Cystamine Potently Suppresses In Vitro HIV Replication in Acutely and Chronically Infected Human Cells

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Abstract

We have investigated the effects of cystamine on the replication of human immunodeficiency virus (HIV) in human lymphocytes and macrophages, the natural targets of HIV in vivo. Treatment of chronically infected macrophages with cystamine, at a concentration (500 μM) that did not show any cytotoxic or cytostatic effects, strongly decreased (> 80%) HIV-p24 antigen production and completely abolished the production of infectious viral particles. Cystamine does not affect viral transcription, translation or protein processing; indeed, all HIV proteins are present in a pattern similar to that of nontreated cells. Instead, cystamine interferes with the orderly assembly of HIV virions, as shown by electron microscopy analysis, that reveals only defective viral particles in treated cells. Moreover, suppression of HIV replication, due to the inhibition of proviral DNA formation was observed in acutely infected lymphocytes and macrophages pretreated with cystamine. These results show that cystamine potently suppresses HIV replication in human cells by contemporaneously blocking at least two independent steps of the viral life cycle, without affecting cell viability, suggesting that this compound may represent a new possibility towards the treatment of HIV-1 infection. (*J. Clin. Invest.* 1994. 93:2251–2257.) Key words: HIV • cystamine • macrophages • lymphocytes

Introduction

A large number of HIV-1-infected patients are now receiving antiretroviral treatment with zidovudine or didanosine (1–3). These compounds work as inhibitors of reverse transcriptase, a viral enzyme acting at an early stage of virus replication (4, 5). This means that such drugs can indeed protect cells that are as yet not infected but are totally inactive against virus replication in cells that are already infected (chronically infected cells). The uselessness of these antivirals on chronically infected cells, together with the development of drug resistance, may in part explain their limited efficacy in patients treated for prolonged periods (6, 7). For these reasons, antiviral drugs able to attack the virus at late stages of its replication cycle (thus potentially active on chronically infected cells) are urgently required to improve the chances of success in the therapy of HIV infection.

It has been reported recently that thiol-group containing compounds such as *N*-acetylcysteine (NAC)[1] and glutathione (GSH) may inhibit a postintegrational stage of the HIV life cycle, and indeed suppress viral replication in chronically infected monocytic cells (8, 9). Due to their ability to scavenge reactive oxidative intermediates (ROI), directly or by replenishing cellular GSH, these compounds inhibit the activity of NFk-B, a transcription factor required for viral gene expression (8–10).

To better explore the role of thiolic compounds on HIV replication, we undertook an in vitro study to evaluate the potential anti-HIV activity of cystamine, a diamine formed by two molecules of cysteamine linked by a disulphide bond. Indeed, cystamine may increase the levels of intracellular GSH (11, 12) and has been shown to act in a nontoxic manner as a scavenger for ROI in rats and mice (13–15). Here we show that cystamine potently inhibits HIV replication in chronically infected human macrophages without affecting cellular functions. Chronically infected macrophages are considered a crucial reservoir of the virus, and one of the major factors responsible for the spreading of HIV throughout the body (16, 17). Thus, inhibition of virus production in these cells represents a major goal in the therapy of HIV related diseases. Interestingly, the inhibition of HIV replication by cystamine in chronically infected cells does not seem to be related to the regulation of gene expression as in the case of NAC and GSH (8, 9), but rather to some interference with the orderly assembly of viral particles.

In addition we found that cystamine may also inhibit HIV replication in de novo infected human lymphocytes and macrophages by restricting the formation of proviral DNA.

The in vitro anti-HIV activity of cystamine in de novo and chronically infected human lymphocytes and macrophages (the natural targets of HIV in vivo) suggests that this substance could be active against viral replication also in vivo.

Methods

Cells. Peripheral blood obtained from healthy HIV-negative donors was enriched for mononuclear cells (PBMC) by centrifugation over Ficoll Hypaque. Mature adherent macrophage populations (called 7 d adherent macrophages) were obtained by incubating 10^6 PBMC/well in 48-well plates (Costar Corp., Cambridge, MA) for 7 d, followed by extensive washing to remove nonadherent cells. Using this method, the yield after removal of the nonadherent cells was 10^5 macrophages per well. Further details of this procedure are described elsewhere (18).

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1. *Abbreviations used in this paper:* MID, minimum infectious dose; NAC, *N*-acetylcysteine; ROI, reactive oxidative intermediates.

Macrophages obtained with this method were < 1% E-rosette positive and > 95% nonspecific esterase positive (Technicon Instruments, Tarrytown, NY).

Lymphocytes were obtained by distributing PBMC into AB-human serum coated T-75 flasks (Falcon, Basel, Switzerland) to a final concentration of 4×10^6 cells/ml of complete medium (containing RPMI-1640, penicillin 100 U/ml, streptomycin 100 μg/ml, L-glutamine 0, 3 mg/ml, and 20% heat inactivated fetal calf serum). After 2 h of incubation in 5% CO_2 at 37°C, nonadherent cells were removed by four gentle washes with phosphate buffered saline (PBS) at 37°C, collected and stimulated with phitohaemoagglutinine (PHA) (5 μg/ml) in T-75 flasks to a final concentration of 2×10^6 cells/ml of complete medium for 3 d in 5% CO_2 at 37°C.

H9, a T4 cell line chronically infected with HIV-1, was used in selected experiments.

Virus. A monocytotropic strain of HIV-1, HTLV-$III_{Ba\text{-}L}$ (gift of Drs. S. Gartner, R. C. Gallo, and M. Popovic, National Cancer Institute), and a lymphocytotropic strain of HIV-1, HTLV-III_B were used. These will be referred to as HIV-$1_{Ba\text{-}L}$ and HTLV-III_B, respectively. Supernatants from infected cultures of fresh macrophages were used as the source of HIV-$1_{Ba\text{-}L}$; these were filtered and stored in liquid nitrogen before use. Titration to determine infectivity was performed in a primary macrophage system as previously described (19). Supernatants of infected H9 cells were used as the source of HTLV-III_B. The minimum infectious dose (MID) of this strain was assessed in the ATH8 cell line (4, 20). Comparable $TCDI_{50}$ of the HTLV-III_B stock were obtained when titered on normal T cells.

Chemicals. Zidovudine was obtained from Wellcome (Pomezia, Italy). Cystamine dihydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO).

Toxicity. Cystamine toxicity in lymphocytes and in H9 cells was evaluated by trypan blue dye exclusion. The effect of cystamine upon viability of uninfected or HIV-infected macrophage cultures was evaluated by counting the number of nuclei (cells were suspended in lysis buffer (10 mM KCl, 2 mM $MgCl_2$, 0.5% Triton X-100 in 10 mM Tris-HCl, pH 7.5) and the nuclei scored in a counting chamber at the phase contrast microscope. Cystamine toxicity upon cellular protein synthesis in macrophages was evaluated as percentage of inhibition of incorporation of [^{35}S]methionine (see below for further details). The effect of cystamine on macrophage function (phagocytosis) was evaluated as the percentage of inhibition of macrophage cells able to phagocyze India ink particles.

Assay of antiviral activity. The anti-HIV efficacy of cystamine was evaluated in macrophages by its addition just after infection (de novo infected cells) or when the infection was already established (chronically infected cells). The assay to evaluate anti-HIV drug efficacy in de novo infected cells has been previously described (18). Briefly, 10^5 7 d adherent macrophages were suspended in complete medium. The cells were challenged with 100 MID/well of HIV-$1_{Ba\text{-}L}$, and incubated at 37°C in a CO_2 incubator. 2 h after viral exposure, the macrophages were extensively washed to remove excess virus, exposed to various concentrations of cystamine and then cultured under the same conditions and drug concentrations as before. Cells were washed and fed every 7 d.

To evaluate drug activity in chronically infected cells, 7 d adherent macrophages were infected as described above and cystamine and AZT were added on day 7 after viral challenge. Cells were then washed and fed every 4 d.

Anti-HIV activity of cystamine in lymphocytes was determined as follows: PHA-stimulated PBMC were washed twice with PBS, counted, distributed into 15-ml polyethylene tubes at a concentration of 6×10^6 PBMC/ml and challenged with 100 MID of HTLV-III_B. The PBMC were incubated in 5% CO_2 at 37°C for 2 h and washed twice in PBS. Then PBMC were inoculated in each well of a 48-well plate, in the presence or the absence of cystamine, at a concentration of 10^6 cells/ml of medium supplemented with 10 U/ml of recombinant Interleukin 2 (IL-2) (Collaborative Research Incorporated, Bedford, MA). Half the volume of supernatant in each well was replaced every 3–4 d.

For the assessment of cystamine activity in H9 T-cells, 2×10^5 cells were seeded in culture tubes (Falcon 2025) in 1 ml of complete medium, then various concentration of cystamine were added to the cultures. Cells were regularly fed every 5 d with fresh medium and replenished with cystamine.

Viral detection. HIV-p24 antigen production in supernatants was assessed at regular time points (see *Results*) by a sandwich ELISA (Abbott, Pomezia, Italy).

HIV infectivity assay. On day 21, the supernatants of control- and cystamine-treated macrophage cultures were collected, filtered, and stored at −70°C. Serially diluted aliquots of each supernatant were then added in quadruplicate to 7 d adherent macrophages. Infection was carried out as described above (see de novo infection). Culture supernatants of these cells were tested for HIV-p24 antigen production 14 d after infection. From these data, the 50% tissue culture infectious dose ($TCID_{50}$) was calculated as previously described (21).

Enzymatic amplification. 7-d adherent macrophages were isolated and purified as described above. Macrophage cultures were challenged with the virus and then incubated without or with 500 μM cystamine as described above (see de novo infection). 24 h after infection, wells were extensively washed, macrophages were detached from the wells, and the DNA was extracted as previously described (22).

After ethidium bromide fluorescent quantitation of the amount of DNA, equivalent amounts of each sample were subjected to 30 cycles of polymerase chain reaction (PCR) amplifications in a total volume of 100 μl using the HIV gag primer pair SK 38/39. The HIV+ control was plasmid DNA (Perkin-Elmer Corp., Norwalk, CT) containing the entire rearranged genome of the HIV-Z6 isolate. Amplified product, a 115-bp sequence in the gag region of the HIV genome was specifically detected by the olygomer-hybridization procedure (23). The SK 19 oligonucleotide probe was end labeled with [^{32}P]adenosine triphosphate as previously described (24). X-ray films of polyacrylamide gels were quantified by analysis with an LKB Ultrascan laser densitometer.

Protein synthesis, immunoprecipitation, and immunoblot analysis. To measure protein synthesis, chronically infected cells either treated or untreated with 500 μM cystamine, were metabolically labeled with [^{35}S]methionine (500 μCi) (Amersham International, Buckingham-Shire, England) in methionine free medium for 5 h. After cell lysis, the radioactivity incorporated into acid-insoluble material was determined (25).

HIV proteins were detected by immunoprecipitation using human serum containing anti-HIV antibodies. Cell lysates containing the same amounts of radioactive material were precipitated with the antibody absorbed to protein A–agarose beads (Pharmacia, Piscataway, NJ). After extensive washing, the precipitates were subjected to SDS-PAGE 10% and the gel was autoradiographed. Autoradiograms were quantitated densitometrically using a laser-beam densitometer (Bio Rad Laboratories, Richmond, CA).

For immunoblot analysis, equal amounts of protein (100 μg) were separated on 10% SDS-PAGE gels and electroblotted to nitrocellulose filters as described (25). For immunostaining of viral proteins, the filters were incubated with human sera containing anti-HIV antibodies.

Electron microscopy analysis. For thin section EM analysis, cystamine-treated and mock-treated HIV-infected cultures were processed as described above. On day 21 after viral challenge, macrophage cells were fixed with 2.5% glutaraldehyde directly in the 48-well plate. Cells were then detached by gentle scraping, collected by low speed centrifugation and postfixed with 1% OsO_4. After dehydration, the specimens were embedded into Epon resin. Thin sections were stained with uranyl acetate and lead nitrate and observed under a Philips electron microscope.

Results

Anti-HIV activity of cystamine in de novo infected cells. In a first set of experiments we evaluated the anti-HIV activity of cystamine in de novo infected macrophages and lymphocytes.

Detectable infection of macrophages and lymphocytes (assessed by HIV-p24 antigen production) was obtained in each experiment performed. HIV was highly cytopathic for cultured lymphocytes therefore these cultures had to be stopped at day 14 after infection. The anti-HIV activity of cystamine was quite similar in lymphocytes and macrophages (Fig. 1). The inhibitory effect by cystamine on p24 antigen production was dose dependent, started at the concentration of 10 μM, and was maximal at 200 μM (Fig. 1). In all of the experiments performed the inhibition of HIV production lasted as long as cystamine was kept in the medium.

Anti-HIV activity of cystamine in chronically infected cells. Macrophages are considered the reservoir of HIV in the body. Indeed, they can be chronically infected with HIV and may produce great amounts of infectious virus for a long time without undergoing cytolysis (16, 26, 27). To assess the ability of cystamine in suppressing HIV production also in chronically infected cells we added it to macrophages once the infection was established. In these experiments we compared the extent of viral inhibition by cystamine with that obtained by AZT, a drug that does not interfere with HIV production in infected cells (4, 5). Cystamine, as opposed to AZT, suppressed HIV replication (as determined by production of HIV-p24 antigen) from chronically infected macrophage cultures (Fig. 2). The inhibitory effect started to manifest itself at 200 μM and was maximal at 500 μM. However, no antiviral activity was seen with concentrations of cystamine below 200 μM and total inhibition of p24 antigen production was never obtained even with a concentration (200 μM) that was completely active on de novo infected cells.

The antiviral activity of cystamine was even stronger in chronically infected T-cells (H9) with respect to macrophage cells (Fig. 3). Indeed, we obtained inhibition of HIV-p24 production > 99% with concentration as low as 200 and 100 μM cystamine (respectively after 5 and 10 d of exposure), and 50% with 50 μM Cystamine. Interestingly, to inhibit virus production on H9 cells are sufficient cystamine concentrations (50 and 100 μM) which are inactive on macrophage cells. These findings could be due to differences in the uptake of cystamine between H9-neoplastic and normal human cells.

It has been reported that interferons may block HIV replication in chronically infected macrophages (28). Macrophages produce interferons and are a major source of these cytokine during any infection (29). Due to these observations, we wondered whether cystamine was acting as an interferon inducer. However, no interferon activity (inhibition of encephalomyocarditis virus-induced cytopathic effect in human foreskin fibroblast) (30) was detected in culture fluid of uninfected or HIV-infected macrophages treated or not with cystamine (not shown).

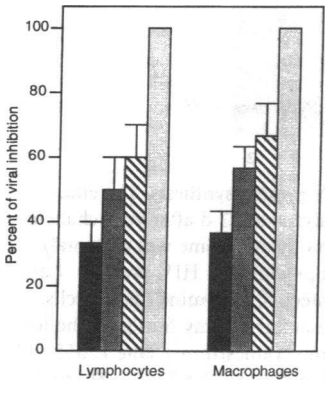

Figure 1. Suppression of p24-antigen production by cystamine in de novo infected lymphocytes and macrophages. (■) Cystamine 10 μM; (■) cystamine 50 μM; (▨) cystamine 100 μM; (▨) cystamine 200 μM. Results are expressed as percent of virus inhibition compared with positive controls. Assessment of virus replication was performed at 21 d after infection in macrophages and at 14 d after infection in lymphocytes. HIV-p24 antigen production in control macrophages (day 21) and lymphocytes (day 14) was 143 (±32) and 47 (±16) ng/ml, respectively. The data represent the averages of four experiments carried out in triplicate. The variability between triplicate results was < 15%.

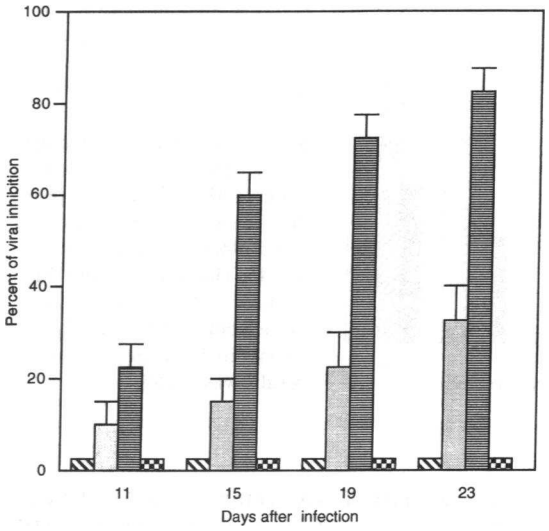

Figure 2. Shut off of viral production in chronically infected macrophages by cystamine. (▨) Cystamine 100 μM; (▨) cystamine 200 μM; (▤) cystamine 500 μM; (■) AZT 10 μM. Cystamine has been added at 7 d after infection. Results are expressed as percent of p24 antigen production compared with positive controls. HIV-p24 antigen production in control macrophages was 131 (±42) ng/ml at day 11, 157 (±38) ng/ml at day 15, 163 (±47) ng/ml at day 19 and 119 (±28) ng/ml at day 23. The data represent the average of six experiments carried out in triplicate. The variability between triplicate results was < 15%.

Toxicity. Toxicity data are reported in Fig. 4. Cystamine is not toxic for cultured cells at the concentration which are active against HIV replication. The ~ 50% toxic doses were 0.5 mM for H9 cells, 2 mM for lymphocytes and uninfected macrophages and 4 mM for chronically infected macrophages. After 14 d of continuous exposure to cystamine 0.5 mM the rate of total protein synthesis (measured as [^{35}S]methionine incorporation) in uninfected or HIV infected macrophages were similar to that of not cystamine-treated controls (1.97×10^6 and 2.1×10^6 CPM/1 μG of protein versus 1.95×10^6 and 1.9×10^6 CPM/1 μG of protein, respectively). Also after 7 d of exposure to cystamine 0.5 mM the percentage of macrophage cells able to phagocyte India ink particles was similar in control and in treated cells (not shown).

Effect of cystamine upon proviral DNA synthesis. PCR analysis was carried out to determine the effect of cystamine upon proviral DNA formation. DNA was extracted from infected cultures 24 h after viral challenge, a period that allows only one cycle of HIV replication (31). Using these experimental conditions, the amount of HIV–DNA amplified by PCR should depend on the uncoating and reverse transcriptase steps, since possible interferences of cystamine with virus binding were excluded by adding the compound after viral challenge (see *Methods*). As shown in Fig. 5, at a concentration of 500 μM,

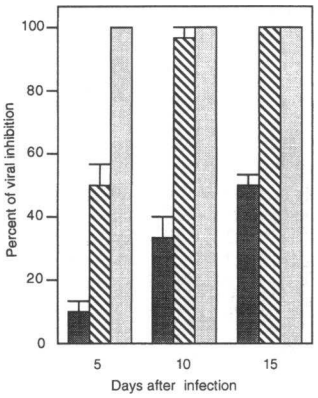

Figure 3. Shut off of viral production in chronically infected H9 cells. (■) Cystamine 50 μM; (▨) cystamine 100 μM; (□) cystamine 200 μM. Results are expressed as percent of p24 antigen production compared to positive controls. HIV-p24 antigen production in control H9 cultures was >300 ng/ml at days 5, 10, and 15. The data represent the average of three experiments carried out in duplicate. The variability between duplicate results was < 15%.

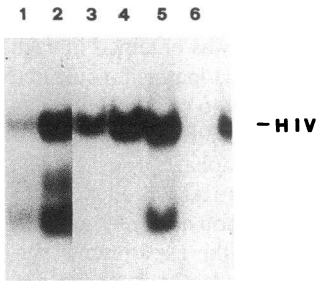

Figure 5. Oligomer-hybridization analysis of HIV gag-amplified (115 bp) products. Lane *1*, amplification of 900 ng of DNA from infected macrophages treated with 500 μM cystamine. Lane *2*, amplification of 900 ng of DNA from nontreated, HIV-infected macrophages. Lanes *3–5*, respectively, 10^2, 10^3, 10^4, copies of the HIV control plasmid diluted in 900 μg of human placental DNA. Lane *6*, amplification of 900 ng of DNA from mock-infected macrophages. Densitometric analysis of this representative x-ray film gave the following results (measured as absorbance values/mm²): lane *1*, 21.141; lane *2*, 89.801; lane *3*, 14.104; lane *4*, 44.343; lane *5*, 119.943; lane *6*, 0.403.

cystamine suppressed HIV–DNA synthesis > 90%. These results indicate that cystamine blocks some early steps of HIV replication.

Effect of cystamine on HIV protein synthesis. The effect of cystamine on the synthesis of viral proteins was studied in chronically infected macrophages. The cells were labeled with [^{35}S]methionine and the immunoreactive proteins were precipitated with a HIV-1 antibody-positive human serum. As determined by scanning of several autoradiographic patterns, identical amounts of p55^{gag}, p24 and p17 viral proteins were detected in untreated as well as in cystamine-treated cells (Fig. 6 *A*), excluding any significant effect of cystamine on viral protein synthesis. Also, these data suggest that cystamine did not inhibit HIV–protease function. Instead, as previously reported, the inhibition of the HIV-1 protease function results in the marked accumulation of HIV-p55^{gag} (its natural substrate) and in the reduction of the p24 and p17 proteolytic fragments (32).

The intracellular levels of viral proteins were analyzed by immunoblot analysis. Greater amounts of viral proteins were detected in cystamine-treated cells with respect to the untreated control (Fig. 6 *B*). This could be due to impaired release of virus particles from cystamine-treated cells.

Electron microscopy analysis. HIV infection of macrophages cultures resulted in the formation of syncytia producing large amounts of viral particles with the typical morphology of HIV. Indeed, viral particles show the triangular shaped inner core and a well formed capside (Fig. 7, *a* and *b*). The addition of cystamine to infected macrophages cultures caused a dramatic change in viral assembly and budding. Indeed, no mature viruses were found inside or outside the cells; instead viral particles lacking the inner core have been detected inside the cells indicating that proper viral assembly could not take place (Fig. 7, *c–e*).

Noninfectious HIV. The titer of infectious HIV in supernatants of cystamine-treated or untreated macrophage cultures was determined at different time points by endpoint dilution. Fig. 8 shows that in control cells, HIV titer from supernatants collected from day 11 to 23 did not change significantly, while it progressively declined to zero in supernatants from cysta-

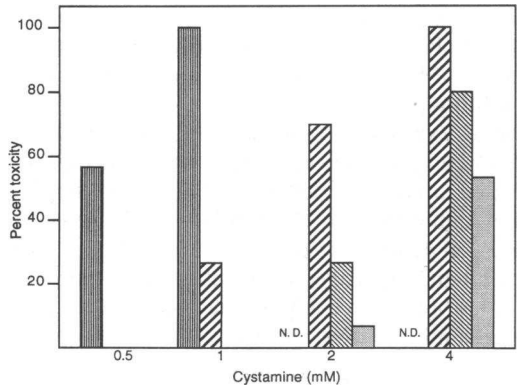

Figure 4. Cystamine toxicity. (▩) H9; (▨) lymphocytes; (▧) uninfected macrophages; (▩) infected macrophages. Cystamine was added at day 0 to H9 cultures, at day 3 to lymphocytes, at day 7 to uninfected macrophages, and at day 14 to chronically infected macrophages. Cells were then continuously exposed to cystamine for 14 d. The toxicity was determined by trypan blue dye exclusion assay in lymphocyte and H9 cultures or by counting the number of nuclei after cell lysis directly in the culture wells in macrophages. The data represent the average of three experiments carried out in triplicate. N.D., not done.

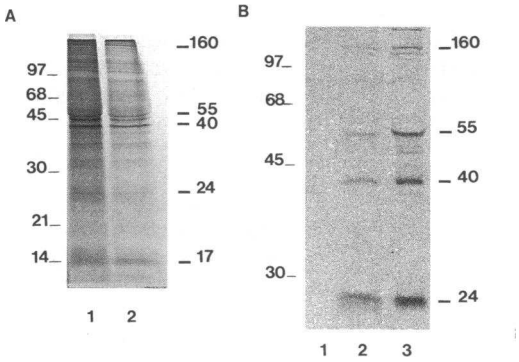

Figure 6. Effect of cystamine on HIV protein synthesis. The effect of cystamine on viral proteins was analyzed at 23 d after viral challenge (when suppression of p24 production by cystamine was maximal). (*A*) SDS-PAGE analysis of immunoprecipitated HIV proteins. Lane *1*, infected, untreated cells; lane *2*, infected, cystamine treated cells. Densitometric analysis of this representative x-ray film gave the following results (measured as absorbance values/mm²): lane *1*, p55 = 3, 09, p24 = 1, 63, p17 = 4, 00; lane *2*, p55 = 3, 10, p24 = 1, 86, p17 = 4, 07. (*B*) Immunoblot analysis of cell lysates. Lane *1*, uninfected control cells; lane *2*, infected, untreated cells; lane *3*, infected, cystamine treated cells. Experiments were repeated three times with identical results.

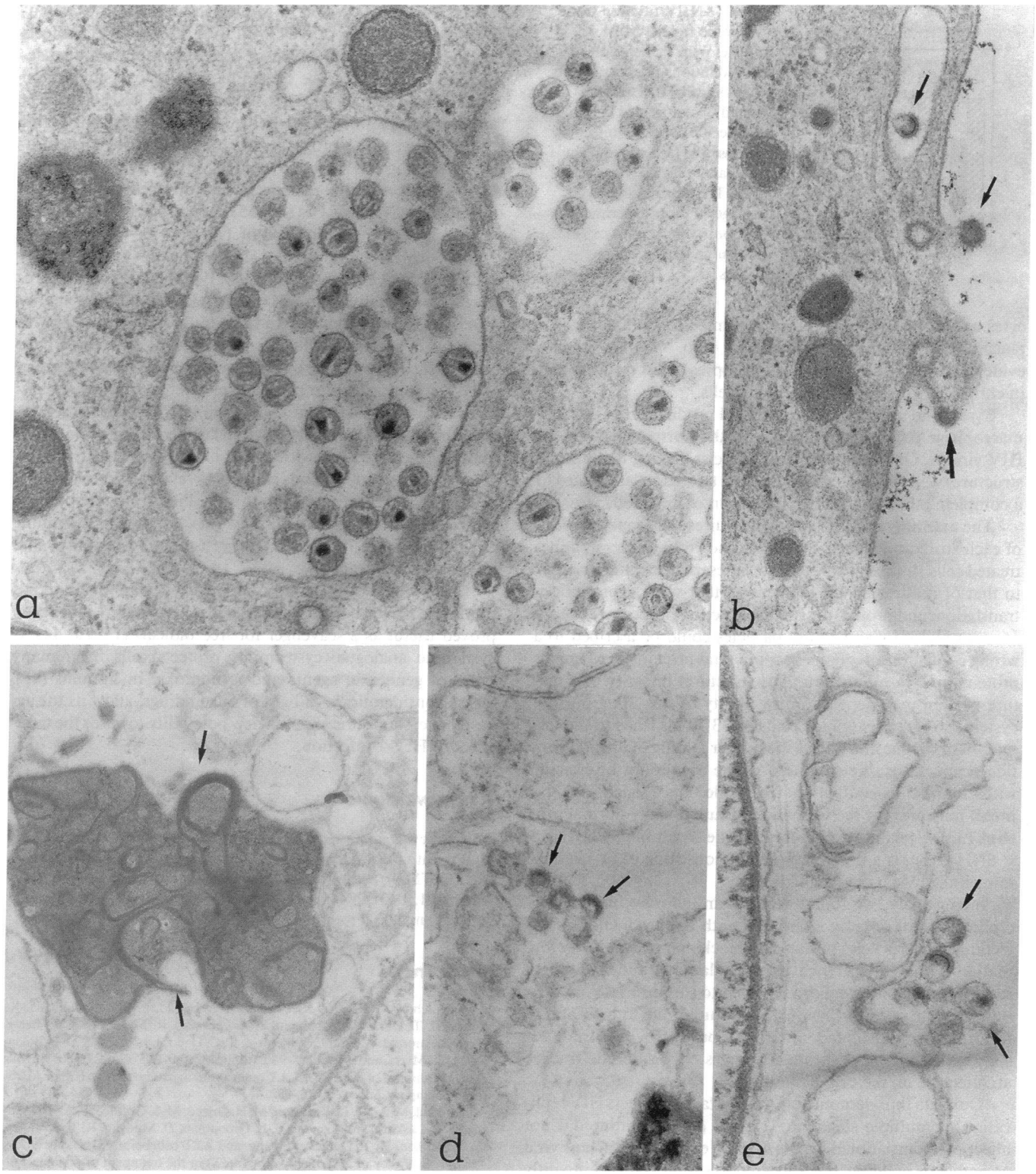

Figure 7. Electron micrographs of infected M/M cultures treated or not with cystamine. (*a* and *b*) Infected M/M 23 d after viral challenge. (*a*) A cytoplasmic vescicle filled with mature virus. (*b*) Efficient intra- and extracellular viral budding (*arrows*). (*c, d,* and *e*) infected M/M after 15 d of exposure to cystamine 500 μM 23 (days after viral challenge). (*c*) Only defective viral particles are found inside the cytoplasmic vescicle. (*d* and *e*) Intra- and extracellular budding of defective virions (*arrows*). (*a* and *c*) ×56,000; (*b* and *d*) ×45,000; (*c*) ×30,000.

mine-treated cultures. The lack of infectious viruses in spite of the residual HIV-p24 antigen found in the supernatants of infected cultures exposed to cystamine (Fig. 2) could be due to the presence of virions that did not properly assemble.

Discussion

In this paper we show that cystamine potently inhibits HIV replication and infectivity in acutely infected human lympho-

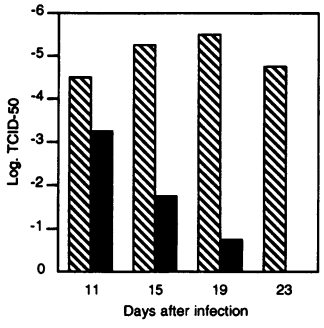

Figure 8. HIV titration from cystamine treated M/M. M/M cultures were challenged with serial dilutions of supernatants of chronically infected M/M treated or not treated with cystamine 500 μM. HIV infection was assessed by a p24 antigen assay and a $TCID_{50}$ was calculated. The data represents the average of three experiments. (-) Control macrophages; (■) cystamine treated macrophages.

cytes and in acutely and chronically infected human macrophages, the natural targets of HIV in vivo. We also provide evidences that cystamine potently suppresses HIV expression in chronically infected, neoplastic T-cells.

Cystamine suppresses chronic viral production in infected macrophage cells by interfering with the normal assembly of HIV virions. Only defective viral particles lacking the internal structures were detectable in cystamine treated cells. This led to a complete inhibition of the production of infectious virus.

The assembly of viral particles requires the proper amount of each structural protein. We have shown that in cystamine-treated cells all capsidic proteins are present in a pattern similar to that of nontreated cells indicating that viral transcription, translation, and protein processing function properly. The lack of assembly of infective viral particles could be ascribed to a wrong configuration of one or more viral proteins as to impair protein–protein interaction. The structural integrity of a mature protein may depend on the ability of free SH groups of cysteine to form disulphide bridges (33). This leads to the formation of cystine and allows the proper folding of the polypeptide chain. Thus, the oxidation of the SH groups of cysteine with compounds containing disulphide bonds (such as cystamine) may prevent the formation of functionally normal proteins (33). Cystamine, (2,2'-dithio-bis[ethylamine], $CH_2H_7NS = SNH_7CH_2$), is a dimeric molecule consisting of two cysteamine linked via a disulphide bond.

Interestingly, we found that cystamine may inhibit HIV replication not only by interfering with the production of infectious viral particles but also by blocking proviral DNA formation. Other thiols, such as penicillamine, and 2,3-dimercapto-l-propanol, have been reported to suppress HIV replication in acute systems (34–36). Such thiols, due to their ability to form mixed disulphide links with proteins, could limit adsorption of the virus through changes on disulphide bridges architecture on the cell membrane.

Our data thus demonstrate that cystamine affects HIV replication at multiple stages. At 25–100 μM cystamine did not affect posttranslational events of HIV expression such as viral assembly or budding, but appeared to suppress predominantly early steps of the viral life cycle. At higher concentrations (200–500 μM), however, cystamine suppressed both early and late steps of HIV replication. Other than a strong and sustained inhibition of HIV replication induced by cystamine, there is an additional reason that makes this therapeutic approach particularly attractive. Recently it has been suggested that tumor necrosis factor-alpha (TNFα) plays a central role in the progression of AIDS (37). This is consistent with evidence showing that TNFα levels are abnormally high in serum from AIDS patients (38, 39). TNFα exerts some of its toxic effects by stimulating the production of ROI (40). Intracellular GSH protects cells by scavenging ROI; however, the oxidant-buffering capacity of cellular GSH can be overcome by excessive stimulation with TNFα (41). Indeed, intracellular levels of GSH have been found decreased in AIDS patients (42, 43); increasing TNFα levels together with a progressive reduction of GSH concentration clearly correlate with the progression of AIDS. Thus, drugs such as cystamine, that either replenish intracellular GSH (12) or directly scavenge ROIs (13–15) may protect HIV-infected patients against the toxic effect of TNFα.

In conclusion, inhibition of HIV replication and infectivity in de novo and chronically infected lymphocytes and macrophages by cystamine can be considered an important step toward a combined therapy. Indeed, multidrug regimens with compounds acting at different stages of viral replication have been advised to improve the chances of success in the therapy of HIV infection and to prevent the emergence of drug-resistant HIV isolates. Cystamine, that inhibits HIV replication interfering with posttranscriptional stages of viral life cycle can be usefully associated with drugs that inhibit RT activity and in general with all compounds active on pretranscriptional stages of HIV cycle. Moreover, due to the ability to contemporary inhibit two independent steps of HIV life cycle, cystamine could limit the emergence of drug-resistant viral strains even if used as monotherapy.

Cystamine may be administered per os in animals and has been shown to be nontoxic in rats and in mice where it has been proved useful as a scavenger for free radicals (13–15, 44). A cystamine analogue, cysteamine, protects cells from many types of genotoxic agents and is nontoxic in humans (45). These considerations, in light of our findings, allow us to suggest that cystamine could be a new possibility towards the treatment of HIV-1 infection.

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