Antiviral Research, 22 (1993) 31–43
Elsevier Science Publishers B.V.

AVR 00647

Modulatory effect of *N*-acetyl-L-cysteine on the HIV-1 multiplication in chronically and acutely infected cell lines

Alessandra Pani, M. Elena Marongiu and Paolo La Colla

Dipartimento di Biologia Sperimentale, Sez. Microbiologia, Università di Cagliari, Cagliari, Italy

(Received 30 September 1992; accepted 1 June 1993)

Summary

N-acetyl-L-cysteine (NAC) is known to antagonize the PMA- or cytokine-stimulated HIV-1 replication in latently and acutely infected monocytic and lymphocytic cell lines, and to reduce the virus multiplication in acutely infected, PHA-stimulated PBMC. We here report on the modulatory effects of NAC on the HIV-1 multiplication in both chronically and acutely infected lymphocytes that produce high virus levels independently from cytokine activation. In both cases, NAC doses of 0.12 and 0.25 mM decreased, whereas doses of 0.5–2 mM increased the infectious HIV-1 yield. At these concentrations, the modulatory effect of NAC on the HIV-1 multiplication paralleled that on cell proliferation, suggesting a close correlation between the two phenomena; in fact, under conditions where NAC could not modulate the cell growth, the drug also failed to modulate the HIV-1 multiplication. High NAC concentrations (4–16 mM), which were able to increase the proliferative rate of both chronically infected H9/III$_B$ and normal T lymphocytes, increased up to 6-fold the virus multiplication in H9/III$_B$ cells but were inhibitory to HIV-1 in acutely infected cells. This inhibition was due to the fact that, like dextran sulfate, NAC interfered with an early event in the virus growth cycle. The finding that high NAC doses were also capable of preventing syncytium formation in H9/III$_B$

Correspondence to: A. Pani, Dipartimento di Biologia Sperimentale, Sez. Microbiologia, Università di Cagliari, V.le Regina Margherita 45, Cagliari, Italy. Fax: 070-67-802.

Abbreviations: AZT, azidothymidine; GSE, glutathione ester; GSH, glutathione; IL-6, interleukin 6; LTR, long terminal repeat; NAC, *N*-acetyl-L-cysteine; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; TNFα, tumor necrosis factor.

and C8166 (or MT-4) cocultures further indicated an interference of the drug with receptor-binding-related events.

N-acetyl-L-cysteine; HIV-1; Infected cell lines

Introduction

N-Acetyl-L-cysteine (NAC), is widely used in humans for the treatment of pulmonary disorders (Moldeus et al., 1986; Olsson et al., 1988; Ventresca et al., 1989; Burgunder et al., 1989) and for counteracting the effects of the oxidative stress induced by acetaminophen overdose (Smilkstein et al., 1988). NAC has also been shown effective in blocking the acute toxicity of TNFα in animal models (Zimmerman et al., 1989) and in protecting cells in vitro against oxidative stress (Moldeus et al., 1985; Simon and Suttorp, 1985). All these properties appear to be related to the capability of NAC to directly scavenge oxidants (Auroma et al., 1989) and to increase intracellular GSH levels (Smilkstein et al., 1988; Burgunder et al., 1989).

Recently, other interesting in vitro effects of NAC have been reported (Roederer et al., 1990 and 1991; Kalebic et al., 1991). NAC antagonizes the PMA- and TNFα-induced stimulation of the LTR-directed HIV-1 gene expression in a cell clone; it counteracts both the TNFα- and PMA-induced stimulation of HIV-1 replication in Molt-4 and PBMC, respectively; it appears to have a direct effect on the HIV-1 replication in PHA-stimulated PBMC and, similarly to GSH and GSE, it suppresses the HIV-1 expression induced by TNFα, PMA or IL-6 in latently infected monocytes and T-lymphocytes.

Because of the above effects, and since increases in TNFα (Lahdevirta et al., 1988; Mintz et al., 1989; Folks et al., 1989) and decreases in GSH levels (Eck et al., 1989; Buhl et al., 1989) seem to correlate with the progression of AIDS, NAC has been proposed for the AIDS therapy, alone or in combination with AZT (Roederer et al., 1990; Kalebic et al., 1991).

The purpose of the present work was to investigate in vitro the NAC effects on the HIV-1 multiplication in chronically infected lymphocytes that spontaneously produce high levels of virus, and to define its mode of action in acutely infected T-cell lines.

Materials and Methods

Chemicals

NAC was supplied by Zambon Group. Stock solutions of the drug (640 mM) were prepared in serum-free RPMI 1640 and their pH was adjusted to 7.2 with NaOH; aliquots were made and stored at −20°C. AZT and Dextran sulfate 5000 were purchased from Sigma. Culture media (RPMI 1640, D-MEM) and

foetal calf serum (FCS) were purchased from Gibco. Penicillin G and streptomycin sulphate were purchased from Squibb.

Cells

The following cells were used. H9, a T4 cell line which is permissive to HIV-1 replication but is partially resistant to its cytopathic effect (Popovic et al., 1984); $H9/III_B$, an H9 cell clone which is persistently infected with HIV-1 (Popovic et al., 1984); C8166, a T4 cell line containing an HTLV-I genome of which only the tat gene is expressed (Sodroski et al., 1984); MT-4, a T4 cell line carrying an HTLV-I genome which is readily susceptible to killing by HIV-1 (Harada et al., 1985); Raji, a human lymphoblast-like cell line from a Burkitt lymphoma (ATCC CCL 86); L1210, lymphocytic mouse leukemic cells (ATCC CCL 219); Vero, african green monkey kidney cells (ATCC CRL 1586); HEp-2, epidermoid carcinoma larynx (ATCC CCL 23).

Lymphoid cells were grown in RPMI-1640, whereas Vero and HEp-2 were propagated in D-MEM. Media were supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin and 100 μg/ml streptomycin (growth medium). The cultures were incubated at 37°C in a 5% CO_2 atmosphere and were mycoplasma-negative, as checked by a MycoTect Kit (Gibco).

Virus

HIV-1 ($HTLV/III_B$ strain) was obtained from supernatants of $H9/III_B$ cells collected at the end of an exponential growth phase; aliquots were made and stored at −70°C. The titre of virus stocks was 2×10^5 cell culture infectious doses fifty ($CCID_{50}$)/ml. A high titre HIV-1 stock (2.5×10^6 $CCID_{50}$/ml) was obtained in MT-4 cells which were acutely infected with the above strain at a multiplicity of infection (m.o.i.) of 0.01.

Cytotoxicity and cell proliferation assays

T- and B-cell lines were seeded in flat-bottomed 24-well plates at a density of 0.5 or 1×10^5 cells/ml. The cultures were incubated in growth medium, in the absence or in the presence of various dilutions of test compounds. Cell numbers were determined with a Coulter counter and were corrected for viability, as determined by trypan blue exclusion. Vero and HEp-2 cells were seeded in 6-well plates at a density of 1×10^5 cells/ml in RPMI supplemented with 10% FCS and were allowed to adhere overnight. The cultures were then reincubated in growth medium, in the absence or in the presence of the test compounds. Cell numbers and viability were determined as described above after trypsinization of the monolayers.

Alternatively, the effects of NAC on cell growth were determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Pauwels et al., 1988) in 96-well microtitre plates seeded at a density of 1×10^5 cells/ml.

Antiviral assays

Persistent infection. $H9/III_B$ cells from an exponentially growing culture were extensively washed to remove the previously produced HIV-1. Unless otherwise stated, the cells were seeded in 24-well plates at a density of 1×10^5 cells/ml and incubated in growth medium in the absence or in the presence of the test drugs. After 3 days, both the infectious HIV-1 titre and the number of viable cells were determined.

Acute infection. C8166 or MT-4 cells from exponentially growing cultures were resuspended at a density of 1×10^6 cells/ml and, unless otherwise stated, were infected with HIV-1 at a m.o.i. of 0.01. After a 1 h incubation at 37°C, the unadsorbed virus was removed by washing three times with RPMI, the cells were seeded in 24-well plates at 1×10^5 cells/ml and were incubated in growth medium in the absence or in the presence of the test drugs. After 3 days, the HIV-1 yield was determined by end point titration of supernatants.

HIV-1 titration

Titrations were performed by the standard limiting dilution method (1:2 ratio, 4 replicas per dilution) in C8166 seeded at 1×10^5 cells/ml. After 4 days at 37°C, syncytia were scored under the light-microscope and virus titres were expressed as $CCID_{50}$/ml according to the Reed and Muench method (Lenette and Schmidt, 1964).

Syncytium assay

Extensively washed $H9/III_B$ (1×10^3 cells) were cocultured with uninfected C8166 or MT-4 (1×10^5 cells) in the absence or in the presence of the test drugs. The number of syncytia was determined under the light microscope after 24 h incubation.

Statistical analysis

The unpaired Student's test was used to determine the statistical significance of the mean values obtained in untreated and drug-treated cultures.

Results

Modulatory effects of NAC on HIV-1 multiplication and cell growth in chronically infected lymphocytes

The effects of single NAC treatments on the HIV-1 multiplication and cell growth in chronically infected $H9/III_B$ cells were evaluated by measuring the infectious virus titre and the viable cell number, respectively. The experimental conditions used allowed untreated cultures to undergo exponential growth for three cell cycles and, thus, to give the maximum virus yield.

The results (Fig. 1) indicated that NAC doses of 0.12 and 0.25 mM decreased, whereas doses comprised between 0.5 and 16 mM stimulated the

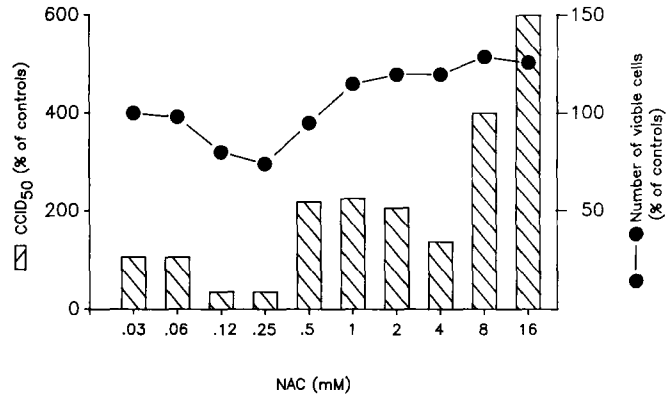

Fig. 1. Effect of NAC on cell growth and HIV-1 multiplication in chronically infected H9/III_B cells. After extensive washing, H9/III_B cells were resuspended at a density of 1×10^5 cells/ml and incubated in the absence or in the presence of serial NAC dilutions. After 72 h, aliquots of the supernatants were collected for virus titration, whereas the rest of the samples was evaluated for viable cell numbers. Virus yield and viable cell numbers in untreated controls were $1.7 \times 10^5 \pm 0.31$ $CCID_{50}$/ml and $8.4 \times 10^5 \pm 0.16$ cells/ml, respectively. Data represent mean values for three separate experiments. $P < 0.005$ for both $CCID_{50}$ and cell number values.

HIV-1 multiplication. The enhancement obtained with 8 and 16 mM NAC was particularly impressive since it led to a 4- and 6-fold increase in virus yield, respectively.

Interestingly, NAC also modulated the proliferation of H9/III_B cells, an effect which paralleled that on HIV-1 multiplication, thus raising the possibility of a close correlation between the two phenomena.

Modulatory effects of NAC on the HIV-1 multiplication in acutely infected lymphocytes and on the growth of mock-infected cells

The effect of single NAC treatments on HIV-1 multiplication in acutely infected C8166 cells was evaluated under conditions of multiple growth cycles of the virus. The NAC effect on cell growth was evaluated in parallel samples of mock-infected cells.

As shown in Fig. 2, NAC doses of 0.12 and 0.25 mM decreased, whereas doses of 0.5–2 mM increased HIV-1 multiplication. Again, the modulatory effect of NAC on the proliferation of mock-infected cells paralleled that on the HIV-1 multiplication.

Since C8166 cells were infected at a low m.o.i., NAC had the chance to modulate the growth of a large proportion of the cells before they became infected. Therefore, it could be postulated that, also in the acute infection, the modulatory effect of 0.12–2 mM NAC on cell proliferation was related to that on HIV-1 multiplication.

Surprisingly, NAC doses comprised between 4 and 16 mM, that were effective in stimulating the growth of mock-infected cells, were found to inhibit HIV-1 multiplication. These results, which were also obtained in acutely

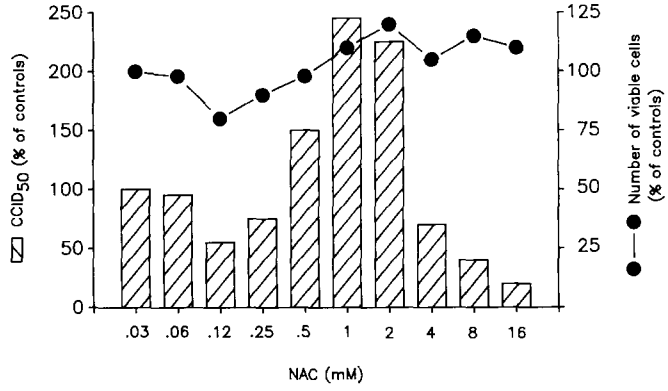

Fig. 2. Effect of NAC on cell growth and HIV-1 multiplication in acutely infected C8166. C8166 cells were infected with HIV-1 at a m.o.i of 0.01 and then resuspended at 1×10^5 cells/ml in the absence or in the presence of serial NAC dilutions. 72 h post-infection, aliquots of the supernatants were collected for virus titration. Duplicate, mock-infected samples of a parallel experiment were evaluated for viable cell numbers. Virus yield and viable cell numbers in untreated controls were $6.2 \times 10^5 \pm 0.25$ $CCID_{50}$/ml and $8.1 \times 10^5 \pm 0.10$ cells/ml, respectively. Data represent mean values for two separate experiments. *P* values ranged between 0.005 and <0.001 for $CCID_{50}$ and <0.005 for cell number values.

infected and mock-infected MT-4 cells (data not shown), contrasted with those obtained in H9/III_B and suggested a direct effect of high NAC doses on early events in the virus growth cycle.

TABLE 1

Influence of various treatment periods with NAC, dextran sulfate (DS) or AZT on their anti-HIV-1 activity in MT4 and H9/III_B cells

Treatment period		[b]$CCID_{50}$/ml (± S.D.)	
[a]Adsorption	Incubation	MT-4	H9/III_B
None	None	$8.0 \times 10^5 \pm 0.27$	$5.3 \times 10^3 \pm 0.34$
NAC 16 mM	None	$1.0 \times 10^5 \pm 0.23$	$1.0 \times 10^4 \pm 0.24$
None	NAC 16 mM	$3.0 \times 10^5 \pm 0.18$	$2.5 \times 10^4 \pm 0.30$
DS 50 μg/ml	None	$8.0 \times 10^3 \pm 0.15$	$4.5 \times 10^3 \pm 0.27$
None	DS 50 μg/ml	$5.4 \times 10^3 \pm 0.30$	$4.8 \times 10^3 \pm 0.29$
AZT 0.1 μg/ml	None	$6.1 \times 10^5 \pm 0.29$	$5.5 \times 10^3 \pm 0.20$
None	AZT 0.1 μg/ml	$<1.0 \times 10^2$	$5.0 \times 10^3 \pm 0.15$

[a]MT-4 cells were infected at a m.o.i of 0.01. Adsorption was at 20°C for 2 h. During this period H9/III_B were resuspended at 1×10^6 cells/ml to simulate an infection.
[b]$CCID_{50}$ (Cell culture infectious dose fifty). Data represent mean value (± S.D.) for two separate experiments.

Influence of various treatment periods with NAC, Dextran sulfate or AZT on their anti-HIV-1 activity

In order to gain more insights on the mode of action of high NAC doses, the effects of different treatment periods with this drug were comparatively evaluated in acutely and chronically infected cells (Table 1). Dextran sulfate and AZT, two drugs targeted at different steps preceding integration and, thus, effective HIV-1 inhibitors in acutely but not in chronically infected cells (Furman et al., 1986; Mitsuya et al., 1985 and 1988), were used as reference compounds.

In MT-4 cells infected at a m.o.i. of 0.01, 16 mM NAC significantly reduced HIV-1 multiplication when present either during or after the adsorption period. Dextran sulfate showed a similar, although more potent inhibitory pattern, whereas AZT was effective only when present after the adsorption period.

These results suggested that, like dextran sulfate (Baba et al., 1988), high NAC doses were effective in interfering with an early step in the HIV-1 growth cycle (see treatment limited to the adsorption period). Moreover, because of the low multiplicity of infection, both compounds reduced the capability of HIV-1 to go through successive growth cycles even when added after the adsorption period, thus explaining the lack of enhancement of HIV-1 multiplication observed with NAC 4–16 mM in acutely infected cells (see Fig. 2).

As expected, none of the compounds inhibited the HIV-1 multiplication in H9/III_B cells, and NAC was the sole drug capable of inducing a significant increase (up to 5-fold) in the virus yield.

Effect of NAC on cell-fusion

Based on the above results, we evaluated the capability of NAC to prevent the formation of syncytia between chronically infected and normal T lymphocytes (Table 2). A 50% inhibition of syncytium formation was observed at 16 mM NAC in H9/III_B-C8166 cocultures, and at 8 mM NAC

TABLE 2

Effect of NAC, dextran sulfate (DS) and AZT on syncytium formation

Compound	% Syncytia at 24 h[a]	
	H9/III_B-C8166	H9/III_B-MT-4
None	100	100
NAC 2 mM	100	100
NAC 4 mM	100	75
NAC 8 mM	100	50
NAC 16 mM	50	0
DS 50 μg/ml	50	0
AZT 0.5 μg/ml	100	100

[a]The inhibitory effect persisted up to 72 h post-infection.

in H9/III_B-MT-4 cocultures. Lower NAC doses were ineffective. Dextran sulfate was also more effective in inhibiting cell fusion in H9/III_B-MT-4 cocultures, whereas AZT was always ineffective.

Since syncytium formation is mediated by a gp-120-CD4 interaction similar to that leading to HIV-1 adsorption to T4 cells (Mitsuya et al., 1988; Moriya et al., 1991), these results strengthened the hypothesis that high NAC doses interfere with a receptor-binding-related event.

Effect of NAC on the HIV-1 multiplication under conditions restrictive for cell growth

In order to substantiate the hypothesis that the modulatory effect of NAC on the HIV-1 multiplication depended on that on cell proliferation, we adopted two different experimental conditions, both restrictive for cell growth (Table 3).

When chronically infected H9/III_B cells were seeded at a high density, i.e., under conditions preventing NAC from modulating their growth, the drug also failed to modulate HIV-1 multiplication.

On the other hand, when C8166 cells were infected at a m.o.i. of 1 (i.e. under conditions resulting in rapid (36 h) cell death), and then were resuspended at a low density, NAC was again unable to modulate HIV-1 multiplication.

Vice versa, when C8166 cells were infected at a m.o.i. of 0.01 and then were seeded at a high density (1×10^6 cells/ml), the virus yield in untreated controls was so low ($< 10^2$ $CCID_{50}$/ml) as to prevent an evaluation of the NAC effects.

Effect of NAC on the growth of lymphoid and non-lymphoid cells

The modulatory effect of NAC on the growth of chronically infected and normal T4 cell lines led us to investigate the effects of the drug on additional lymphoid and non-lymphoid cell lines. To this end, conditions were used allowing untreated controls to undergo exponential growth for three/four cell cycles.

For cytotoxicity evaluation (Table 4), cultures were seeded at 1×10^5 cells/

TABLE 3

Effect of NAC on the HIV-1 multiplication under conditions preventing cell growth

NAC (mM)	[a]$CCID_{50}$/ml (± S.D.)	
	[b]H9/III_B	[c]C8166
None	$1.9 \times 10^4 \pm 0.1$	$2.9 \times 10^5 \pm 0.3$
0.2	$1.8 \times 10^4 \pm 0.3$	$3.1 \times 10^5 \pm 0.2$
2	$2.3 \times 10^4 \pm 0.3$	$2.5 \times 10^5 \pm 0.3$
16	$1.5 \times 10^4 \pm 0.2$	$2.9 \times 10^5 \pm 0.1$

[a]$CCID_{50}$ (Cell culture infectious dose fifty). Data represent mean value (± S.D.) for two separate experiments.

[b]H9/III_B were incubated at 1×10^6 cells/ml and were found at the same density 72 h later, irrespective of the treatment.

[c]C8166 cells, infected at a m.o.i. of 1 were seeded at a density of 1×10^5/ml and then incubated in the absence or in the presence of NAC.

TABLE 4

Cytotoxicity of NAC for different cell lines

	NAC (mM)							
	H9/III$_B$	H9	MT-4	C8166	Raji	L1210	HEp2	Vero
[a]MNTD	37	31	25	19	33	25	17	15
[b]CC_{50}	60	65	55	51	60	58	36	30

[a]MNTD is the maximum NAC concentration (mM) allowing cell growth at control rates for three-four cell cycles.

[b]CC_{50} is the NAC concentration (mM) required to reduce the number of viable cells by 50%.

Data represent mean value for two separate experiments; variability among duplicate samples was less than 10%.

ml, in the absence or in the presence of NAC. After 48 (L1210), 72 (Raji) or 96 h (Vero, HEp-2 and T cell lines), cell number and viability were determined. The results indicated that NAC was cytotoxic for lymphoid cell lines at concentrations higher than 19 mM and that the drug was slightly less cytotoxic for lymphoid than for Vero and HEp-2 cells.

At concentrations below the MNTD (Fig. 3), NAC showed a modulatory

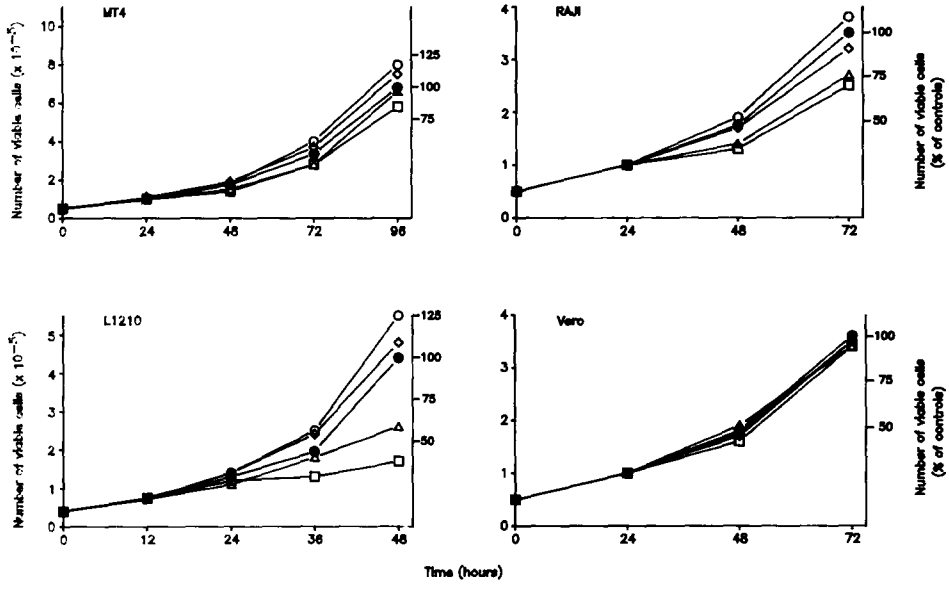

Fig. 3. Modulatory effect of NAC on the growth of lymphoid and non-lymphoid cells. Cultures, seeded at an initial density of 5×10^4 cells/ml, were incubated in the absence or in the presence of serial NAC dilutions. At 24 h intervals (12 h for L1210), the number of viable cells was determined. Untreated controls (closed circles); NAC, 0.12 mM (squares); NAC, 0.25 mM (triangles); NAC 1.0 mM (rhombs); NAC, 2.0 mM (open circles). Variability among triplicate sample was less than 10%. *P* values ranged between 0.01 (MT-4) and 0.001 (L1210).

effect also on the growth of the other lymphoid cell lines tested while leaving unaltered the proliferative rate of both Vero and HEp-2 (not shown) cells. Overall, NAC doses of 0.12 and 0.25 mM decreased, whereas doses of 1 mM and higher stimulated the growth rate of lymphoid cell lines.

Comparable results for both cytotoxic and modulatory effects of NAC on cell growth were obtained with the MTT method (data not shown).

Discussion

In a variety of latently and acutely infected cell lines, NAC inhibits the HIV-1 replication stimulated by PMA or inflammatory cytokines. The antiviral activity of the drug is exerted through the inhibition of the PMA- or cytokine-mediated activation of NF-kB (Staal et al., 1990), a cellular transcriptional factor which also increases the promoter activity of the HIV LTR (Nabel & Baltimore, 1987; Duh et al., 1989; Griffin et al., 1989; Osborn et al., 1989).

In the present study, we have investigated the effects of NAC in both chronically and acutely infected cells which produce high levels of virus independently from the activation by chemical or biological inducers. The results indicate that NAC modulates the HIV-1 multiplication through a modulatory effect on cell growth.

In several lymphoid and non-lymphoid cell lines, CC_{50} and MNTD values were in the range 30–60 mM and 15–37 mM, respectively, and the drug was slightly less cytotoxic for lymphoid than for epithelial and fibroblast cells. Over a wide concentration range, NAC was able to selectively modulate the growth of lymphoid cell lines while leaving unaltered that of fibroblast and epithelial cells: low doses (0.12–0.25 mM) decreased, whereas higher doses (1–16 mM) increased the proliferative rate of T and B lymphocytes. The modulatory effect of NAC on cell growth is consistent with its role as a precursor in the biosynthesis of GSH (Smilkstein et al., 1988; Burgunder et al., 1989); with the demonstration that many of the NAC effects are related to its capability to influence intracellular GSH levels, and with the finding that the latter thiol modulates the proliferation of highly purified T-cells, possibly through a regulation of the DNA synthesis (Suthanthiran et al., 1990).

Under experimental conditions allowing NAC to modulate cell growth (i.e., low cell density), we found that low doses decrease, whereas higher doses increase the infectious HIV-1 yield. The modulatory effect on HIV-1 multiplication parallels that on cell proliferation in the range 0.12–16 mM in chronically infected cells, and in the range 0.12–2 mM in C8166 or MT-4 cells acutely infected at a low m.o.i. In the latter model, NAC doses of 4–16 mM, though capable of stimulating cell growth, were inhibitory to HIV-1. This finding, which contrasted with the considerable increase in HIV-1 yield observed in $H9/III_B$ cells, suggested a direct effect of the drug on early events in the HIV-1 multiplication cycle. As a matter of fact, we demonstrated that, at high doses, NAC behaves like dextran sulfate and interferes with an early step

common to both virus adsorption and syncytium formation. Therefore, what appeared to be an inverse correlation between modulation of cell growth and HIV-1 multiplication (Fig. 2) was, on the contrary, the result of the NAC interference with the HIV-1 progression through successive growth cycles because of the low m.o.i. used.

When the NAC effects were evaluated under conditions apt to prevent modulation of cell growth, either because of the high density of the cultures ($H9/III_B$) or because of the rapid CPE development (C8166 infected at a high m.o.i.), the drug was unable to modulate HIV-1 multiplication. These findings suggest a close correlation between the NAC effects on HIV-1 multiplication and cell proliferation and a common modulatory mechanism.

Overall, these data give a picture of the NAC effects on HIV-1-infected cells which is different from that emerged from previous work. An explanation that could account for this discrepancy may come from the observation that, under the various experimental conditions used, neither PMA nor TNFα were able to enhance the HIV-1 yield (data not shown). This would suggest that our cell lines were expressing high NF-kB levels.

The usefulness of drugs, such as NAC, that interfere with induction of HIV from latency is even more obvious following recent reports indicating the presence of large reservoirs of latently infected lymphocytes and macrophages in secondary lymphoid organs, from early to late stages of the HIV infection (Embreston et al., 1993; Pantaleo et al., 1993).

However, the evidence presented here suggests that, whenever the HIV-1 replication occurs independently from cytokine-induction, the main anti-HIV-1 effect of NAC would be an interference with virus adsorption and syncytium formation. Since this NAC effect, as well as that preventing induction from latency, is neither potent nor very selective, the beneficial effects of NAC in the AIDS therapy should be weighed against the risk of an enhancement of HIV-1 multiplication.

Acknowledgement

This work was supported by grants from Istituto Superiore di Sanità (Progetto AIDS 1991 n. 6206 064), CNR (Progetto FATMA n. 91.00179.PF41) and Zambon Research, Zambon Group, Milano. The authors wish to thank P. Atzei and E. Sais for excellent technical assistance and P. Melis for skillful secretarial help.

References

Auroma, O.I., Halliwell, B., Hoey, B.M. and Butler, J. (1989) The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. Free Radic. Biol. Med. 6, 593–597.

Baba, M., Pauwels, R., Balzarini, J., Arnout, J., Desmyter, J. and De Clercq, E. (1988) Mechanism

of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus in vitro. Proc. Natl. Acad. Sci. USA 85, 6132–6136.

Buhl, R., Holroyd, K.J., Mastrangeli, A., Cantin, A.M., Jaffe, H.A., Wells, F.B., Saltini, C. and Crystal, R.G. (1989) Systemic glutathione deficiency in symptom free HIV-seropositive individuals. Lancet ii, 1294–1298.

Burgunder, J.M., Varriale, A. and Lauterburg, B.H. (1989) Effect of *N*-acetylcysteine and glutathione following paracetamol administration. Eur. J. Clin. Pharmacol. 36, 127–131.

Duh, E.J., Maury, W.J., Folks, T.M., Fauci, A.S. and Rabson, A.B. (1989) Tumor necrosis factor alpha activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF-kappaB sites in the long terminal repeat. Proc. Natl. Acad. Sci. USA 86, 2336.

Eck, H.P., Gmunder, H., Hartmann, M., Petzoldt, D., Daniel, V. and Droge, W. (1989) Low concentration of acid-soluble thiol (cysteine) in the blood plasma of HIV-1 infected patients. Biol. Chem. Hoppe-Seyler 370, 101–108.

Embretson, J., Zupancic, M., Ribas, J.L., Burke, A., Racz, P., Tenner-Racz, K. and Haase, A.T. (1993) Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 362, 359–362.

Folks, T.M., Clouse, K.A., Justement, J., Rabson, A., Duh, E., Kehrl, J.H. and Fauci, A. (1989) Tumor necrosis factor induces expression of human immunodeficiency virus in a chronically infected T-cell clone. Proc. Natl. Acad. Sci. USA 86, 2365–2368.

Furman, P.A., Fyfe, J.A., St.Clair, M.H., Weinhold, K., Rideout J.L., Freeman, G.A., Nusinoff-Lehrman, S., Bolognesi, D.P., Broder, S., Mitsuya, H. and Barry, D.W. (1986) Phosphorylation of 3′-azido-3′-deoxythymidine and selective interaction of the 5′-triphosphate with human immunodeficiency virus reverse transcriptase. Proc. Natl. Acad. Sci. USA 83, 8333–8337.

Griffin, G.E., Leung, K., Folks, T.M., Kunkel, S. and Nabel, G.J. (1989) Activation of HIV gene expression during monocyte differentiation by induction of NF-kappa B. Nature 339, 70.

Harada, S., Koyanagi, Y. and Yamamoto, N. (1985) Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. Science 229, 563–566.

Kalebic, T., Kinter, A., Poli, G., Anderson, M.E., Meister, A. and Fauci, A. (1991) Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester and *N*-acetylcysteine. Proc. Natl. Acad. Sci. USA 88, 986–990.

Lahdevirta, J., Maury, C.P.J., Teppo, A.-M. and Repo, H. (1988) Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am. J. Med. 85, 289–291.

Lenette, E.H. and Schmidt, N.J. (1964) In: Diagnostic Procedures of Virus and Rickettsial Disease. New York, American Public Health Association.

Mintz, M., Rapaport, R., Oleske, J.M., Connor, E.M., Koenigsberger, M.R., Denny, T. and Epstein, L.G. (1989) Elevated serum levels of tumor necrosis factor are associated with progressive encephalopathy in children with Acquired Immunodeficiency Syndrome. Am. J. Dis. Chil. 143, 771–774.

Mitsuya, H., Weinhold, K.J., St.Clair, M.H., Lehrman, S.N., Gallo, R.C., Bolognesi, D., Barry, D.W. and Broder, S. (1985) 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy associated virus in vitro. Proc. Natl. Acad. Sci. USA 82, 7096–7100.

Mitsuya, H., Looney, D.J., Kuno, S., Ueno, R., Wong-Staal, F. and Broder, S. (1988) Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. Science 240, 646–648.

Moldeus, P., Berggren, M. and Grafstrom, R. (1985) *N*-acetylcysteine protection against the toxicity of cigarette smoke and cigarette condensates in various tissues and cells in vivo. Eur. J. Respir. Dis. 66 Suppl. 139, 123–129.

Moldeus, P., Cotgreave, I.A. and Berggren, M. (1986) Lung protection by a thiol-containing antioxidant: *N*-acetylcysteine. Respiration 50 Suppl. 1, 31–42.

Moriya, T., Kurita, H., Matsumoto, K., Otake, T., Mori, H., Morinoto, M., Ueba, N. and Kunita, N. (1991) Potent inhibitory effect of a series of cyclodextrin sulfates (mCDS) on the replication of HIV-1 in vitro. J. Med. Chem. 34, 2301–2304.

Nabel, G. and Baltimore, D. (1987) An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature 326, 711–713.

Olsson, B., Johansson, M., Gabrielsson, J. and Bolme, P. (1988) Pharmacokinetics and bioavailability of reduced and oxidated *N*-acetylcysteine. Eur. J. Clin. Pharmacol. 34, 77–82.

Osborn, L., Kunkel, S. and Nabel, G.J. (1989) Tumor necrosis factor alpha and interleukin I stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. Nature 86, 2336.

Pantaleo, G., Graziosi, C., Montroni, M., Demarest, J.F., Butini, L., Kotler, D.P. and Fauci, A. (1993) HIV infection is active and progressive in lymphoid tissue during the clinical latent stage of disease. Nature 362, 355–358.

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J. and De Clercq, E. (1988) Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods 20, 309–321.

Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. (1984) Detection, isolation and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science 224, 497–500.

Roederer, M., Staal, F.J.T., Raju, P.A., Ela, S.W., Herzenberg, L.A. and Herzenberg, L.A. (1990) Cytokine-stimulated human immunodeficiency virus replication is inhibited by *N*-acetylcysteine. Proc. Natl. Acad. Sci. USA 87, 4884–4888.

Roederer, M., Raju, P.A., Staal, F.J.T., Herzenberg, L.A. and Herzenberg, L.A. (1991) *N*-Acetylcysteine inhibits latent HIV expression in chronically infected cells. AIDS Res. Hum. Retroviruses 7 (6), 563–567.

Simon, L.M. and Suttorp, N. (1985) Lung cell oxidant injury: decrease in oxidant mediated cytoxicity by *N*-acetylcysteine. Eur. J. Respir. Dis. 66, Suppl. 139, 132–135.

Smilkstein, M.J., Knapp, G.L., Kulig, K.W. and Rumack, B.H. (1988) Efficacy of oral *N*-acetylcysteine in the treatments of acetaminophen overdose. N. Engl. J. Med. 319, 1557–1562.

Sodroski, J.G., Rosen, C.A. and Haseltine, W.A. (1984) Trans-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science 225, 381–385.

Staal, F.J.T., Roeder, M., Herzenberg, L.A. and Herzenberg, L.A. (1990) Intracellular thiols regulate activation of nuclear factor kB and transcription of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA 87, 9943.

Suthanthiran, M., Anderson, M.E., Sharma, V.K. and Meister, A. (1990) Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. Proc. Natl. Acad. Sci. USA 87, 3343–3347.

Ventresca, G.P., Cicchetti, V. and Ferrari, V. (1989) In: P.C. Braga and L. Allegra (Eds.), Drugs in Bronchial Mucology, pp. 77–102. Raven Press.

Zimmerman, R.J., Marafino, B.J. Jr., Chan, A., Landre, P. and Winkelhake, J.L. (1989) The role of oxidant injury in tumor cell sensitivity to recombinant human tumor necrosis factor in vivo. J. Immunol. 142, 1405–1409.