV-SK-1-infected cells expressed ACTH compared to 25% and 30% of the HIV-MCK1- and HIV-213-infected cultures, respectively. ACTH expression peaked at 35 days after infection, with approximately 80% of cells infected with HIV-MCK-expressing ACTH compared to 65% of cells in the HIV-SK-1-infected cultures and 60% in the HIV-213-infected cultures. In contrast, 42 days postinfection, p24 expression reached a maximum of 95% in cultures infected with HIV-SK-1 and HIV-213 and decreased dramat-

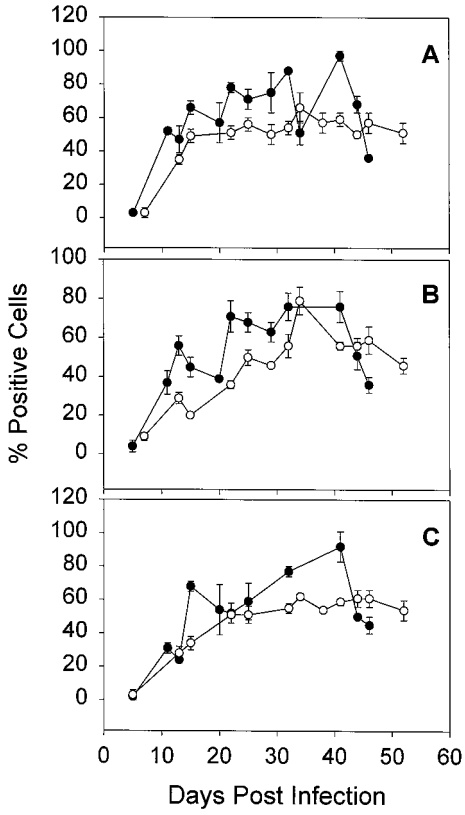

FIG. 1. Correlation of ACTH expression and p24 production in HIV-infected H9 cells. Cells were infected with three strains of HIV-1: A) SK-1, B) MCK, and C) 213. Immunofluorescent staining was an indirect technique using a polyclonal rabbit anti-ACTH antiserum (*open circles*) or a murine monoclonal anti-p24 antibody (*closed circles*), as primary antibodies. H9 cells (3.5×10^5) were inoculated with HIV-1 at an approximate MOI of 0.1. At the indicated times, aliquots were removed and stained by immunofluorescence. Staining controls were all less than 5% and included noninfected cells, normal rabbit or mouse serum, or anti-ACTH absorbed with ACTH. The data are mean values of triplicate samples from a representative experiment.

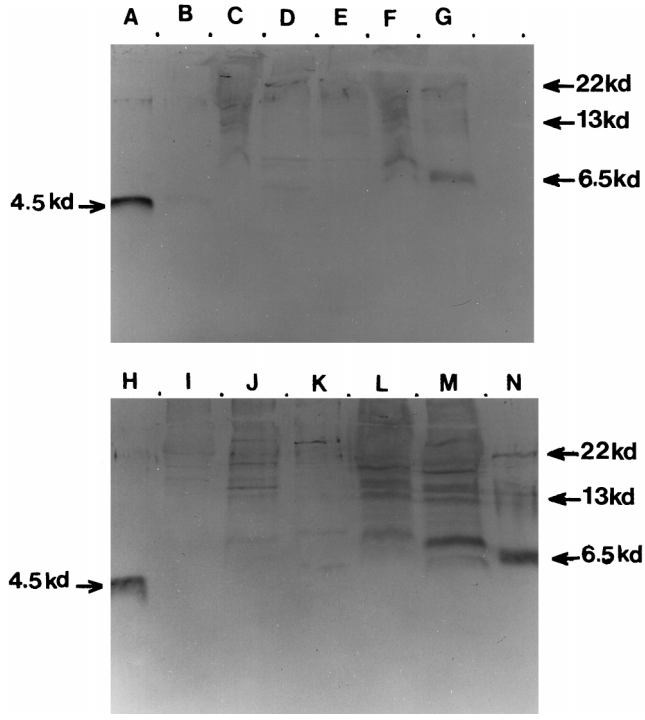

FIG. 2. Western immunoblot analysis of ACTH production by HIV-SK-infected H9 cell lysates (2×10^7 cells). After detergent (Nonidet P-40) lysis of cells, 10 μL of the lysates were run on a 20% discontinuous SDS-polyacrylamide gel. After electrophoretic transfer to a nitrocellulose membrane, rabbit anti-ACTH was added. The membrane was washed and treated with alkaline phosphatase-conjugated goat antirabbit antiserum and subsequently exposed to substrate. Normal rabbit serum was used as a negative control. Lanes A and H, Purified porcine 4.5-kDa ACTH (2.5 μg/mL). Lane B, Purified porcine 4.5-kDa ACTH (1 μg/mL). Lanes C and D, H9 cells grown in RPMI and serum-free RPMI. Lanes E, F, J, K, L, and M, Infected H9 cells from days 5, 9, 13, 26, 30, and 38 after infection, respectively. Lane I, Negative control (primary human fibroblast). Lanes G and N, Positive control (pituitary cell line AtT20).

ically to about 35% by the end of the experiment. The results were similar in HIV-MCK cultures; however, HIV-p24 peak expression was present in 70% of the cells.

Characterization of HIV-induced ACTH

Next, studies were performed to verify that HIV induces *bona fide* bioactive ACTH as seen in other viral systems (37, 38). A Western blot analysis was performed on HIV-infected H9 cell lysates for ACTH (Fig. 2). This confirmed the IF results by showing the consistent presence of an immunoreactive 6.5-kDa ACTH band that corresponded to the major band detected in the pituitary cell lysate. Qualitative comparison of color intensity between HIV-infected samples at various time points during infection revealed an increasing trend in the amount of ACTH produced by H9 cells as the HIV infection progressed.

Mechanisms of HIV induction of ACTH

As a means to determine whether ACTH was produced directly by the HIV-infected cells rather than by indirect induction, by a HIV-induced cytokine for instance, HIV-infected cells were dually stained for ACTH and p24 immunoreactivity. Figure 3 demonstrates that 13 days after infection by HIV some of the p24-positive H9 cells (Fig. 3B) did not express ACTH (Fig. 3A), whereas almost all cells expressing ACTH also stained positively for HIV-p24. Similar studies, 27 days after infection, indicated that eventually all p24-positive cells would also express ACTH (data not shown).

Experiments were then performed to determine whether a productive HIV infection was required to stimulate ACTH production in H9 cells. Results in Fig. 4 show that a 4-h incubation of UV-inactivated HIV with H9 cells was adequate in stimulating ACTH production in these cells. Furthermore, this effect was partially blocked by anti-gp120 antiserum. Control samples, which were H9 cells treated with anti-gp120 and cells stained with normal rabbit serum were negative. Figure 5 reflects similar results when infectious HIV was used as the inducer of ACTH production. Anti-gp120 antibodies again partially inhibited the ACTH induction.

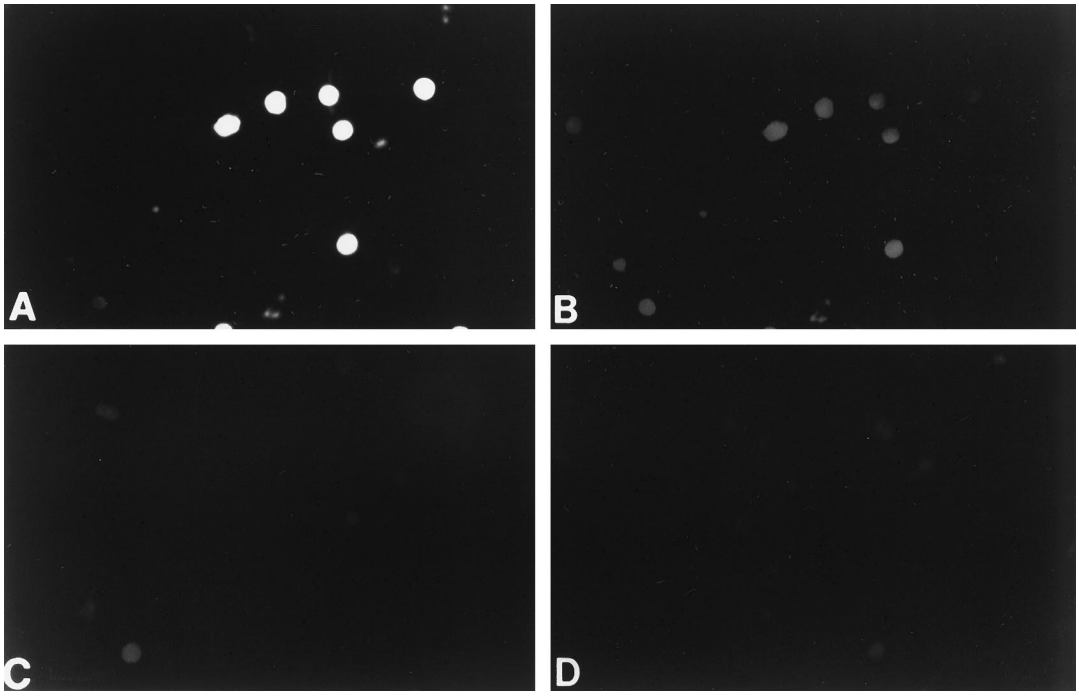

FIG. 3. Dual immunofluorescent staining of H9 cells infected with HIV (SK-1; MOI, 1). Fourteen days after infection, cells were harvested, fixed, and stained for both ACTH (green fluorescence) and p24 antigen (red fluorescence), simultaneously. A and B show ACTH and p24 expressions, respectively. The original photographs were in color and are reproduced here as black and white images. Negative control samples included HIV-infected cells stained using normal rabbit (C) and mouse (D) sera. Photomicrographs are representative of three to five fields observed for three experiments.

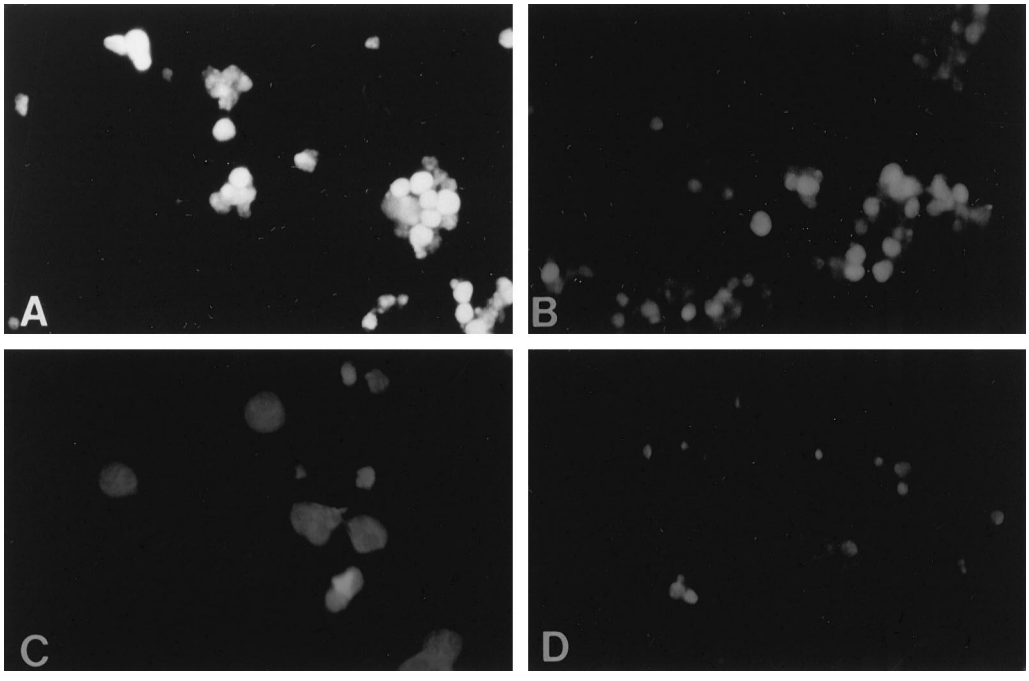

FIG. 4. IF detection of ACTH in H9 cells treated with UV-inactivated HIV. In A, H9 cells were incubated with UV-inactivated HIV for 4 h at 37 C and then fixed and stained for ACTH. B presents similarly treated cells with the addition of anti-gp120 antibodies. Controls consisted of H9 cells treated with anti-gp120 alone (C) as well as cells treated with inactive HIV and stained with normal rabbit serum (D). Photomicrographs are representative of three to five fields observed for three experiments.

ACTH induction by soluble gp120

To determine whether interaction with the CD4-binding site on the T cell was mediating the induction of ACTH, soluble gp120 was used to treat H9 cells. Lymphoid ACTH was induced rapidly within 1 h in a dose-dependent manner (Fig. 6). This was more rapid than with infectious virus, but

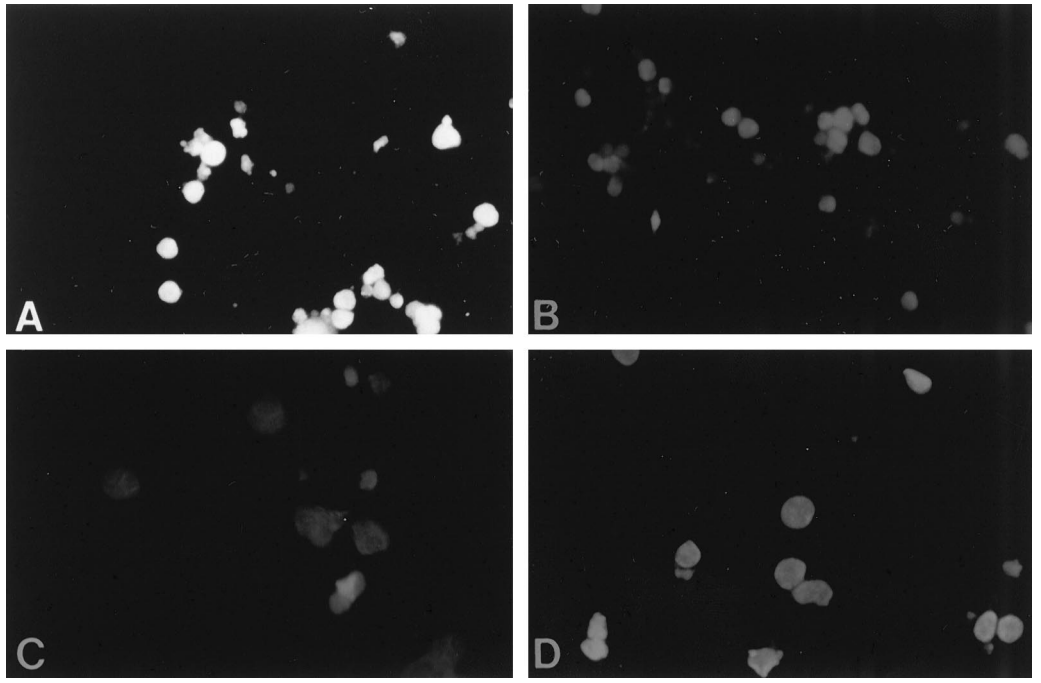

FIG. 5. IF detection of ACTH in H9 cells treated with infectious HIV-1. A shows H9 cells that were incubated with HIV-1 for 4 h at 37 C and then fixed and stained for ACTH. B presents similarly treated cells with the addition of anti-gp120 antibodies. Controls consisted of H9 cells treated with anti-gp120 alone (C) as well as cells treated with HIV-1 and stained with normal rabbit serum (D). Photomicrographs are representative of three to five fields observed for three experiments.

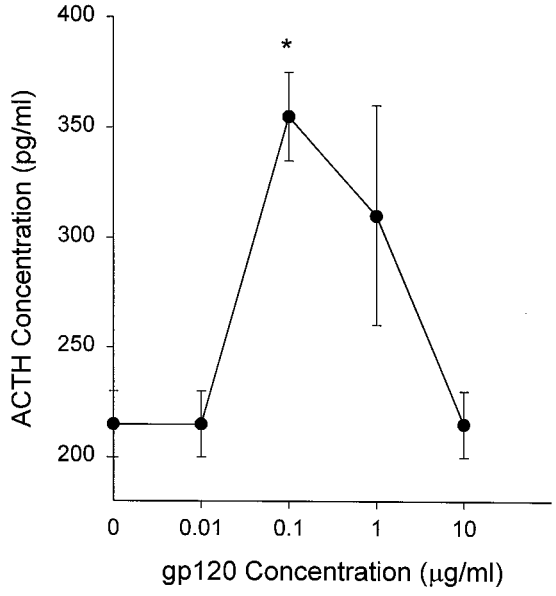

FIG. 6. Dose-dependent effect of gp120 on ACTH production by H9 cells. Cultures were treated with the designated concentration of gp120 (mock-treated cells received medium alone). After 48 h, cell lysates were prepared and assayed by RIA. The data are presented as the averages (±SEM) of two experiments run as duplicates. *, $P \leq 0.04$, by paired t test.

was similar to the kinetics of induction with whole, UV-inactivated virus (Fig. 4).

Our approach to testing whether gp120 induces *de novo* synthesis of lymphoid ACTH was to stimulate H9 cells with gp120 in the presence of ^{3}H-labeled amino acids and compare the amounts of radioactively labeled ACTH in the supernatant fluid of gp120-treated and mock-treated cultures. Figure 7 shows that by SDS-PAGE analysis of affinity-purified eluates from gp120-treated cultures, there was a marked increase in the *de novo* synthesis of two ACTH-related molecular species, namely the 13- and 17-kDa proteins. To show that the gp120-induced intracellular lymphoid ACTH is bioactive and thus physiologically relevant, Y-1 cell rounding assays for ACTH bioactivity were used. Figure 8 compares the bioactivities of lysates from H9 cells treated with gp120 *vs.* mock-treated H9 cells. The biological activities of these lysates appear primarily due to ACTH, as anti-ACTH antibodies inhibited this activity. Thus, although we primarily detected higher mol wt forms of immunoreactive ACTH, the bioactivity suggests that a processed form is produced. Our inability to see the processed form (lower mol wt species) in Fig. 7 may be due to the inefficiency of the intrinsic radiolabeling or the low specific activity of the labeled ACTH peptide.

Effect of ACTH on HIV replication

Two approaches were taken to determine the roles of ACTH and MSH produced by HIV-infected cells in viral replication. One was to treat HIV-infected cells with exogenous neuropeptides and then monitor p24 expression. ACTH and MSH tended to be inhibitory when they had a significant effect, but there was too much variation in the results for definitive conclusions (data not shown). The second approach was to block endogenous ACTH by the addition of antiserum against ACTH-(1–24) to the HIV-infected PBL cultures. Figure 9 shows in four separate, replicate experiments that anti-ACTH-(1–24) enhanced viral p24 expression. Although the degree of enhancement varied between

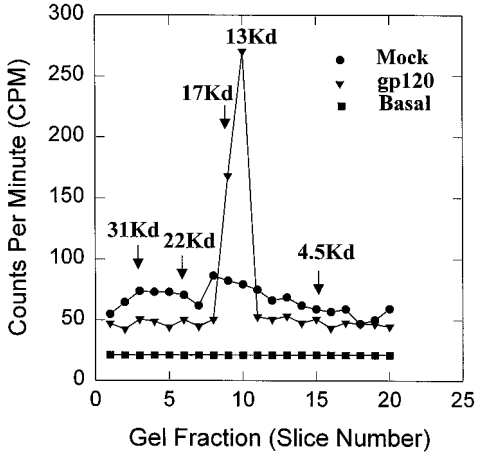

FIG. 7. Comparison of the affinity-purified, intrinsically labeled H9 culture supernatant fluids from gp120-treated *vs.* nontreated cells. Eluted samples from the two cultures were pH adjusted, and 10 μL of each sample were run on a 20% discontinuous polyacrylamide gel. The gel was then sliced (0.3 cm), and each slice was counted individually in a liquid scintillation counter.

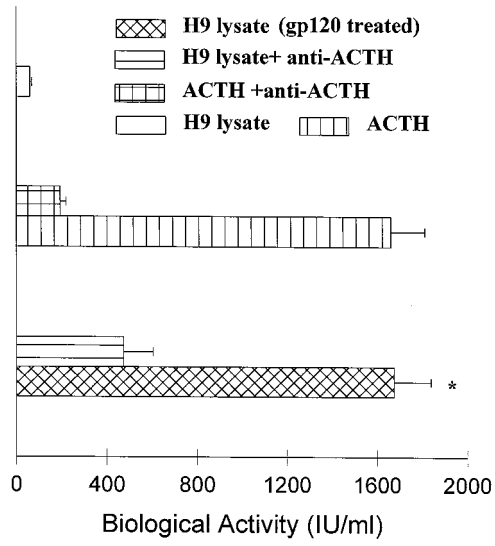

FIG. 8. ACTH-specific biological activity of the gp120-induced H9 cell lysates. H9 cells (2×10^7) were treated with 2 μg/mL gp120 for 4 h. After low speed centrifugation ($1000 \times g$), cells were lysed, and 1:3 dilutions of the lysates were incubated with or without anti-ACTH (1:500) in Y-1 cultures. Morphological changes were assessed, and the end-point dilution was compared to those of ACTH standard samples run simultaneously. Data are the average of duplicate samples and are representative of three or more experiments.

experiments, it generally reached its maximal level 8 days after infection by HIV-1. The anti-ACTH enhanced HIV-1 replication by up to 6 times over the control values. In all four experiments, the effect of anti-ACTH declined during the second week of infection, but it did not decrease to the virus control level.

Discussion

Numerous recent studies have identified endocrine changes in AIDS from both pathogenesis and treatment perspectives (1–3, 5, 8, 10, 13, 23, 39–41). The present study examines how the POMC-related hormones may be involved. HIV induced production of *bona fide* ACTH in H9, T lymphocytes. Complete replication of the virus was not necessary, as UV-inactivated HIV or soluble gp120 also induced ACTH production. The lymphoid ACTH appeared to play a role, at least locally, on viral replication because the addition of anti-ACTH antiserum enhanced the expression of the p24 capsid antigen.

The observation that some of the H9 cells that express the HIV-p24 antigen do not produce ACTH suggests that perhaps p24 production, and therefore HIV replication, precedes the expression of ACTH in these cells. The kinetics of ACTH production and its correlation to p24 production confirm this observation. The Western blot analysis of the lysates from HIV-infected H9 cells complemented our results from the IF studies by showing the mol wt of the molecules detected by immunofluorescence. The consistent presence of an immunoreactive ACTH band corresponding to the intracellular pituitary ACTH (42) indicates that the species detected in the HIV-infected cells is very similar, if not identical, to pituitary ACTH. Furthermore, the finding that a higher percentage of cells from the later stages of HIV-infected H9 cultures expressed ACTH is suggestive of an increasing trend in ACTH induction as the infection progresses. This is possibly a secondary induction by cytokines produced during the infection. The molecular mass difference between secreted ACTH (4.5 kDa) and the ACTH detected in the H9 lysates (6.5 kDa) is explained by the fact that intracellular ACTH migrates as a higher molecular mass on SDS-PAGE than secretory ACTH (42). The presence of higher molecular mass bands that were similar to other ACTH precursor molecules (*i.e.* 35, 22, and 13 kDa) was also seen in the lysates of pituitary cells. However, since the pituitary lysates were diluted for comparative purposes, only the predominant 6.5-kDa band was in sufficient quantity to appear in the Western blot.

The different species of ACTH-related peptides may affect HIV replication. The antiserum to ACTH-(1–24) recognizes full-length ACTH-(1–39), but not αMSH or endorphins. The antiserum may also neutralize 13-kDa or larger ACTH-related peptides, and this antiviral activity would be a novel function for these molecules. Opioids and opiates have been shown to affect HIV replication (43). Although these current experiments do not address the role of endorphins induced by HIV (23), it will be important in the future for understanding the full implications of lymphoid POMC production on HIV replication.

Since its discovery (44), several groups have explored the possible roles for lymphoid-derived ACTH. This study confirms and extends observations by ourselves (22) and others (23) of POMC induction in HIV-infected lymphocytes. It also appears to be consistent with the 1981 report (44) that first documented the induction of lymphocyte ACTH production by Newcastle disease virus. Moreover, it agrees with the studies that have demonstrated the induction of ACTH by other viruses and infectious agents (45, 46). However, our findings were in contrast to the report by Oates *et al.* (46, 47) that did not find POMC messenger ribonucleic acid levels elevated in HIV-infected cells. This discrepancy may be due

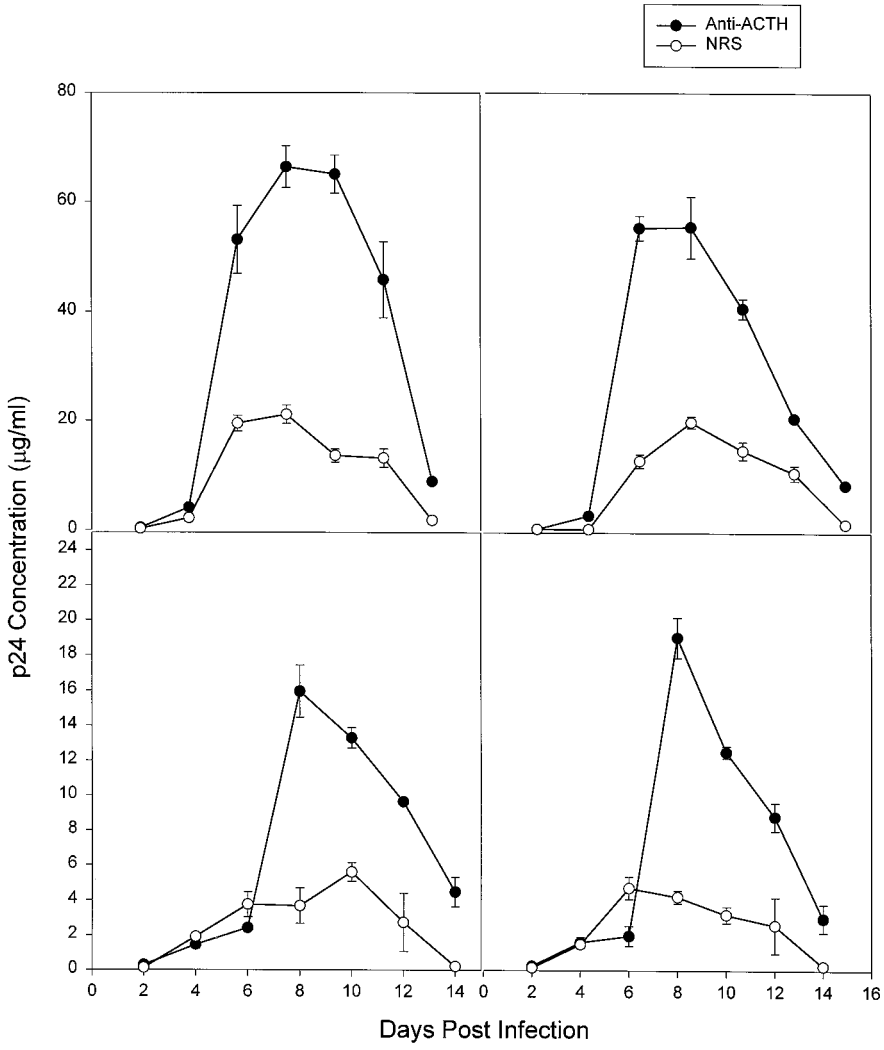

FIG. 9. Enhancement of HIV-1 replication by ACTH antiserum. PBLs were infected with HIV-1 (strain SK) and treated with anti-ACTH (1:500) every other day. The concentration of p24 in the culture supernatant fluids was measured by enzyme-linked immunosorbent assay as described in *Materials and Methods*. Control wells received normal rabbit serum (1:500). Each *point* represents the average (±SEM) of four samples. Each panel is a separate experiment, using PBLs from different individuals.

to factors such as differences in the length and multiplicity of infection as well as cell lineage (B cell line) or possibly HIV strain.

This present study extends upon the previous studies by examining possible mechanisms for HIV's induction of ACTH. The finding that three strains of HIV, with different biological properties, have similar effects on ACTH production by H9 cells implies that the ACTH induction process involves mechanisms common to different HIV strains. This led to examining whether HIV attachment to H9 cells, in the absence of an infection, would induce ACTH production. Consequently, H9 cell cultures were treated with the preparations of UV-inactivated HIV-1 and monitored for ACTH expression. Interestingly, IF showed that the majority of the cells were expressing immunoreactive ACTH. This suggested that initial HIV attachment to H9 cells and intracellular events subsequent to the binding of the gp120 molecule to the cell surface CD4 receptors are involved in the ACTH induction process. Thus, it appears that HIV-1 replication is sufficient, but not required, for triggering this activation. The anti-gp120 antibodies significantly blocked the effect of HIV-1, confirming the specificity of the induction. The facts that ACTH production can be detected as early as 4 h after treatment with HIV-1 and that there was low ACTH expression in the first 2 weeks of HIV infection imply that perhaps the ACTH induction by this virus involves a rapid transient phase (hours) and a slower steady production phase (days), as seen in the preliminary IF studies. In addition, differences in the virus to cell ratio (MOI, 0.1 *vs.* 1.0) may also explain differences in the rate of HIV induction in these cultures. In our previous study (22), HIV infection of H9 cells induced levels of ACTH and MSH in the range of 30–54 pg/mL, which are roughly comparable to levels of the endorphin measured by Barcellini *et al.* (23), and no ACTH was detected in noninfected cells.

These data indicate that viral gp120 is able to stimulate ACTH production. Presumably it mediates the induction through binding to CD4. Interaction between gp120 and CD4 are thought to be responsible for many effects of HIV infection, and *in vitro* gp120 will modulate many cellular functions (48). Although it is typically considered an accessory molecule for T cell receptor binding and recognition of antigens, CD4 binding does activate intracellular signaling pathways. The tyrosine kinase encoded by the *lck* protooncogene, $p56^{lck}$ has been found to be associated noncovalently with CD4 (49). It has been reported that gp120 has different activities in T

cells depending upon their activation state. In activated cells, gp120 blocks phosphatidylinositol 4,5-biphosphate hydrolysis and Ca^{2+} mobilization (50). Conversely, in resting T cells and monocytes, gp120 stimulates these activities (51, 52). Intracellular Ca^{2+} mobilization is one effect of CRF stimulation of pituitary POMC expression, and there are several lines of converging evidence to suggest that the pathways for POMC and HIV expression may be linked at the transcriptional level (53).

There is an increasing awareness of the role that host factors play in AIDS (54). For instance, it has recently been recognized that chemokine receptors serve as secondary binding sites for HIV (55–60). There is also evidence that hCG (61, 62) or a related factor (63) inhibits HIV replication. These and the data presented in this report raise the question of whether the production of lymphoid ACTH is a cofactor and whether it is to the benefit of the virus or the host. ACTH is generally thought to be suppressive to immune responses, both directly on lymphocytes and through the induction of adrenal glucocorticoids (25). The later, of course, are widely acknowledged to be antiinflammatory and are thought to be a major negative feedback mechanism for immune activation (64). By induction of corticosteroids, lymphoid ACTH production could affect the host resistance, and this induction has been found previously in a Newcastle disease virus model (38). In support of this, a combination of hypercortisolemia and elevated Th2 cytokines has been shown to be prognostically unfavorable in AIDS (65). Conversely, the experiment in which antibody to ACTH enhanced p24 expression suggests that lymphoid ACTH might be antiviral to a degree and therefore protective for the host. ACTH acts most like a Th2 cytokine, and most of its immune effects are inhibitory. It modulates an *in vitro* antibody response (66), interferon-γ production (26), phagocytic cell activation (22), and chemotaxis (67). Especially germane to these activities are our recent findings on the relationships that ACTH has to interleukin-10, a prototypical Th2 cytokine, in both immune and neuroendocrine activities that directly impinge on HIV status (68–70).

As ACTH is constantly present in serum and is constitutively produced by some lymphoid cell types (*i.e.* macrophages), our current hypothesis concerning its role in the immune system is that it is a tonic inhibitor (71). That is, it sets a threshold that needs to be surmounted for activation to occur. This would prevent minor stimuli from activating the immune system and possibly causing an inappropriate response. For HIV, this inhibition by ACTH could work to a virus's advantage by limiting damage for prolonged viral replication, but it could also be to the host's advantage to slow viral replication to allow the immune system a greater opportunity to respond. This may be an important part of the equilibrium in virus production and cell destruction postulated by Coffin (72).

In fact, a growing number of researchers suspect that imbalances in the network of cytokines may ultimately trigger the immune system collapse associated with HIV (73). Thus, considering that it has not been possible to attribute the degree of immune suppression to any single factor, the interaction of immune and neuroendocrine [for example, our findings with IL-10 and ACTH (68–70)] components may represent a mechanism to mediate a part of this suppression.

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