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Cytokine-stimulated human immunodeficiency virus replication is inhibited by *N*-acetyl-L-cysteine

(thiols/signal transduction/glutathione/tumor necrosis factor α/AIDS)

MARIO ROEDERER, FRANK J. T. STAAL, PAUL A. RAJU, STEPHEN W. ELA, LEONORE A. HERZENBERG, AND LEONARD A. HERZENBERG*

Department of Genetics, Stanford University, Stanford, CA 94305

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ABSTRACT **We show that the stimulation of human immunodeficiency virus (HIV) brought about by tumor necrosis factor α and phorbol 12-myristate 13-acetate can be inhibited by adding *N*-acetyl-L-cysteine (NAC). NAC, which replenishes intracellular glutathione, effectively inhibits the tumor necrosis factor α- or phorbol ester-stimulated replication of HIV in acutely infected cell cultures. NAC also inhibits the cytokine-enhanced HIV long terminal repeat-directed expression of β-galactosidase in *in vitro* HIV model systems. These results show that intracellular thiol levels influence HIV production. Furthermore, because NAC reverses tumor necrosis factor α toxicity both in cells and in animals and is a well-known drug that can be administered orally without known toxicity in humans, these results suggest that NAC is a possible therapeutic agent in AIDS.**

Droge and colleagues (1) have demonstrated that human immunodeficiency virus (HIV)-infected individuals have lower levels of serum acid-soluble thiols and lower levels of intracellular glutathione (GSH) in peripheral blood mononuclear cells (PBMCs). Crystal and colleagues (2) have confirmed and extended these findings by showing that asymptomatic HIV-seropositive individuals have dramatically reduced GSH levels in lung epithelial lining fluid and in blood plasma. Data presented here relate these findings to the regulation of HIV replication by showing that increased intracellular thiol levels block the stimulation of HIV by phorbol 12-myristate 13-acetate (PMA) and tumor necrosis factor α (TNF-α).

Fauci and colleagues (3) have suggested that TNF-α plays a central role in the progression of AIDS. This is consistent with evidence showing that TNF-α levels are abnormally high in serum samples from AIDS patients. These levels rise as opportunistic infections become frequent in patients with AIDS-related complex and AIDS. Furthermore, TNF-α levels are high in all individuals with Communicable Disease Center (CDC) stage IV AIDS (4, 5).

TNF-α exerts some of its toxic effects by stimulating production of reactive oxidative intermediates (ROIs) (6, 7). PMA, which mimics lymphokine activities, also stimulates ROI production (7, 8). Intracellular GSH protects cells by scavenging ROIs; however, the oxidant-buffering capacity of cellular GSH can be overcome by excessive stimulation with TNF-α (9). Thus, drugs that either replenish intracellular GSH or directly scavenge ROIs protect against the toxic effects of TNF-α and other agents that cause oxidative damage.

N-Acetyl-L-cysteine (NAC), which is used clinically to counteract the effects of oxidative stress induced by acetaminophen overdose (10), has been shown to scavenge oxidants directly and to increase intracellular GSH (11, 12). In this report, we show that NAC counteracts the *in vitro* stimulatory effects of TNF-α or PMA on HIV-directed gene expression and inhibits HIV replication in cell lines and PBMC. Thus, we suggest that NAC may be valuable as a therapeutic agent in treating AIDS.

EXPERIMENTAL METHODS

Derivation of an HIV-*lacZ* Reporter Cell Line. The pNAZ construct used here contains the HIV long terminal repeat (LTR) fused to the bacterial β-galactosidase (*lacZ*) gene (Fig. 1), the simian virus 40 promoter fused to the neomycin phosphotransferase gene, and the prokaryotic ampicillin resistance gene. This plasmid was introduced into human embryonic kidney-derived 293S cells by using a calcium phosphate transfection procedure. G418-resistant cells were selected and maintained in G418 at 1 mg/ml (GIBCO). Fluorescence activated cell sorter (FACS)-Gal assays (15) showed a broad distribution of basal expression of HIV-*lacZ* in this population. Clones with different basal expression levels were sorted by a FACS and grown. The clone chosen for further work, 293.27.2, has a relatively high basal expression level of β-galactosidase. Responses to TNF-α and PMA were similar in other clones.

Stimulation of Cells and Measurement of β-Galactosidase Activity. Cells were plated in individual wells of a 96-well Falcon microtiter plate at 2000 per well in 200 μl of Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal calf serum, L-glutamine at 290 μg/ml, penicillin at 100 units/ml, and streptomycin at 70 μg/ml. The next day, PMA (Sigma), recombinant TNF-α (Cetus), or NAC (Aldrich) was added to the desired concentration, and incubations were carried out a variety of times in an atmosphere of 5% CO_2 humidified at 37°C. At the end of the incubation, the medium was aspirated, and the cells were extracted with 120 μl of 0.1% Triton X-100 in Z buffer (60 mM Na_2HPO_4/40 mM NaH_2PO_4/10 mM KCl/1 mM $MgSO_4$, pH 7.0). 4-Methylumbelliferyl β-D-galactoside (MUG) in Z buffer (diluted from 30 mM stock in dimethylformamide) was added to a final concentration of 0.6 mM (30 μl), and the incubations were carried out for 30 min at room temperature. Reactions were stopped by adding 75 μl of Stop buffer (15 mM EDTA/300 mM glycine, pH 11.2). High pH is necessary for optimal fluorescence of 4-methylumbelliferone, hydrolyzed from MUG. Fluorescence was determined in a Fluoroskan micro-

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Abbreviations: GSH, glutathione; HIV, human immunodeficiency virus; LTR, long terminal repeat; NAC, *N*-acetyl-L-cysteine; PBMC, peripheral blood mononuclear cell(s); PMA, phorbol 12-myristate 13-acetate; TNF-α, tumor necrosis factor α; ROI, reactive oxidative intermediates; MUG, 4-methylumbelliferyl β-D-galactoside; AZT, 3′-azido-3′-deoxythymidine; FACS, fluorescence activated cell sorter.

*To whom reprint requests should be addressed.

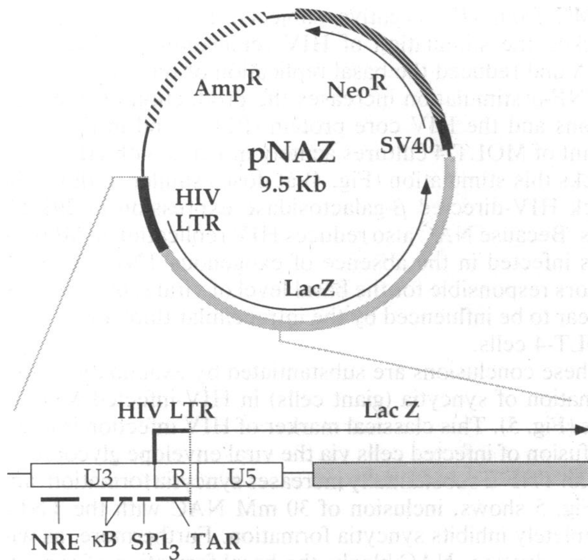

FIG. 1. HIV LTR-*lacZ* reporter gene construct (pNAZ). The HIV LTR derived from the ARV-2 HIV strain (13) contains the transcription enhancer elements, including the negative regulatory element (NRE), two NF-κB-binding sites, and three SP1-binding sites. In addition, it contains the TAR mRNA expression-enhancing element (14) and sequences 3′ up to and including the *Hin*dIII site at nucleotide 534. For selection purposes, pNAZ contains a Simian virus 40 (SV40) promoter/enhancer directing expression of neomycin phosphotransferase, which confers resistance to G418.

titer plate reader (Flow Laboratories). This MUG assay is specific for bacterial galactosidase because the endogenous mammalian galactosidase is inactive at pH 7.0.

Monitoring HIV Infections. MOLT-4 cells were preincubated 45 min with Polybrene, pelleted, and resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum, L-glutamine at 290 μg/ml, penicillin at 100 units/ml, and streptomycin at 70 μg/ml (complete RPMI medium). One million cells per sample were infected with HTLV-III$_B$ at a nominal multiplicity of infection of 3 for 2 hr. Cells were washed twice to remove free virus and cultured in complete RPMI medium with or without TNF-α (10 ng/ml) and with or without 3′-azido-3′-deoxythymidine (AZT) (0.1 μM). Because of the instability of NAC in solution, cells were centrifuged and resuspended in fresh medium supplemented with these molecules daily. Cell number and HIV core protein (P24) level determinations were made daily with ELISA (Abbott); P24, therefore, represents the amount of antigen accumulated during each day. For microscopy, cells were fixed by adding $HgCl_2$ to a final concentration of 1 mM.

For infection of PBMC, cells were isolated from heparinized blood of HIV-negative individuals by Ficoll-Paque density centrifugation and were stimulated for 3 days in RPMI 1640 medium supplemented with 20% fetal calf serum, L-glutamine at 290 μg/ml, penicillin at 100 units/ml, streptomycin at 70 μg/ml, 5% (vol/vol) purified human interleukin 2 (Pharmacia), and phytohemagglutinin at 2 μg/ml. After stimulation, 10^6 cells per sample were infected with HTLV-III$_B$ at nominal multiplicity of infection of 3 and incubated similarly to the MOLT-4 infections.

RESULTS

NAC Blocks PMA and TNF-α Stimulation of HIV LTR-Directed Gene Expression. To study the effects of cytokines, drugs, and other potential modulators on HIV-directed gene expression, we use a cell line, 293.27.2, that has a stably integrated HIV-LacZ fusion construct. In this cell line, the HIV LTR directs expression of the bacterial *lacZ* gene (Fig. 1), which is measured as β-galactosidase activity either in individual viable cells by FACS-Gal or in cell lysates by the MUG fluorometric assay.

Either TNF-α or PMA stimulates β-galactosidase expression directed by the HIV LTR in 293.27.2 cells (Fig. 2). As Fig. 3 shows, these stimulations are inhibited by NAC, an acetylated form of cysteine that scavenges ROIs and supplies cysteine for GSH synthesis *in vivo* (11, 12). A similar antagonism between NAC and TNF-α or PMA occurs in a clone of H9, a $CD4^+$ T-cell line, stably transfected with pNAZ (data summarized in Table 1).

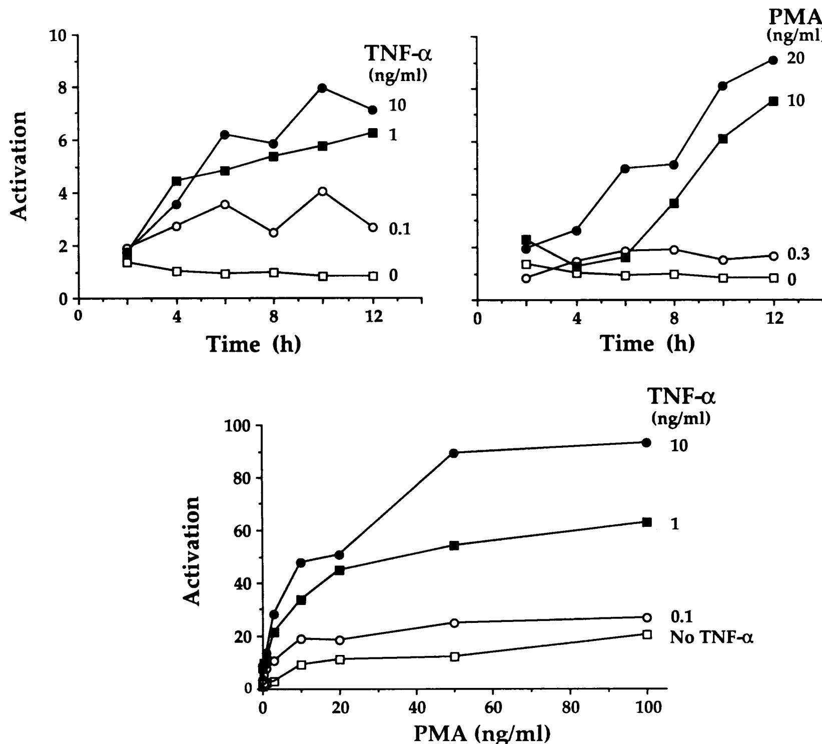

FIG. 2. TNF-α and PMA act synergistically to stimulate the HIV LTR. Plotted is the activation of β-galactosidase activity relative to the unstimulated control (activation = 1). Results from a typical experiment (out of nine) are shown. (*Upper*) Stimulation by incubation with three concentrations of TNF-α or PMA for 8 hr. Concentrations of TNF-α as low as 100 pg/ml or of PMA at 1 ng/ml resulted in stimulation. Magnitude of the observed stimulations agrees with previous data for other cell lines (16, 17). (*Lower*) TNF-α and PMA were added simultaneously to evaluate synergistic activation. The 8-hr coincubation resulted in substantially greater activation than that by either agent alone (note the 10-fold difference in scale between *Upper* and *Lower*).

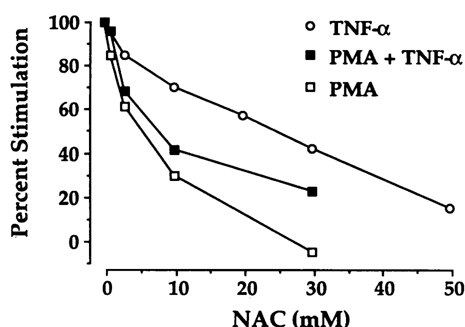

FIG. 3. NAC inhibits the stimulation of HIV LTR by TNF-α and PMA. Assays were done as in Fig. 2 but with the addition of various concentrations of NAC. In this experiment (representative of nine experiments), stimulation with TNF-α (10 ng/ml) and PMA (20 ng/ml) (with or without NAC) was for 8 hr. Preincubation of cells with NAC for an additional 2 hr slightly increased inhibition of stimulation. The PMA-induced activation is markedly more sensitive than the TNF-α-induced stimulation of the HIV LTR to NAC inhibition. Synergistic stimulation is also inhibited effectively by NAC. Note, however, that at 30 mM NAC this inhibition is not as effective as against PMA alone, suggesting that TNF-α stimulation with PMA was not blocked more effectively than without PMA.

High concentrations of NAC (>20 mM) are required to significantly inhibit the TNF-α stimulation. Similarly high concentrations of NAC are required in other systems to protect cells *in vitro* against oxidative stress (18, 19). The inhibition of PMA stimulation, in contrast, occurs at lower levels of NAC—e.g., 1 mM NAC inhibits 15%, and complete inhibition occurs above 10 mM; this may be from a differential sensitivity of the TNF-α and PMA signaling pathways to NAC. Alternatively, it may reflect differences in the rate of oxidant generation by the two stimulants.

Synergy Between TNF-α and PMA. At maximum, the stimulation by TNF-α and PMA together is substantially higher than for either of the factors used individually (Fig. 2). This synergistic activation is effectively blocked by NAC (Fig. 3). These findings demonstrate (*i*) that the mechanism ultimately responsible for viral stimulation is not saturated by either TNF-α or PMA acting alone; (*ii*) that TNF-α and PMA stimulate via at least partially independent pathways; and (*iii*) that NAC inhibits the independent segments of each of these pathways (because the two pathways are differentially sensitive to NAC).

The DNA-binding protein complex NF-κB plays a major role in regulating HIV transcription (16, 20). Furthermore, NF-κB has been shown to be an important factor in the stimulation of HIV by TNF-α and PMA. The NAC inhibition of this stimulation is consistent with the idea that intracellular thiol levels influence the regulation of genes whose expression is modulated by NF-κB or other transcription factors.

NAC Inhibits TNF-α and PMA Stimulation of HIV Replication in T Cells. We examined the influence of NAC on viral replication during acute HIV infection in a transformed $CD4^+$ T cell line (MOLT-4) and in phytohemagglutinin-stimulated PBMC from HIV-negative subjects. In both cases, NAC blocked the stimulation of HIV replication by TNF-α and PMA and reduced the basal replication of the virus.

TNF-α stimulation increases the production of infectious virions and the HIV core protein (P24) found in the supernatant of MOLT-4 cultures acutely infected with HIV. NAC blocks this stimulation (Fig. 4) at doses similar to those that block HIV-directed β-galactosidase expression in 293.27.2 cells. Because NAC also reduces HIV replication in MOLT-4 cells infected in the absence of exogenous TNF-α (Fig. 4), factors responsible for the basal level of viral replication also appear to be influenced by the intracellular thiol levels of the MOLT-4 cells.

These conclusions are substantiated by examination of the formation of syncytia (giant cells) in HIV-infected MOLT-4 cells (Fig. 5). This classical marker of HIV infection is due to the fusion of infected cells via the viral envelope glycoprotein gp120. TNF-α substantially increases syncytia formation, and, as Fig. 5 shows, inclusion of 30 mM NAC with the TNF-α completely inhibits syncytia formation. Furthermore, as with P24 production, NAC blocks the basal formation of syncytia in the absence of TNF-α (data not shown).

NAC also complements the antiviral activity of AZT, a reverse transcriptase inhibitor used widely to combat HIV infection in AIDS patients. At optimal levels, AZT blocks HIV replication in MOLT-4 cells, even with TNF-α (data not shown); however, at suboptimal AZT levels, TNF-α readily stimulates HIV replication, and NAC blocks this stimulation (Fig. 4). These findings suggest that elevated serum levels of TNF-α in AIDS patients may counter the therapeutic effectiveness of AZT. Thus, raising depleted GSH levels in AIDS patients (e.g., with NAC) may provide an effective adjunct to AZT therapy.

NAC Inhibits HIV Replication in PBMC. PMA or PMA plus TNF-α are good stimulators of viral production in PBMC (isolated from HIV-negative individuals, activated with phytohemagglutinin, and then infected with $HTLV-III_B$). Very low concentrations of NAC (<3 mM) completely inhibit these stimulations (Fig. 6). Furthermore, higher concentrations of NAC reduce P24 production in both stimulated and unstimulated cultures—i.e., in cultures infected with the virus in the absence of TNF-α or PMA. Because PMA mimics interleukin-mediated activation of T cells and such stimulation is thought to be responsible for evolution of HIV infection from latency to the symptomatic stages of AIDS, these findings with PBMC suggest that NAC may be broadly effective in maintaining latency or in preventing further progression of disease in a symptomatic individual.

Specificity of the Physiological Effects of NAC. Stimulations by TNF-α and PMA that induce HIV replication have been shown to induce oxidative stress (6–8). We have shown here that HIV stimulation by these agents is strongly inhibited by NAC, a thiol-containing antioxidant that replenishes GSH, the major physiologic antioxidant in cells. Thus, NAC can be thought of as blocking HIV replication by a mechanism that serves generally to block the effects of oxidative stress.

Table 1. Summary of model HIV systems

Model/assay	Cell type	Inhibition by 30 mM NAC: Of TNA-α stimulation, %	Of PMA stimulation, %	Of long-term growth rate*
Transcription and replication after acute infection with HIV/P24 ELISA	PBMC	100	100	None
	MOLT-4 T cells	85	NA	+
LTR-directed β-galactose expression/MUG hydrolysis	293.27.2	55	100	++
	H9 T cells	25	100	None

NA, not applicable (PMA is toxic).
* +, 20–40% reduction in growth rate; ++, ≈50% reduction in growth rate.

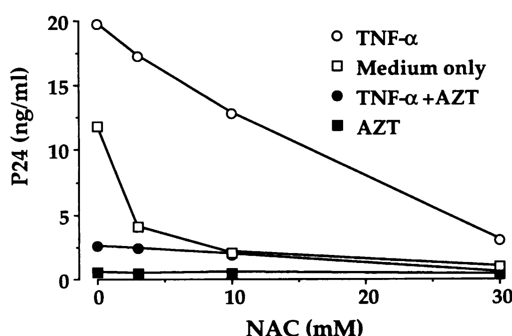

FIG. 4. TNF-α (10 ng/ml) stimulates viral replication in MOLT-4 cells. The amount of P24 synthesized between days 1 and 2 after HIV infection is shown for different culture conditions (AZT, 0.1 μM). At this point, cell counts were identical for all samples, and viability was >95% (as assayed by trypan blue exclusion). The highest concentration of NAC (30 mM) slowed growth of MOLT-4 cells over longer time periods (4–5 days) but did not decrease viability.

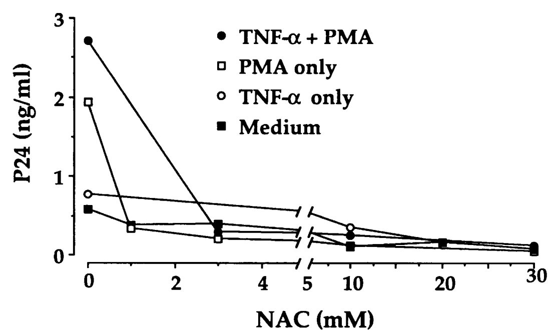

FIG. 6. NAC blocks PMA and TNF-α stimulation of viral replication in PBMC. TNF-α (10 ng/ml) stimulated the rate of viral replication in PBMC as assayed by P24 levels less than 2-fold; PMA (20 ng/ml) stimulated replication ≈4-fold. Note that the stimulation obtained with TNF-α alone is substantially lower than that seen in MOLT-4 cells, perhaps because monocytic cells in PBMC culture are already producing significant levels of TNF-α (21). In a representative experiment (of four experiments), cell viability was >95% (as assayed by trypan blue exclusion), and cell growth was identical at all NAC concentrations for at least 8 days. Shown are the P24 levels on day 5 after infection, representing the amount of P24 synthesized between days 4 and 5.

NAC has pleiotropic effects, however, which complicate the evaluation of its specificity in inhibiting HIV expression. As Table 1 shows, the highest concentration of NAC used in our experiments partially inhibited the growth of some of the transformed cell lines. This inhibition is completely reversed upon removal of NAC and does not cause cell death, as viability is always the same as control cultures (>95%; data not shown). Similarly, culturing HIV-infected MOLT-4 cells with 30 mM NAC significantly reduces P24 production and abolishes syncytia formation before the effects of NAC on growth become detectable. Lower concentrations of NAC (e.g., 10 mM) do not affect growth in any of the cultures but still inhibit HIV (albeit less effectively in some cases). Thus, in these models, NAC is specific in the sense that it affects HIV expression more strongly than it affects cell activation and growth.

The specificity of NAC is unequivocal in PBMC. The growth of activated PBMC is unaffected by 30 mM NAC, a concentration far above that which effectively blocks the PMA-stimulated replication of HIV. Thus, in this most physiological comparison, NAC inhibits HIV replication without hampering growth of HIV-infected cells.

DISCUSSION

TNF-α, which stimulates HIV replication, is also known as cachectin, a molecule at least partially responsible for cachexia or wasting, a condition characterized by anorexia, loss of muscle strength, and dramatic weight loss (22). Continuous low-level administration of TNF-α has been shown to induce cachexia in mice (23) similar to that seen in the terminal stages of AIDS (24). High TNF-α levels are frequently found in AIDS patients, although the relationship between the elevated TNF-α levels and weight loss is unclear (4, 5). In any event, increases in TNF-α levels (4) and decreases in GSH levels (1) clearly correlate with the progression of AIDS.

We suspect that these changes are tightly coupled. That is, the declining GSH levels in AIDS patients may contribute to the increasing TNF-α levels and vice versa because a decline in intracellular GSH increases sensitivity to TNF-α (9), and TNF-α decreases intracellular GSH. Furthermore, because TNF-α production is stimulated by bacterial and parasitic infections, this positive feedback loop between TNF-α and GSH may explain the rapid progression of the disease once opportunistic infections begin.

The decreased GSH levels in asymptomatic patients may explain another well-known aspect of the progression of AIDS. Because the lungs are highly sensitive to oxidative stress (25), the frequent and early appearance of lung disorders in AIDS (26–28) could be due to GSH deficits (2), which reduce protection against oxidants and thereby increase the vulnerability of the lungs. Thus, whether TNF-α plays a primary role in stimulating viral production or merely contributes to AIDS pathology by exacerbating "wasting," there

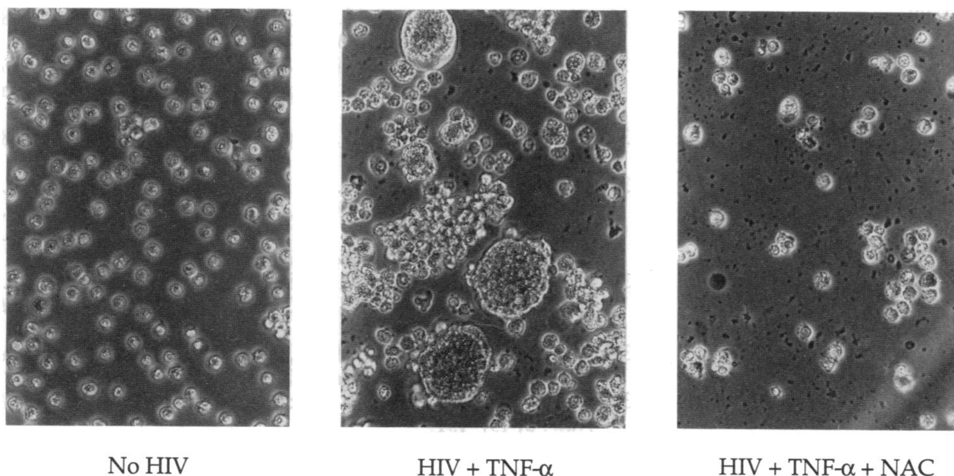

FIG. 5. TNF-α stimulates and NAC inhibits syncytia formation in MOLT-4 cells. MOLT-4 cells were infected as for Fig. 4. Formation of cell aggregates and syncytia (giant cells), mediated by the fusogenic envelope glycoprotein, can be seen in infected cultures. Formation of syncytia was dramatically increased with TNF-α at 10 ng/ml; addition of 30 mM NAC completely blocked their appearance. Apparent cell density differences are from fixation and preparation steps, as cell densities in the cultures were identical.

is good reason to attempt to counter TNF-α toxicity in HIV-infected individuals.

Thiol Therapy for AIDS. NAC may provide a safe and effective defense against detrimental depletions of thiol stores and increases in TNF-α levels. This cysteine derivative has been orally administered for nearly 20 yr in the treatment of pulmonary disorders. Its pharmacokinetics and safety are well documented (see, e.g., refs. 12 and 29–31), and it has been shown to completely block acute TNF-α toxicity *in vivo* in rats (9). Furthermore, NAC is routinely used in humans to overcome the otherwise lethal oxidative stress caused by acetaminophen overdose by increasing depleted GSH levels (10, 12).

Treatment of HIV-infected individuals with NAC may serve both to restore lowered thiol levels to counter the TNF-α–GSH spiral, and, as shown here, to inhibit viral replication stimulated by TNF-α and other cytokines. Such treatment could be valuable for maintaining latency in asymptomatic patients, perhaps in conjunction with more aggressive anti-infectious agent chemotherapies that use pentamidine, AZT, and dideoxyinosine.

Thiols other than NAC have also been shown to strongly inhibit HIV. The cysteine analogue penicillamine completely inhibits HIV replication *in vitro* (32–34). In addition, 2,3-dimercapto-1-propanol inhibits HIV production through inhibition of the HIV transactivating protein, Tat (35). However, although these drugs show strong antiviral activity, their toxicity to humans precludes long-term *in vivo* use.

Several chemicals and drugs that deliver cysteine or GSH to deficient cells *in vivo* (cysteine and GSH pro-drugs) are potentially useful (36); however, all have some disadvantages. L-Cysteine administered parenterally or orally is quickly oxidized to cystine and is toxic in animals (36). GSH itself can be administered as an aerosol but does not enter cells except when degraded (36). Nonetheless, aerosolized GSH and its esters (which can enter cells) have been proposed recently to specifically correct the observed GSH deficiency in lungs in AIDS patients and may be effective for this purpose (2).

In summary, we have demonstrated that NAC blocks the TNF-α stimulation of HIV replication *in vitro* in normal PBMC. In addition, we have shown that NAC blocks viral stimulation by PMA, which mimics the activities of interleukins in leukocyte activation. At a basic level, these findings suggest that ROI production and intracellular thiol levels are important in the regulation of gene expression. Furthermore, at a clinical level, they suggest that NAC may block HIV stimulation *in vivo* by lymphokines produced during leukocyte activation. The question of how well NAC, alone or in combination with other antiretroviral drugs, can inhibit viral production and decrease wasting in AIDS patients could be resolved by an appropriate clinical trial.

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1. Eck, H.-P., Gmunder, H., Hartmann, M., Petzoldt, D., Daniel, V. & Droge, W. (1989) *Biol. Chem. Hoppe-Seyler* **370**, 101–108.
2. Buhl, R., Holroyd, K. J., Mastrangeli, A., Cantin, A. M., Jaffe, H. A., Wells, F. B., Saltini, C. & Crystal, R. G. (1989) *Lancet* **ii**, 1294–1298.
3. Folks, T. M., Clouse, K. A., Justement, J., Rabson, A., Duh, E., Kehrl, J. H. & Fauci, A. S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2365–2368.
4. Lahdevirta, J., Maury, C. P. J., Teppo, A.-M. & Repo, H. (1988) *Am. J. Med.* **85**, 289–291.
5. Mintz, M., Rapaport, R., Oleski, J. M., O'Connor, E. M., Koenigsberger, M. R., Denny, T. & Epstein, L. G. (1989) *Am. J. Dis. Child.* **143**, 771–774.
6. Klebanoff, S. J., Vadas, M. A., Harlan, J. M., Sparks, L. H., Gamble, J. R., Agosti, J. M. & Waltersdorph, A. M. (1986) *J. Immunol.* **136**, 4220–4225.
7. Yoshie, O., Majima, T. & Saito, H. (1989) *J. Leukocyte Biol.* **45**, 10–20.
8. DeChatelet, L. R., Shirley, P. S. & Johnston, R. B. (1976) *Blood* **47**, 545–554.
9. Zimmerman, R. J., Marafino, B. J., Jr., Chan, A., Landre, P. & Winkelhake, J. L. (1989) *J. Immunol.* **142**, 1405–1409.
10. Smilkstein, M. J., Knapp, G. L., Kulig, K. W. & Rumack, B. H. (1988) *N. Engl. J. Med.* **319**, 1557–1562.
11. Aruoma, O. I., Halliwell, B., Hoey, B. M. & Butler, J. (1989) *Free Radical Biol. Med.* **6**, 593–597.
12. Burgunder, J. M., Varriale, A. & Lauterburg, B. H. (1989) *Eur. J. Clin. Pharmacol.* **36**, 127–131.
13. Kao, S.-Y., Calman, A. F., Luciw, P. A. & Peterlin, B. M. (1987) *Nature (London)* **330**, 489–493.
14. Berkhout, B., Silverman, R. H. & Jeang, K.-T. (1989) *Cell* **59**, 273–282.
15. Nolan, G. P., Fiering, S., Nicolas, J.-F. & Herzenberg, L. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2603–2607.
16. Duh, E. J., Maury, W. J., Folks, T. M., Fauci, A. S. & Rabson, A. B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5974–5978.
17. Tong-Starksen, S. E., Luciw, P. A. & Peterlin, B. M. (1989) *J. Immunol.* **142**, 702–707.
18. Moldeus, P., Berggren, M. & Grafstrom, R. (1985) *Eur. J. Respir. Dis.* **66**, Suppl. 139, 123–129.
19. Simon, L. M. & Suttorp, N. (1985) *Eur. J. Respir. Dis.* **66**, Suppl. 139, 132–135.
20. Osborn, L., Kunkel, S. & Nabel, G. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2336–2340.
21. Molina, J.-M., Scadden, D. T., Byrn, R., Dinarello, C. A. & Groopman, J. E. (1989) *J. Clin. Invest.* **84**, 733–737.
22. Beutler, B. & Cerami, A. (1986) *Nature (London)* **320**, 584–588.
23. Oliff, A., Defeo-Jones, D., Boyer, M., Martinez, D., Kiefer, D., Vuocolo, G., Wolfe, A. & Socher, S. H. (1987) *Cell* **50**, 555–563.
24. Chlebowski, R. T. (1985) *Nutr. Cancer* **7**, 85–91.
25. Cantin, A. M., North, S. L., Hubbard, R. C. & Crystal, R. G. (1987) *J. Appl. Physiol.* **63**, 152–157.
26. Chayt, K. J., Harper, M. E., Marselle, L. M., Lewin, E. B., Rose, R. M., Oleske, J. M., Epstein, L. G., Wong-Staal, F. & Gallo, R. C. (1986) *J. Am. Med. Assoc.* **256**, 2356–2359.
27. Suffredini, A. F., Ognibene, F. P., Lack, E. E., Simmons, J. T., Brenner, M., Gill, V. J., Lane, H. C., Fauci, A. S., Parrillo, J. E., Masur, H. & Shelhamer, J. H. (1987) *Ann. Intern. Med.* **107**, 7–13.
28. Guillon, J.-M., Autran, B., Denis, M., Fouret, P., Plata, F., Mayaud, C. M. & Akoun, G. M. (1988) *Chest* **94**, 1264–1270.
29. Moldeus, P., Cotgreave, I. A. & Berggren, M. (1986) *Respiration* **50**, Suppl. 1, 31–42.
30. Olsson, B., Johansson, M., Gabrielsson, J. & Bolme, P. (1988) *Eur. J. Clin. Pharmacol.* **34**, 77–82.
31. Ventresca, G. P., Cicchetti, V. & Ferrari, V. (1989) in *Drugs in Bronchial Mucology*, eds. Braga, P. C. & Allegra, L. (Raven, New York), pp. 77–102.
32. Chandra, A., Demirhan, I., Arya, S. K. & Chandra, P. (1988) *FEBS Lett.* **236**, 282–286.
33. Schulof, R. S., Scheib, R. G., Parenti, D. M., Simon, G. L., DiGioia, R. A., Paxton, H. M., Sztein, M. B., Chandra, P., Courtless, J. W., Taguchi, Y. T., Sun, D. K., Goldstein, A. L. & Sarin, P. S. (1986) *Arzneim.-Forsch.* **36**, 1531–1534.
34. Scheib, R. G., Parenti, D. M., Simon, G. L., Courtless, J. W., Schulof, R. S., Sarin, P. S. & Chandra, P. (1987) *Am. J. Med.* **83**, 608.
35. Kubota, S., El-Farrash, M. A., Maki, M., Harada, S. & Hatanaka, M. (1990) *AIDS Res. Human Retroviruses*, in press.
36. Meister, A., Anderson, M. E. & Hwang, O. (1986) *J. Am. Coll. Nutr.* **5**, 137–151.