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Norepinephrine Accelerates HIV Replication Via Protein Kinase A-Dependent Effects on Cytokine Production¹

Steve W. Cole,^{2*‡§} Yael D. Korin,^{*¶} John L. Fahey,^{*‡§} and Jerome A. Zack^{*‡§}

To explore the role of sympathetic nervous system activation in HIV pathogenesis, we examined the effect of the neuroeffector molecule norepinephrine (NE) on HIV-1 replication in quiescently infected PBMCs that were subsequently activated with Abs to CD3 and CD28. NE accelerated HIV-1 replication at concentrations ranging from 10^{-8} to 10^{-5} M. This effect could be mimicked by protein kinase A (PKA) activators (forskolin or dibutyryl-cAMP) and abrogated by β -adrenoreceptor antagonists or the PKA inhibitor rp-cAMP, indicating transduction via the adrenoreceptor signaling pathway. NE reduced cellular activation and altered the production of several HIV-modulating cytokines: IL-10 and IFN- γ were markedly suppressed; TNF- α , IL-1 β , IL-2, IL-4, and IL-6 were mildly suppressed; and levels of IL-12 were not significantly altered. The addition of either exogenous IFN- γ or IL-10 abrogated the effect of NE on virus production. Thus PKA-dependent suppression of cytokine production appears to mediate the enhancement of HIV-1 replication by NE. *The Journal of Immunology*, 1998, 161: 610–616.

Stress has long been believed to influence viral infections, but the biologic mechanisms underlying these effects remain largely unknown (1, 2). One possible mode of interaction involves sites of direct contact between cells of the peripheral nervous system and cells of the immune system. All primary and secondary lymphoid organs contain the termini of neurons from the sympathetic division of the autonomic nervous system (SNS)³ (3, 4). When activated by stress or other stimuli, these neurons release micromolar quantities of norepinephrine (NE) into sites that are rich in T lymphocytes and macrophages (3, 5). β_2 -adrenoreceptors on the lymphocyte plasma membrane bind NE and signal the cellular interior via the $G_{\alpha S}$ subunit of a heterotrimeric G protein linked to the adenylyl cyclase-cAMP-protein kinase A (PKA) signaling cascade (6–8). Adrenoreceptor ligation alters several aspects of lymphocyte function in vitro, including cellular activation, cytokine production, cell traffic and adhesion, and cytotoxic activity (6, 9–15). However, little is known about the effects of SNS activation on disease pathogenesis.

The innervation of lymphoid tissue by the SNS may be particularly relevant to HIV infection, since lymphoid organs represent the primary site of HIV pathogenesis. Long before the onset of clinical illness, high concentrations of virus and large numbers of infected CD4⁺ T lymphocytes are present in the lymph node paracortex, splenic periarteriolar lymphoid sheath, and a variety of other secondary lymphoid structures (16–18). These sites are also

richly innervated by SNS neurons (3, 4), raising the possibility that SNS activation could conceivably influence HIV pathogenesis. In the present study, we examine the effect of the SNS neuroeffector molecule NE on HIV-1 replication in an in vitro model of CD4⁺ T lymphocyte infection.

In vivo the vast majority of T lymphocytes are quiescent. These cells are vulnerable to infection by HIV-1 but are incapable of fully reverse-transcribing the viral genome (19, 20). Activation of infected T lymphocytes leads to complete reverse transcription and rapid viral replication (20, 21, 73). To model this process, we infected quiescent PBMCs with a CXCR-4-tropic strain of HIV-1 and activated those cells with Abs to CD3, the signaling component of the T cell receptor, and Abs to CD28, a critical costimulatory molecule (22–25). To examine the effect of SNS activation on HIV-1 replication, NE was added simultaneously with CD3/CD28 costimulation. Previous research indicates that the pharmacologic inducers of cAMP can increase HIV-1 replication in continuously cycling cell lines (26, 27). However, it is not known whether physiologic cAMP inducers such as NE can increase HIV-1 replication or whether such effects occur in normal lymphocytes that are activated in a physiologically relevant manner. In addition, the mechanism by which cAMP affects HIV-1 replication remains unknown. Here, we demonstrate that NE accelerates HIV-1 replication in freshly infected PBMCs. We also show that this effect is transduced via the β -adrenoreceptor-adenylyl cyclase-cAMP-PKA signaling pathway and is mediated by the pronounced suppression of IFN- γ and IL-10.

Materials and Methods

Infection, activation, and cell culture

Healthy donor PBMCs were infected with HIV-1_{NL4-3} (0.05 infectious units/cell) for 2 h in the presence of 10 μ g/ml polybrene. Following infection, cells were washed and costimulated with Abs to CD3 (0.1 μ g/ml adhered to flask by goat anti-mouse Ab; Southern Biotechnology Associates, Birmingham, AL) and CD28 (0.1 μ g/ml soluble; Biodesign, Kennebunkport, ME). Unstimulated control cells received no exogenous Abs. Cells were cultured for 6 to 8 days at 3×10^5 /ml in RPMI 1640 supplemented with 10% (v) human AB serum, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 2 mM glutamine at 37°C in an atmosphere of 5% CO₂. NE ([-]-Arterenol; Sigma, St. Louis, MO) and other reagents were added once, at activation. Other reagents added at activation included the adenylyl cyclase activator forskolin (Sigma) (28); the membrane-permeable

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³ Abbreviations used in this paper: SNS, sympathetic nervous system; NE, norepinephrine; PKA, protein kinase A; db-cAMP, dibutyryl-cAMP; rp-cAMP.

cAMP analogue $N^6, 2'$ -*O*-dibutyryl adenosine-3', 5'-cyclic monophosphate (db-cAMP; Sigma) (29); the membrane permeable PKA antagonist adenosine-3', 5'-cyclic monophosphothioate, rp isomer (rp-cAMP; Calbiochem, La Jolla, CA) (30); human IFN- γ (R&D Systems, Minneapolis, MN), IL-2 (Genzyme, Cambridge, MA), IL-4 (Genzyme), and IL-10 (R&D Systems). To determine whether the effects of NE were mediated by β -adrenoreceptors, infected PBMCs were incubated in the presence of the receptor antagonist sotalolol (ICN Biomedicals, Costa Mesa, CA) or dl-propranolol HCl (ICN Biomedicals) for 1 h before activation in the presence of NE. Cell viability was assessed by trypan blue exclusion and did not differ between NE-treated and untreated cultures at 2, 4, 6, or 8 days postinfection. Cell viability was also not altered by the addition of adrenoreceptor blockers (propranolol, sotalolol, phentolamine) or PKA antagonists (rp-cAMP) to NE-treated cultures. Apoptosis rates were assessed by a flow cytometric measurement of annexin V-FITC (Brand Applications) and propidium iodide binding. Apoptosis rates did not differ across cultures of costimulated cells, cultures costimulated in the presence of NE, or NE supplemented by adrenoreceptor blockers or PKA antagonists.

HIV-1 quantification

Virus production was quantified by ELISA for HIV-1 p24 core protein (Coulter, Maastricht, The Netherlands). p24 levels were compared across treatments by ANOVA followed by Duncan's multiple range test for specific comparisons. Dose-response and kinetic profiles were analyzed by linear regression on log p24 values. p24 levels were graphed as the mean plus SE. To confirm the equality of viral inoculation, we also examined the proviral load at 14 h postinfection using PCR (AA55 and M667 primer sequences for the R/U5 region of the viral long terminal repeat, quantified relative to input cell number as indicated by β -globin primers LA1 and LA2) (20). Briefly, one primer of each pair was radio end-labeled, and amplification was conducted for 25 cycles in parallel with control standards consisting of linearized cloned HIV-1 DNA and known quantities of cellular DNA. Radiolabeled, amplified products were resolved on a 6% polyacrylamide gel and quantified by radioanalytic image analysis in comparison with standard curves.

Cytokine quantification and identification of cytokine-producing cells

ELISAs were used to measure the supernatant concentrations of human IL-1 β (R&D Systems), IL-2 (Genzyme), IL-4 (Genzyme), IL-6 (Innogenetics, Zwijndrecht, Belgium), IL-10 (Immunotech, Westbrook ME), TNF- α (Genzyme), IFN- γ (Biosource International, Westlake Village, CA), and IL-12 p70; capture Ab 12H4 and detection Ab C8.6 were kindly provided by G. Trinchieri, (Wistar Institute, Philadelphia, PA). Concentrations of each cytokine were determined in parallel at 0, 2, 4, and 6 days postinfection across three independent experiments and graphically expressed as the mean plus SE. Differences in cytokine production were analyzed by the Student *t* test. Total cytokine production was quantified as the area under the curve of the cytokine level over time (as in Fig. 3); the statistical significance of differences in total cytokine production was determined by the Student *t* test.

Cells producing IFN- γ were identified by flow cytometric analysis of intracellular binding of anti-cytokine Abs following a 6-h incubation in the presence of 10 μ g/ml brefeldin A (following the protocol of the Ab manufacturer). The CD4⁺/- and CD8⁺/- phenotype of cytokine-producing cells was determined by surface staining with anti-CD4 and anti-CD8 Abs (Becton Dickinson, Mountain View, CA) before intracellular cytokine staining. All flow cytometric data were acquired using a FACScan instrument (Becton Dickinson) and analyzed using CellQuest software (Becton Dickinson); dead cells and debris were excluded on the basis of forward scatter vs side scatter profiles.

Assessment of cellular activation

At 3 days postinfection, cellular activation was quantified by 1) the flow cytometric analysis of cell cycle stage via intracellular staining of RNA and DNA, 2) the cell surface expression of CD25 (IL-2R; expressed early in the cell cycle) and CD71 (transferrin receptor; expressed late in the cell cycle) by flow cytometry (Becton Dickinson Immunocytometry protocol), and 3) 6 h of [³H]thymidine uptake. In cell cycle staging, cells were permeabilized with saponin (0.004% w) and stained with 7-amino actinomycin D (Sigma) and pyronin Y (Calbiochem) to quantify intracellular DNA and RNA, respectively (31, 73). Cycling cells move from a 2N DNA/RNA^{low} state (G_0/G_{1A}) through a 2N DNA/RNA^{high} state (G_{1B}) to a 4N DNA/RNA^{high} state (G_2/M). DNA/RNA quadrants were set on the basis of a 2N DNA/

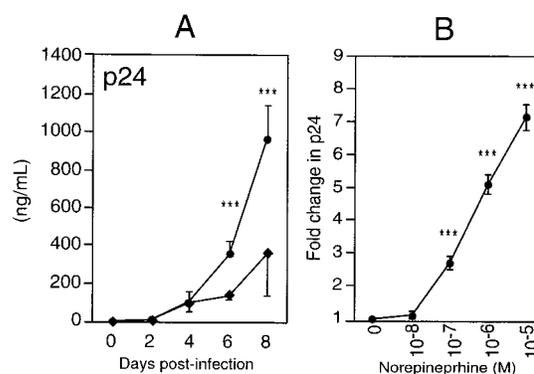


FIGURE 1. Effect of NE on HIV-1 replication. **A**, Kinetics of the effect of NE on HIV-1 replication was assessed by ELISAs of supernatant p24 levels at 0, 2, 4, 6, and 8 days following the infection of quiescent PBMCs and subsequent costimulation by Abs to CD3 and CD28. The simultaneous addition of 10 μ M NE (●) at costimulation significantly accelerated viral replication relative to costimulated controls (◆); $d\text{ p24}/dt = 100.8\text{ ng/ml}^{-1}\text{ day}^{-1}$ vs $48.9\text{ ng/ml}^{-1}\text{ day}^{-1}$ in costimulated controls ($p = 0.03$). Data represent the mean plus SE of 3 experiments and are representative of 15 experiments in which day 6 p24 levels in NE-treated cultures averaged 337% of costimulated controls ($p = .0002$). The statistical significance of comparisons at specific time points is indicated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. **B**, Dose-dependence of the effects of NE on HIV-1 replication was assessed by supernatant p24 levels at 6 days following the infection of quiescent PBMCs and subsequent costimulation in the presence of varying concentrations of NE. Regression analysis indicated a significant dose-response relationship, with p24 levels increasing 2.1-fold per log increase in NE concentration over the range 0.01 to 10 μ M ($p = 0.002$).

RNA^{low} negative control that had been established by activating PBMCs in the presence of 5 mM *N*-butyrate (Sigma), which blocks cell cycle progression in G_{1A} (32). The identification of cycling cells via RNA/DNA staining was highly specific, with <1% of cells falling in the cycling quadrant in the absence of costimulation (vs >65% of costimulated cells). The results of cell cycle staging correlated closely with the expression of the CD25 and CD71 activation Ags and with [³H]thymidine uptake. Dose-response relationships between NE concentration and the fraction of cycling cells were evaluated by linear regression.

Results

NE accelerates HIV-1 replication

To examine the effects of NE on HIV-1 replication, p24 levels were assessed at 2, 4, 6, and 8 days postinfection (Fig. 1A). As expected, costimulating HIV-1_{NL4-3}-infected PBMCs with Abs to CD3 and CD28 markedly increased viral replication relative to unstimulated cultures. Identical costimulation in the presence of 10 μ M NE led to a significant additional increase in HIV replication. This effect emerged reliably across 15 independent experiments, with NE increasing day 6 p24 levels to an average of 337% of costimulated control levels ($p < 0.0001$). In some donors, NE increased day 6 p24 levels by >11-fold. The effect of NE on HIV-1 replication was dose-dependent, with day 6 p24 levels increasing approximately twofold with each log increase in NE concentration over the range 10⁻⁸–10⁻⁵ M (Fig. 1B) ($p = 0.002$). These differences emerged despite the fact that a PCR analysis of the HIV-1 proviral load confirmed equivalent initial viral inoculation (no difference in proviral penetrance at 14 h postinfection; data not shown). PCR analysis corroborated p24 ELISA results in documenting increased proviral spread in NE-treated cultures at 6 to 8 days postinfection (data not shown).

Table I. *Effects of PKA-inducing and-blocking agents on HIV replication^a*

Agent	p24 at 6 Days Postinfection	Difference from Costimulated Control
NE (10 ⁻⁵ M)	337.3% (62.5)	<i>p</i> < .05
NE + β -adrenergic blocker		
Sotalol (10 ⁻⁵ M)	100.4% (16.7)	NS
Propranolol (10 ⁻⁷ M)	87.9% (8.7)	NS
NE + α -adrenergic blocker		
Phentolamine (10 ⁻⁵ M)	327.6% (70.7)	<i>p</i> < .05
PKA-activating agents		
Forskolin (10 ⁻⁵ M)	283.7% (184.7)	<i>p</i> < .05
db-cAMP (10 ⁻⁴ M)	358.4% (291.8)	<i>p</i> < .05
NE + PKA inhibitor		
rp-cAMP (10 ⁻⁴ M)	109.7% (6.8)	NS

^a PKA inducers or inhibitors were added postinfection and prior to CD3/CD28 costimulation. Data represent the mean (SE) percent change in p24 levels relative to costimulated controls (equaling 100%) at 6 days postinfection. NS, not significant.

Effect of NE on HIV-1 replication is mediated by the β -adrenoreceptor-adenylyl cyclase-cAMP-PKA signaling pathway

In the classical NE signaling pathway, ligation of the cell surface β -adrenoreceptor activates adenylyl cyclase, which triggers increased synthesis of cAMP and thereby activates the cAMP-dependent kinase PKA. To determine whether the effect of NE on HIV-1 replication is transduced by ligation of the β -adrenoreceptor, infected PBMCs were incubated in the presence of the β antagonists sotalol (10 μ M) or propranolol (0.1 μ M) for 1 h before activation in the presence of 10 μ M NE. Both adrenoreceptor antagonists abrogated the effects of NE on HIV-1 replication, reducing p24 concentrations to levels that were statistically indistinguishable from costimulated control cultures (Table I). In contrast, the α -adrenoreceptor antagonist phentolamine (10 μ M) failed to block the enhancement of HIV replication by NE (Table I).

To determine whether activation of the adenylyl cyclase-cAMP-PKA signaling pathway is capable of accelerating HIV-1 replication, infected PBMCs were costimulated in the presence of the adenylyl cyclase activator forskolin (10 μ M) or the membrane-permeable cAMP analogue db-cAMP (100 μ M). Both of these PKA activators increased p24 concentrations to levels similar to those observed in NE-treated cultures (Table I).

To determine whether the effects of NE on HIV-1 replication were mediated by increased activation of PKA, infected PBMCs were incubated in the presence of the PKA inhibitor rp-cAMP (100

μ M) for 1 h before costimulation in the presence of NE. PKA blockade strongly inhibited the effect of NE on HIV-1 replication (Table I), reducing p24 concentrations to levels that were statistically indistinguishable from costimulated control cultures. Thus, the activation of the PKA signaling cascade via the cell surface β -adrenoreceptor appears to mediate the effects of NE on HIV-1 replication.

Effect of NE on HIV-1 replication is not mediated by increased cellular activation

Cellular activation is critical to efficient HIV-1 replication (20, 21). NE and other cAMP-inducing agents partially suppress cellular activation in uninfected PBMCs (9), but their effect on cellular activation in HIV-infected lymphocytes is unknown. A flow cytometric assessment of cell cycling by analysis of intracellular DNA and RNA indicated that NE decreased cellular activation in HIV-1-infected PBMCs (Fig. 2). These effects were dose-dependent, with the fraction of cycling cells declining by an average of 15.3% per log increase in NE concentration over the range 10⁻⁷ to 10⁻⁵ M (*p* < 0.0001 across four experiments). Similar effects emerged in flow cytometric analyses of the early CD25 and the late CD71 activation Ags and in measurements of 6-h [³H]thymidine uptake (data not shown). Thus the effect of NE on HIV-1 replication does not appear to be mediated by increased cellular activation in HIV-infected cultures.

NE alters cytokine production profiles

Previous research indicates that NE can suppress the production of IL-2 and IFN- γ by activated (HIV-uninfected) PBMCs and mouse Th1 clones while sparing or enhancing IL-4 production by activated PBMCs and Th2 clones (13, 33). The effects of NE on cytokine production in HIV-infected PBMCs are unknown. However, effects on cytokine production could play a role in altered viral replication, since HIV-1 production can be suppressed by several immunoregulatory cytokines (e.g., IL-10, and IL-12) and enhanced by several other proinflammatory cytokines (e.g., TNF- α , IL-6, and IL-1 β) (34–36).

Consistent with the partial suppression of cellular activation by NE, ELISAs of supernatant cytokine concentrations showed a moderate suppressive effect of NE on the production of IL-2 and IL-4 (Fig. 3). The total production of each cytokine over time (area under curve) was reduced by 49% and 64% for IL-2 and IL-4, respectively, with neither suppression reaching statistical significance (*p* = 0.32 and 0.10, respectively). NE similarly suppressed the proinflammatory cytokines IL-1 β (by 48% over time, *p* = 0.017), IL-6 (48%, *p* = 0.008), and TNF- α (41%, *p* = 0.052) (Fig. 3). However, the most pronounced

FIGURE 2. Effect of NE on cellular activation in HIV-1-infected PBMCs. Cell cycle stage was determined by flow cytometric assessment of intracellular DNA (vertical axis) and RNA (horizontal axis). Costimulation increased the fraction of cycling lymphocytes (i.e., those outside the G₀/G_{1A} quadrant that are identified by arresting costimulated cells at G_{1A} with 5 mM *N*-butyrate) from a mean of 0.3% (A) to 65.2% (B). Identical costimulation in the presence of 10 μ M NE reduced the cycling fraction to 44.5% (C) across four experiments (*p* < 0.01).

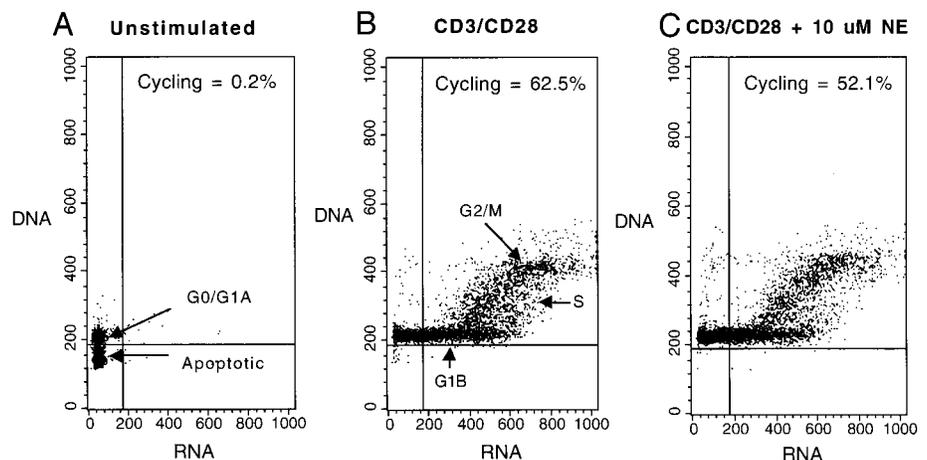
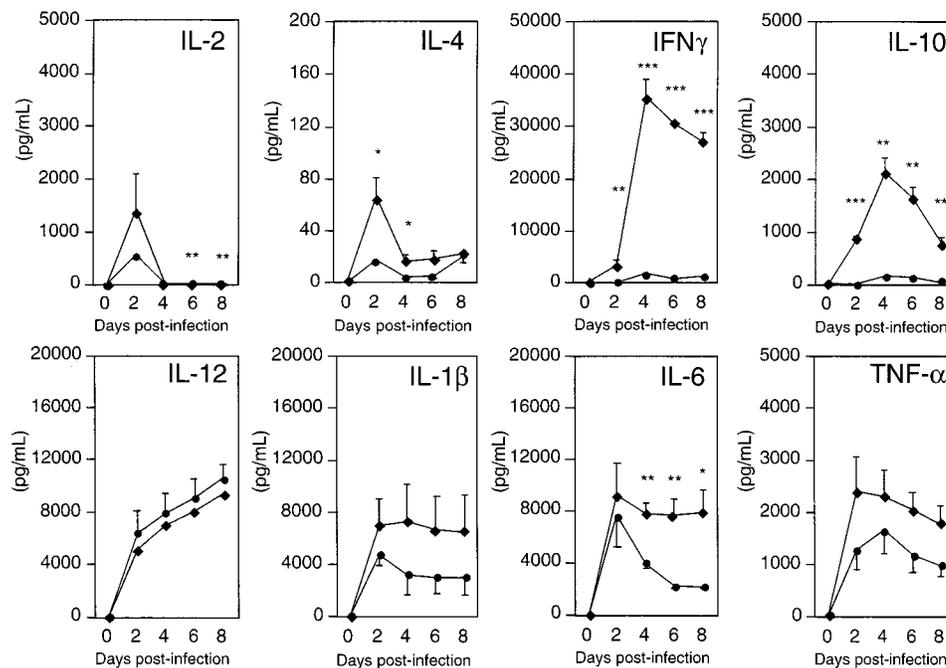


FIGURE 3. Effect of NE on cytokine production. Cytokine levels were determined by ELISA at 0, 2, 4, 6, and 8 days following the infection of quiescent PBMCs and subsequent costimulation in the presence (10 μ M) (●) or absence (◆) of NE. NE suppressed the total production of IFN- γ and IL-10 by 96% ($p = 0.006$) and 94% ($p = 0.014$), respectively. NE moderately suppressed the total production of TNF- α , IL-6, and IL-1 β ($p \lesssim .05$) and did not significantly alter the total production of IL-2, IL-4, or IL-12 (all $p > 0.10$). The statistical significance of comparisons at specific time points is indicated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.



suppressive effects of NE were on IFN- γ and IL-10 (Fig. 3), with the total production of IFN- γ suppressed by 96% ($p = 0.006$) and the total production of IL-10 suppressed by 94% ($p = 0.014$). In contrast to its suppressive effects on other cytokines, NE did not significantly alter the level of IL-12 produced by HIV-infected PBMCs (Fig. 3) ($p = 0.610$).

To determine whether the pronounced effects of NE on IFN- γ and IL-10 production were mediated by activation of the PKA signaling pathway, we examined the ability of the PKA antagonist *rp*-cAMP to abrogate NE-induced cytokine suppression. Preincubation with *rp*-cAMP blocked the NE-induced suppression of IFN- γ by 79% and the NE-induced suppression of IL-10 by 92% across three independent experiments (both $p = 0.008$; data not shown). Concentrations of both cytokines were restored to levels that were statistically indistinguishable from those of costimulated control cultures (both $p > 0.41$). Thus, the suppression of IFN- γ and IL-10 by NE appears to be mediated by the cAMP-PKA signaling pathway.

To determine which cellular sources of IFN- γ were affected by NE, cytokine-producing cells were identified by flow cytometric analysis of intracellular cytokine staining. As shown in Figure 4, NE suppressed IFN- γ production by both CD4⁺ and CD8⁺ lymphocytes (A and B), with 10 μ M NE almost totally abrogating the induction of IFN- γ -producing cells (C–E).

Effects of NE on HIV-1 replication are mediated by IFN- γ and IL-10

To determine whether altered cytokine production mediates the effects of NE on HIV-1 replication, NE-treated cultures were supplemented with either 500 pg/ml of exogenous IFN- γ or 500 pg/ml of exogenous IL-10 (concentrations similar to those found in costimulated control cultures). The restoration of either cytokine alone was sufficient to completely abrogate the effect of NE on p24 levels (Fig. 5), indicating that the acceleration of HIV-1 replication by NE is mediated by the simultaneous suppression of both IFN- γ and IL-10. This effect is specific to IFN- γ and IL-10, in that the addition of either 500 pg/ml of IL-2 or 50 pg/ml of IL-4 to NE-treated cultures did not suppress viral replication (Fig. 5).

Discussion

In the present study, we find that the SNS neuroeffector molecule NE can accelerate HIV-1 replication by up to 11-fold in acutely infected PBMCs. The effect of NE on viral replication is transduced via the β -adrenoreceptor-adenylyl cyclase-cAMP-PKA signaling cascade and is mediated by a pronounced suppression of IFN- γ and IL-10. The present results confirm previous observations that cAMP can increase HIV-1 replication (26, 27) and identify a basis for those effects in the suppression of HIV-limiting cytokines. Consistent with its partial suppression of cellular activation, NE also moderately suppressed the production of several proinflammatory cytokines that are known to increase HIV-1 replication (IL-1 β , IL-6, and TNF- α). However, any replication-retarding effects of reduced cellular activation or proinflammatory cytokine production appear to be offset by the replication-enhancing effects of suppressing IFN- γ and IL-10. The effects of NE on viral replication were dose-dependent over a physiologically relevant range (3, 5), raising the possibility that the activation of SNS neurons innervating lymphoid tissue could conceivably influence HIV disease pathogenesis.

The simultaneous suppression of both IFN- γ and IL-10 was necessary for accelerated HIV-1 replication. Replacement of either cytokine alone abrogated the effects of NE. In humans, both IFN- γ and IL-10 regulate cellular immune responses (37–40). However, altered cellular immunity is not likely to explain the present data, because viral replication differences emerged over periods of time that were too short to support the development of an acquired immune response *in vitro* (41–43). Both IFN- γ and IL-10 are produced at high rates throughout HIV infection (44–48). Both of these cytokines also regulate HIV-1 replication (35, 49–53), although the mechanisms by which they do so remain incompletely understood (54–57). In the present studies, virus production kinetics lagged behind IFN- γ and IL-10 expression kinetics, even though these cytokines were shown to mediate the effects of NE on viral replication. Because the HIV life cycle takes \sim 2 days under optimal conditions, effects on virus production can lag up to two

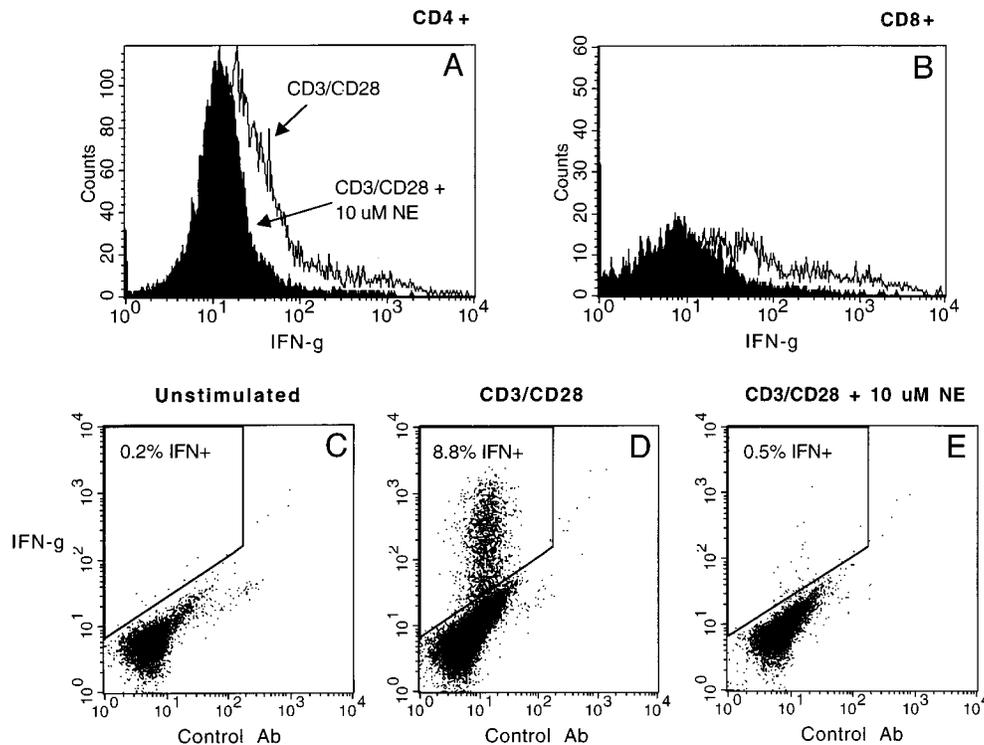


FIGURE 4. Effect of NE on IFN- γ production by CD4⁺ and CD8⁺ cells. Cytokine-producing lymphocytes were identified by intracellular staining with anti-cytokine Abs at 3 days following the infection of quiescent PBMCs and subsequent costimulation. *A*, Relative to costimulated controls (open histogram) 10 μ M NE (solid histogram) significantly reduced the fraction of CD4⁺ lymphocytes expressing IFN- γ . *B*, NE similarly reduced IFN- γ expression in CD8⁺ lymphocytes. Data are representative of three experiments in which NE reduced the fraction of CD4⁺ cells staining positive for IFN- γ by an average of 68% ($p = 0.016$) and reduced the fraction of CD8⁺ cells staining positive for IFN- γ by an average of 66% ($p = 0.023$). Because histogram comparisons can confound alterations in cytokine production with alterations in cell size, we used anti-IFN- γ (FL-2) and an irrelevant, isotype-matched control Ab (FL-1) to spatially separate the effects on cell size from effects on cytokine induction. Relative to unstimulated cells (*C*), costimulation markedly increased IFN- γ expression (*D*). Identical costimulation in the presence 10 μ M NE (*E*) suppressed the fraction of lymphocytes staining positive for IFN- γ by an average of 82% across three experiments ($p = 0.006$). The higher suppression rate in the later analyses stems from the greater number of costimulated cells identified as IFN- γ ⁺; this increased identification is the result of the capacity of two-color analyses to distinguish between small IFN- γ ⁺ cells and nonspecific staining of large cells.

days behind any potential cytokine influences. In addition, cytokine alterations may influence the viral life cycle via downstream events that require further time to develop. The identification of

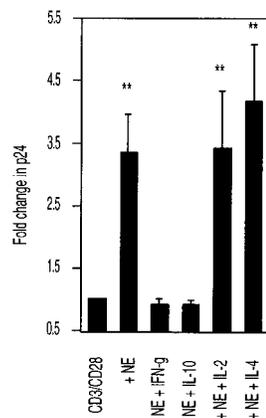


FIGURE 5. Effect of exogenous IL-10 or IFN- γ on NE-induced acceleration in HIV-1 replication. Quiescently infected PBMCs were costimulated in the presence of 10 μ M NE or NE plus the indicated cytokines (500 pg/ml human IFN- γ , IL-10, or IL-2; 50 pg/ml human rIL-4). Data represent the mean fold increase in supernatant p24 levels at 6 days postinfection relative to costimulated controls; the statistical significance of comparisons at specific time points is indicated as ** $p < 0.01$.

such downstream mediators represents an important focus for further research.

The suppression of IFN- γ by NE appears to occur at the level of the cytokine-producing cell itself (primarily CD8⁺ and CD4⁺ T lymphocytes), because the production of IL-12, the major exogenous trigger for IFN- γ production (58–60), was not altered. A direct effect on the cytokine-producing cell is consistent with the presence of a cAMP response element in the IFN- γ promoter (61–63) as well as with the NE-induced suppression of IFN- γ production in isolated murine T cell clones (13). Direct suppressive effects of NE on human IL-10 production have not been demonstrated previously, but the IL-10 promoter does contain a cAMP response element (64). The presence of cAMP-linked β_2 -adrenoreceptors on both CD4⁺ and CD8⁺ T lymphocytes provides a structural basis for direct effects of NE on cytokine-producing cells (8, 13).

The simultaneous suppression of both IFN- γ and IL-10 by NE is consistent with an emerging body of evidence suggesting that the innervation of lymphoid tissue by SNS may function as a natural mechanism for selectively suppressing cellular immune responses while sparing or enhancing other aspects of immune system function (e.g., humoral immune responses) (33, 65–69). As such, analyzing the mechanisms by which the SNS alters lymphocyte function may lead to novel strategies for selectively manipulating immune responses in HIV infection and other disease settings.

Our interest in the effects of SNS activation on the basic HIV life cycle led us to examine a model of CD4⁺ T cell infection in the absence of acquired immune responses (e.g., CD8 antiviral factors or HIV-specific CTLs) (41–43). Basic viral replication rates play a critical role in driving HIV pathogenesis and long-term disease progression (70). The present data indicate that the products of SNS activation can alter HIV-1 replication in activated PBMCs. Such effects underscore the critical role of immunomodulatory factors in altering HIV replication (35) and raise the possibility that the course of HIV could conceivably be affected by factors associated with SNS activation (e.g., physical or psychological stress) (71, 72). Such results also suggest that it may be productive to address immunoregulatory influences from outside the Ag-immune system axis in attempts to suppress HIV replication over the extended periods of time that are necessary for sustained clinical benefit.

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