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Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells

(human retrovirus/acquired immune deficiency syndrome/vitamin C/anti-human immunodeficiency virus agent)

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ABSTRACT **We have studied the action of ascorbate (vitamin C) on human immunodeficiency virus type 1 (HIV-1), the etiological agent clinically associated with AIDS. We report the suppression of virus production and cell fusion in HIV-infected T-lymphocytic cell lines grown in the presence of nontoxic concentrations of ascorbate. In chronically infected cells expressing HIV at peak levels, ascorbate reduced the levels of extracellular reverse transcriptase (RT) activity (by >99%) and of p24 antigen (by 90%) in the culture supernatant. Under similar conditions, no detectable inhibitory effects on cell viability, host metabolic activity, and protein synthesis were observed. In freshly infected $CD4^+$ cells, ascorbate inhibited the formation of giant-cell syncytia (by ≈93%). Exposure of cell-free virus to ascorbate at 37°C for 1 day had no effect on its RT activity or syncytium-forming ability. Prolonged exposure of virus (37°C for 4 days) in the presence of ascorbate (100–150 μg/ml) resulted in the drop by a factor of 3–14 in RT activity as compared to a reduction by a factor of 25–172 in extracellular RT released from chronically infected cells. These results indicate that ascorbate mediates an anti-HIV effect by diminishing viral protein production in infected cells and RT stability in extracellular virions.**

Previous studies demonstrated the antiviral activity of ascorbate against a broad spectrum of RNA and DNA viruses *in vitro* (1–4) and *in vivo* (5, 6). It has been claimed that ascorbate inhibited the activation of a latent human retrovirus (human T-cell leukemia virus 1) induced by 5-iodo-2′-deoxyuridine and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (7). However, it was not established whether ascorbate exerted a virus-specific effect or interacted directly with the activating substances. In addition, the effects of ascorbate on acute infection by human retroviruses have not been determined. *In vivo*, oral, and intravenous administration of ascorbate is said to have produced clinical improvements in patients afflicted with influenza, hepatitis, and herpes virus infections, including infectious mononucleosis (5, 6). Clinical improvement was claimed in AIDS patients who voluntarily ingested high doses of ascorbic acid (8).

Because human immunodeficiency virus 1 (HIV-1) is consistently associated with AIDS (9–12), we investigated the action of ascorbate on HIV infection under controlled conditions *in vitro*. Here, we report the effects of ascorbate on acutely and chronically HIV-infected T-lymphocytic cell lines grown continuously in the presence of nontoxic concentrations of the compound. In addition, we report the action of ascorbate on cell-free virus particles *in vitro*.

MATERIALS AND METHODS

Cells and Cell Viability. H9 and H9/HTLV-III_B cells (13) were originally obtained from H. Streicher (National Cancer Institute). In some experiments, batches of the same cell lines provided by M. McGrath (University of California, San Francisco) were also used. Cells were grown in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum, 2 mM L-glutamine, 1 mM pyruvate, and gentamycin at 50 μg/ml. The CD4-positive VB cell line (14) (provided by M. McGrath) was propagated in RPMI 1640 complete growth medium. Cell viability was determined by using the trypan blue exclusion method.

Ascorbate. The stock solution of 0.06 M L-ascorbate was made by dissolving L-ascorbic acid (tissue-culture grade from Sigma) in RPMI 1640 medium and was stored at −20°C.

Experimental Protocol. Fresh working solutions (10× strength) of ascorbate were prepared daily by diluting the stock in complete growth medium. For cytotoxicity assay, 3×10^5 cells were suspended in 0.9 ml of growth medium and seeded in 24-well microtiter plates. Fresh solutions of ascorbate (0.1 ml of 10× strength) were added daily to obtain final concentrations of 10, 25, 50, 75, 100, 150, 200, 300, and 400 μg/ml. The controls received 0.1 ml of growth medium. Plates were incubated at 37°C in a humidified 5% CO_2/95% air atmosphere for various time intervals. At periodic intervals, 0.5 ml of cell suspension was collected and mixed with 50 μl of trypan blue, and cells were tested for viability.

For quantitation of viral and cellular parameters, cell suspensions (in triplicate) were collected, pooled, and centrifuged at 1400 × *g* for 10 min at 4°C. Supernatant was used for assays of extracellular reverse transcriptase (RT) activity and p24 antigen. Cell pellets were used for the determination of cellular metabolic activity and protein synthesis rates.

Assay of RT. Virus particles in supernatant were pelleted by centrifugation in a refrigerated microfuge (13,500 rpm, 2 hr) and then resuspended in 2% of the original volume (30 μl) of TNE buffer (15). Samples (10 μl) were assayed for RT activity, as described by Hoffman *et al.* (15), by using fresh batches of [*methyl*-^{3}H]dTTP (specific activity, ≈80 Ci/mmol; 1 Ci = 37 GBq; NEN/DuPont). RT activity was expressed as the amount of [^{3}H]dTMP incorporated (cpm per 10^6 cells).

Assay of p24. Levels of p24 antigen in supernatant were assayed using the Abbott HIV antigen enzyme immunoassay (16). The p24 value was expressed as ng per 10^6 cells.

Assay of Protein Synthesis. For radiolabeling, H9 cells (3×10^5 cells per well in microtiter plates) were grown in the presence of ascorbate at 0, 75, 100, and 150 μg/ml as described earlier. On days 1, 2, and 4, cells were harvested, washed, and resuspended in methionine- and cysteine-free medium and then incubated at 37°C for 30 min in 0.5 ml of the same medium supplemented with 50 μCi of Tran^{35}S-label (specific activity, 1013 Ci/mmol; ICN). Labeled cells were pelleted, washed in isotonic phosphate-buffered saline, resuspended in lysis buffer containing 1% Nonidet P-40, and

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Abbreviations: HIV-1, human immunodeficiency virus 1; RT, reverse transcriptase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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stored at −70°C. Lysate was thawed and incubated at 100°C for 3–5 min to remove aminoacyl moieties from RNA. Proteins were precipitated with trichloroacetic acid in the presence of bovine serum albumin (0.2 mg/ml), transferred to nitrocellulose filters (0.45 μm), dried, and suspended in β Blend (ICN), and radioactivity was measured in a scintillation counter. Protein synthesis was determined on duplicate samples of cells independently grown in the presence of ^{35}S-labeled amino acids (17).

Metabolic Activity Assayed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Determination. For metabolic activity assay, 3×10^5 cells per well were seeded in 24-well microtiter plates and grown in ascorbate at 0, 75, 100, and 150 μg/ml. On days 1, 2, and 4, cells were pelleted, resuspended in 1.0 ml of growth medium supplemented with 500 μg of MTT (Sigma), incubated for 4 hr, and treated with acidified isopropanol, and the absorbance at 570 nm was measured as described by Mossman (18).

Inhibition Assay for the Cytopathic Effect of HIV-1 Strain HTLV-III$_B$. Infectious HIV stock was obtained from supernatant fluid of H9/HTLV-III$_B$ cells cocultivated with VB cells at a 1:7.5 ratio for 3.5 days. To quantitate syncytium formation, 2.5×10^5 VB cells in 0.4 ml of growth medium were mixed with 0.5 ml of HIV stock and seeded in 24-well microtiter plates. Then 0.1 ml of either growth medium or 10× strength fresh L-ascorbate solution was added daily and the cells were incubated. On specific days after infection, total number of giant cell syncytia in each well were counted at ×100 magnification. A giant cell was defined as a cell >4 diameters larger than a single uninfected cell.

RESULTS

Cytotoxicity of Ascorbate. Before determining the effect on HIV production, we evaluated the cytotoxicity of ascorbate on H9/HTLV-III$_B$ cells, which are T-lymphocytic H9 cells infected with the AIDS virus (13). Ascorbate is unstable in solution as in conventional culture conditions, with a short half-life (4). Therefore, an experimental protocol was adopted in which cell cultures were given daily additions of fresh solutions of ascorbic acid prepared in buffered growth medium (pH 7.3 ± 0.1). Cells were grown in the continuous presence of various ascorbate concentrations (0–400 μg/ml) for a period of 4 days. Viability of control and ascorbate-treated cultures was determined using the trypan blue exclusion test. No toxicity was observed when cultures were grown in the presence of ascorbate at 5–150 μg/ml (Fig. 1). A slight inhibition of cell growth (73–75% survival) was seen on day 4 of incubation in medium containing ascorbate at 200–300 μg/ml. Cytotoxicity became prominent (≥50% cell death) on day 4 at ascorbate concentration of 400 μg/ml and higher. A slight increase in cell number was noted at concentrations ranging from 10 to 400 μg/ml on the first 2 days and at 5–75 μg/ml ascorbate on day 4. Based on these data, further experiments evaluating ascorbate effects on HIV production were carried out at noncytotoxic concentrations of the compound.

Effects of Ascorbate on HIV Released from Chronically Infected Cells. *Extracellular RT activity in supernatant.* We first assayed RT activity in cell-free supernatant (15) harvested from cultures grown in nontoxic ascorbate concentrations (0–150 μg/ml). Fig. 2 shows the average of RT values of ascorbate-treated cultures and controls from three independent experiments. In the controls, RT titer manifested a peak of virus production on day 4. In contrast, ascorbate-treated cultures showed a striking inhibition of RT production. The first noticeable drop (64% inhibition) in RT titer occurred on day 2 with ascorbate at 50 μg/ml, followed by a progressive decline in a dose-responsive manner. Further decreases in RT level were seen with increase in both ascorbate concentration and time of exposure. On day 4, >99% inhibition in RT titer was seen with ascorbate at 150 μg/ml. A noticeable increase in RT titer consistent with stimulation of cell growth was noted at low concentrations of ascorbate (from 5 to 25 μg/ml) on day 2. However, increase in virus production was transient, as these effects did not persist on day 4 of incubation.

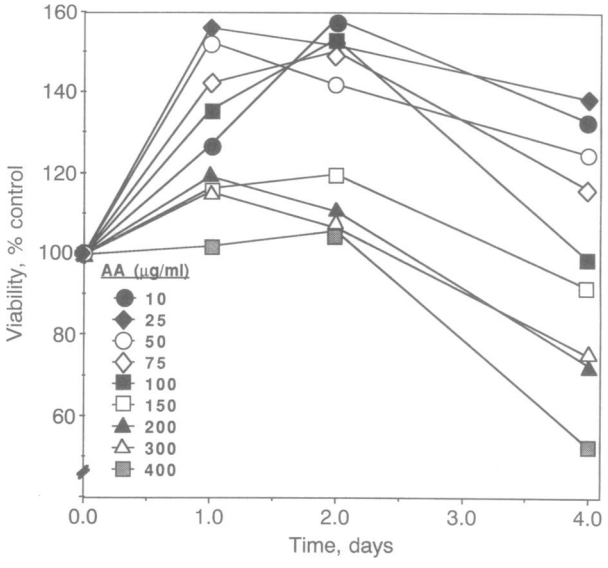

FIG. 1. Analysis of cytotoxicity of ascorbate (AA) for HTLV-III$_B$-infected H9 cells, as determined by trypan blue dye exclusion. Each point is the mean of four cell counts.

p24 levels in supernatant. Another parameter of HIV production is the expression of p24 core antigen. Average values from three experiments are presented in Fig. 3. Control cultures showed a rise in p24 antigen levels at day 2, reaching maximum levels on day 4. In contrast, p24 antigen expression was blocked in ascorbate-treated cultures. Concentrations of ascorbate required to inhibit p24 synthesis were higher than those effective in inhibiting RT production. Thus, the first significant reduction in p24 levels was seen with ascorbate at 150 μg/ml on day 2. Higher declines in p24 values were observed with increased exposure to ascorbate.

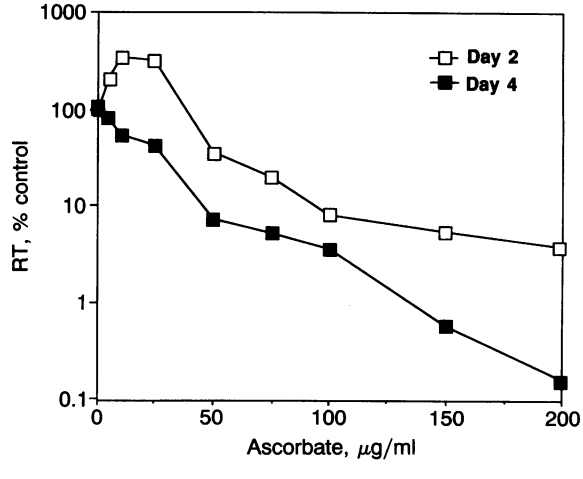

FIG. 2. Effect of ascorbate on RT activity in supernatant harvested from H9/HTLV-III$_B$ cultures. Extracellular RT was assayed as described by Hoffman *et al.* (15). In control samples, the RT values on day 2 and day 4 were, respectively, 55×10^4 and 267×10^4 cpm per 10^6 cells; average background value in blanks (i.e., reactions without enzyme) was 1530 cpm/ml of culture supernatant. In each experiment, the mean of three samples was determined and compared as a percentage of control (taken as 100%).

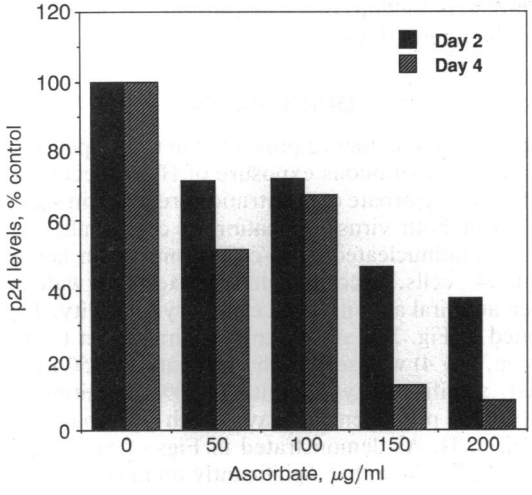

FIG. 3. Effect of ascorbate on HIV p24 antigen levels in supernatant harvested from H9/HTLV-III$_B$ cultures. Extracellular p24 was assayed by Abbott HIV antigen enzyme immunoassay (16). In control samples, the p24 levels on days 2 and 4 were, respectively, 244 and 45 ng per 10^6 cells. The p24 values of ascorbate-treated cultures are compared as a percentage of control.

On day 4, p24 levels in cultures treated with ascorbate at 150 μg/ml were reduced to 13% of the control.

Effect of Ascorbate on Cell Metabolism. We addressed the question of whether ascorbate-induced suppression of RT and p24 production in H9/HTLV-III$_B$ cells was a virus-specific effect or an indirect effect due to inhibition of cellular metabolism or protein synthesis. The metabolic activity of uninfected H9 cells in the presence and absence of ascorbate was determined by using a quantitative colorimetric assay that utilizes the tetrazolium salt MTT (18). MTT is used to measure the activity of various dehydrogenases in viable cells (18, 19). H9 cells grown in the presence of various concentrations of ascorbate (0–150 μg/ml) showed an increase in cellular metabolic activity on day 1 (Fig. 4). This correlated with stimulation of cell proliferation by ascorbate. On days 2 and 4, no significant change in metabolic activity was noted between control cultures and those exposed to ascorbate at 75, 100, and 150 μg/ml.

Effect of Ascorbate on Cellular Protein Synthesis. The effect of ascorbate on cellular protein synthesis was determined by growing uninfected H9 cells for 4 days with ascorbate at 0, 75, 100, and 150 μg/ml (17). On day 1, ascorbate stimulated protein synthesis, consistent with stimulation of metabolic activity and cell growth. On days 2 and 4, the difference in the apparent rates of cellular protein synthesis between ascorbate-treated and control cultures was less than a factor of 2 (Fig. 5). Thus the suppressive effects on HIV production could not be ascribed to a general inhibition of cellular metabolism or protein synthesis.

Effect of Ascorbate on Virus Replication in Freshly Infected Cells. To extend these findings to freshly infected cells, we investigated the effects of ascorbate on acute HIV infection of susceptible CD4$^+$ T lymphocytes. Viral infectivity and cytopathic effect in these cells have been correlated with formation of giant cell syncytia mediated by interaction of HIV envelope glycoprotein with CD4$^+$ cell surface receptor (14, 20, 21). In controls, multinucleated syncytia became visible by day 4 and reached high levels on day 6. The continuous presence of ascorbate caused a dose- and time-dependent decrease in syncytium formation. On day 4, ≈93.3% inhibition in syncytia number was seen with ascorbate at 100 μg/ml (Fig. 6). At this concentration, ascorbate did not inhibit the growth of uninfected VB cells (99% survival by trypan blue dye exclusion), indicating that the

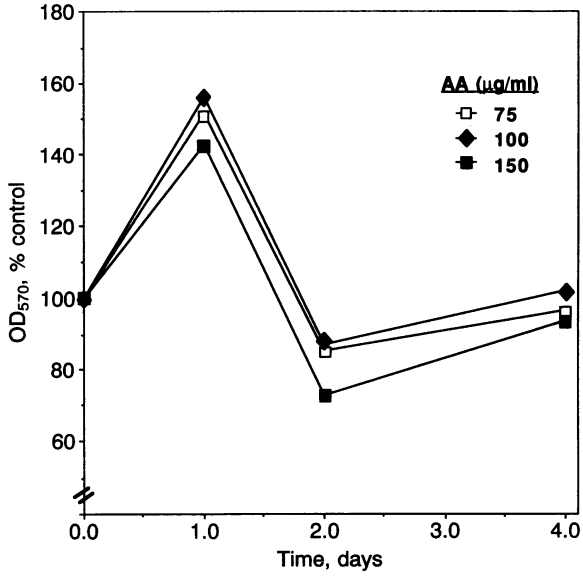

FIG. 4. Analysis of metabolic activity in H9 cells, as determined by MTT assay, in the presence and absence of ascorbate (AA), as described by Mossman (18). Each point is the mean of four OD_{570} readings. Data are plotted as percentage of control.

inhibition of virus replication was not due to cytotoxic effect of the compound.

Direct Inactivation of Virus Particles in Supernatant. We then determined whether decreases in RT titer and syncytium formation were due to direct inactivation of virus particles by ascorbate *in vitro*. Cell-free supernatant containing infectious virus was incubated in the presence and absence of ascorbate at 37°C for 8 and 18 hr. Samples were tested for RT activity and syncytium formation was measured in VB cells. After incubation at 37°C for 18 hr, there was no detectable difference in RT activity between ascorbate-treated virus preparations and controls (Table 1). Syncytium-forming titer of infectious virus of ascorbate-treated and untreated preparations after incubation at 37°C for 1 day was also approximately equal ($2.34–2.70 \times 10^3$ $TCID_{50}$ per ml; where $TCID_{50}$ is the tissue culture 50% infective dose). When chronically infected cells were exposed to ascorbate at 150 μg/ml for 18

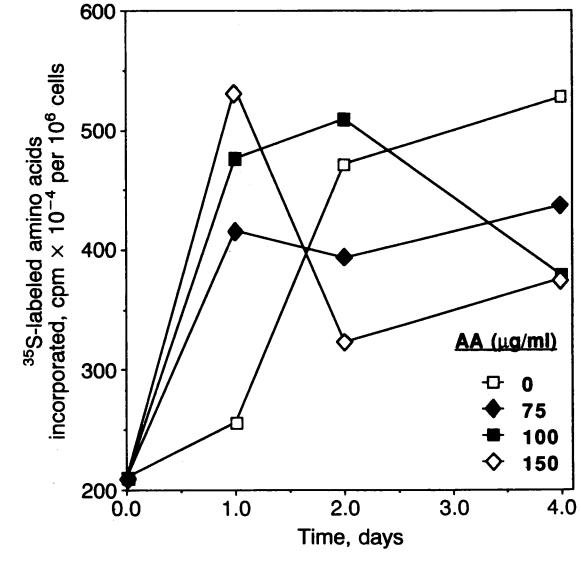

FIG. 5. Determination of protein synthesis rates in H9 cells in the presence and absence of ascorbate (AA). Protein synthesis was assayed as described by Somasundaran and Robinson (17). Each point is the mean of ^{35}S-labeled amino acid incorporation per 10^6 cells.

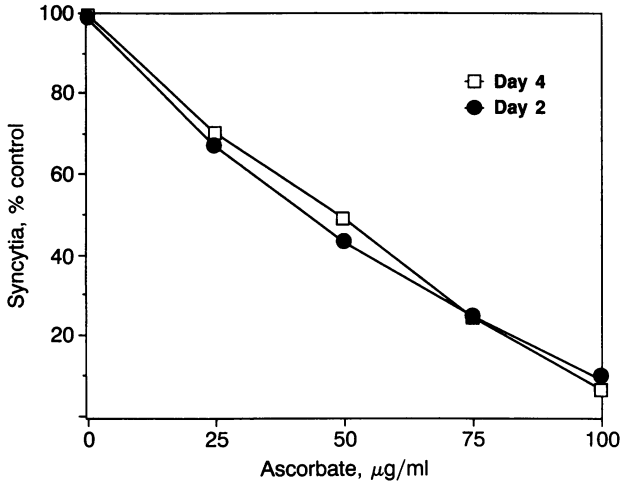

FIG. 6. Dose-dependent decrease in HIV-induced syncytium formation with ascorbate. Syncytia were counted in CD4+ VB cells by using a light microscope. Each point represents the mean of at least four samples and is compared as a percentage of the control infected cultures from the same experiment.

hr at 37°C, the RT titer in culture supernatant was reduced to 11.2% of the control (Table 1). These results indicate that the decrease in extracellular RT titer by ascorbate was not due to direct inactivation of virus after overnight incubation.

To study RT stability in the presence of ascorbate upon prolonged incubation (37°C for several days), the following experiment was carried out. Since thermal inactivation of cell-free virus occurs upon extensive incubation at 37°C, uninfected cells were used to protect virus from heat inactivation. Accordingly, HIV supernatant was mixed with uninfected VB cells and incubated with ascorbate for 4 days, with daily addition of fresh compound. Supernatants were harvested and assayed for RT activity. After 4 days in the presence of ascorbate at 100 and 150 μg/ml, RT activity was reduced, respectively, to 31.5% and 7.0% of control (Table 1). In parallel experiments, chronically infected cells were exposed to ascorbate at 100 and 150 μg/ml for 4 days. The RT levels in supernatant were reduced to 4.0% and 0.6% of control.

After incubation of cell-free virus at 37°C for 4 days with ascorbate at 150 μg/ml, the concentration of p24 protein in the ascorbate-treated preparation (283 ng/ml) was not significantly different from that of the control (263 ng/ml). At the same ascorbate concentration, chronically infected cells exhibited a reduction by a factor of ≈8 in p24 antigen production after 4 days at 37°C (Fig. 3).

DISCUSSION

The experimental evidence presented in this paper has demonstrated that continuous exposure of HIV-infected cells to noncytotoxic ascorbate concentrations resulted in significant inhibition of both virus replication in chronically infected cells and multinucleated giant-cell formation in acutely infected CD4+ cells. Ascorbate dosage exerted a wide margin between antiviral activity and cellular cytotoxicity. Thus, as illustrated in Fig. 2, a significant antiviral effect (>90% RT inhibition, day 4) was seen with ascorbate at ≥50 μg/ml. In contrast, significant cytotoxicity (≥50% inhibition of cell viability) was prominent on day 4 with ascorbate at ≥400 μg/ml (Fig. 1). As demonstrated in Figs. 4 and 5, concentrations of ascorbate that significantly diminished virus titer did not exert inhibitory effects on host metabolic activity and apparent rate of protein synthesis. These findings lend support to a virus-specific action of ascorbate rather than a more general effect on cellular metabolism.

In chronically infected cells, RT activity exhibited a greater sensitivity to ascorbate than p24 antigen expression. This difference can be attributed to the distinct extracellular forms of these two proteins. After intracellular synthesis, a fraction of p24 becomes encapsidated into the virions and some molecules are secreted extracellularly in a free form (16). The p24 antigen capture assay measures both free and virion-bound proteins in the culture supernatant. In contrast, active RT in cell-free supernatant is found only in virion-bound form. Additionally, the quantitative difference between p24 and RT levels may be due to the differential stability of these proteins in the presence of ascorbate. Whereas p24 antigenicity was not lost upon exposure of extracellular virus to ascorbate at 150 μg/ml at 37°C for 4 days, RT activity was reduced by a factor of 14 under similar conditions.

Experiments comparing the kinetics of RT suppression in chronically infected cells with RT stability of cell-free virus provide insight into the mechanism by which ascorbate inhibits HIV. Treatment of chronically infected cells with ascorbate at 150 μg/ml at 37°C for 18 hr reduced RT release to 11.2% of control (Table 1). In contrast, incubation of cell-free virus under the same conditions did not diminish RT activity or syncytium-forming titer of infectious virus. These observations of short-term ascorbate treatment indicate that the compound by itself does not directly inhibit RT activity or functional integrity of envelope glycoprotein involved in syncytium formation (14, 20, 21). The reduction of these viral

Table 1. Analysis of RT stability and RT production in the presence of and absence of ascorbate

			RT activity after incubation at 37°C					
			8 hr		18 hr		4 days	
Virus	Cell source	Ascorbate, μg/ml	cpm × 10^{-4} per 10^6 cells	% control	cpm × 10^{-4} per 10^6 cells	% control	cpm × 10^{-4} per 10^6 cells	% control
HIV	Supernatant	0	6.68	100	6.06	100	ND	ND
		100	7.00	105	6.17	102	ND	ND
		150	7.40	111	6.81	112	ND	ND
HIV-VB	Suspension	0	ND	ND	16.4	100	5.86	100
		100	ND	ND	12.9	78.4	1.85	31.5
		150	ND	ND	7.84	47.8	0.41	6.96
H9/HTLV-III_B	Supernatant	0	79.2	100	56.7	100	267	100
		100	81.9	103	12.7	22.4	10.6	3.96
		150	67.5	85.2	6.33	11.2	1.54	0.58

HIV virus supernatant was prepared from H9/HTLV-III_B cells. Virus supernatant alone or a suspension of supernatant and uninfected VB cells (3×10^5 cells per ml) was exposed to ascorbate at 0, 100, or 150 μg/ml and incubated at 37°C with daily addition of fresh compound. In a parallel experiment, chronically infected H9/HTLV-III_B cells were grown under similar conditions. Supernatants were collected and assayed for RT activity. ND, not done.

parameters in infected cells therefore represent inhibition of a step or steps in HIV replication. Prolonged ascorbate treatment of virus (37°C, 4 days) in the presence of VB cells resulted in a drop in RT activity of a factor of 3–14 with ascorbate at 100 and 150 μg/ml. Under similar conditions, chronically infected cells exhibited a reduction of a factor of 25–172 in extracellular RT activity. These findings are consistent with a combined suppressive action of ascorbate, operating at increasing contact time, on RT production in infected cells and RT stability in extracellular virus particles.

The molecular mechanism by which ascorbate suppresses HIV is not yet fully understood. In earlier studies, ascorbic acid caused degradation of single- and double-stranded genomes of RNA and DNA phages (22–24). Site-specific cleavage of phage DNA occurring at unique sites due to redox reactions involving copper and ascorbate was reported (25). Hydroxyl radicals ($OH^{\cdot}$) generated from hydrogen peroxide were implicated as the reactive species mediating scission of nucleic acid (24–26). In lymphocytes, ascorbate is present in unusually high concentration, as much as 50 times the blood plasma level and >10 times that in nonlymphoid cells (27, 28). Therefore, one possible mechanism of ascorbate action on infected lymphocytic cells is that free unintegrated viral DNA or newly synthesized viral RNA formed during each cycle of HIV replication becomes susceptible to ascorbate-mediated damage, resulting in reduced viral protein production. Alternatively, ascorbate may suppress HIV production by inhibiting the activity of viral enzymes involved in protein processing (e.g., HIV protease). Ascorbate was shown to inhibit the activity of proteolytic enzymes in fish (29). Once HIV components are packaged within the virion, they may become resistant to ascorbate inactivation. However, upon prolonged *in vitro* exposure, virion components may become susceptible to further attack by metabolites of ascorbate generated from its oxidative degradation. Autooxidation of ascorbic acid is associated with the formation of highly reactive breakdown products including furan-type compounds that form adducts with amino and hydroxyl groups of proteins resulting in site-specific cleavage or cross-linking of protein (30–32). Such protein modifications could contribute to inactivation of virion-associated enzyme detected upon prolonged incubation of virus *in vitro*. Further studies of the physical state of HIV nucleic acids and proteins in ascorbate-treated cells and virions could provide insight into its mechanism of action.

Inhibitors of RT activity have been the focus of intensive investigation for the design and development of antiretroviral agents. Among these, 3′-azido-3′-deoxythymidine (AZT) (33), the first drug approved for AIDS treatment, blocks *de novo* HIV infection effectively but was shown not to inhibit virus production in cells containing integrated HIV genomes (34). In the same study, interferon-α inhibited the budding and release of HIV from chronically infected cells but did not suppress intracellular production of viral proteins. The ability of ascorbate to inhibit acute HIV infection and to suppress RT levels in chronically infected cells indicates that the compound acts at a different stage in the HIV life cycle and may, thereby, provide a rationale for developing more effective combination therapy with other anti-HIV agents.

The concentrations at which the anti-HIV effect of ascorbate was seen in this *in vitro* study are physiologically attainable in human blood plasma. For instance, in a clinical trial on terminal cancer patients, E. Cameron (personal communication) showed that oral administration of 10 g of ascorbate resulted in mean plasma ascorbate levels of 28.91 μg/ml (range 17.2–63.6 g). B. Jaffe (personal communication), who is using ascorbate for the treatment of AIDS patients, indicated that ascorbate at 93 ± 29 μg/ml was attained in plasma in people consuming oral ascorbate to achieve urinary levels >1 mg/ml. These findings are consistent with a high bowel tolerance reported for AIDS patients (8). Intravenous infusion of 50 g of ascorbate a day resulted in peak plasma levels of 796 ± 111 μg/ml.

Note Added in Proof. Roederer *et al.* (35) reported inhibition of cytokine-stimulated HIV replication by *N*-acetyl-L-cysteine, another reducing agent like ascorbate.

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1. Murata, A., Kitagawa, H., Inmaru, H. & Saruna, R. (1972) *Agric. Biol. Chem.* **36**, 1065–1067.
2. Murata, A., Kitagawa, H., Inmaru, H. & Saruna, R. (1972) *Agric. Biol. Chem.* **36**, 2597–2599.
3. Schwerdt, P. R. & Schwerdt, C. E. (1975) *Proc. Soc. Exp. Biol. Med.* **148**, 1237–1243.
4. Bissell, M. J., Hatie, C., Farson, D. A., Schwarz, R. I. & Soo, W-J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2711–2715.
5. Klenner, F. R. (1971) *J. Appl. Nutr.* **23**, 61–87.
6. Cathcart, R. F. (1983) *Biol. Med. (Stockholm)* **3**, 6–8.
7. Blakeslee, J. R., Yamamoto, N. & Hinuma, Y. (1985) *Cancer Res.* **45**, 3471–3476.
8. Cathcart, R. (1984) *Med. Hypotheses* **14**, 423–433.
9. Barré-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220**, 868–870.
10. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markham, P. E. (1984) *Science* **224**, 500–503.
11. Levy, J. A., Hoffman, A. D., Kramer, S. M., Landis, J. A., Shimabukuro, J. M. & Oshiro, L. S. (1984) *Science* **225**, 840–842.
12. Blattner, W., Gallo, R. C. & Temin, H. M. (1988) *Science* **241**, 515–516.
13. Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. (1984) *Science* **224**, 497–500.
14. Lifson, J. D., Reyes, G. R., McGrath, M. S., Stein, B. S. & Engleman, E. G. (1986) *Science* **232**, 1123–1127.
15. Hoffman, A. D., Banapour, B. & Levy, J. A. (1985) *Virology* **147**, 326–335.
16. Goudsmit, J., Paul, D. A., Lange, J. M. A., Speelman, H., Van Der Noorda, J., Van Der Helm, H. J., de Wolf, F., Epstein, L. G., Krone, W. J. A., Wolters, E. Ch., Oleske, J. M. & Coutinho, R. A. (1986) *Lancet* **ii**, 177–180.
17. Somasundaran, M. & Robinson, H. L. (1988) *Science* **242**, 1554–1557.
18. Mossman, T. (1983) *J. Immunol. Methods* **65**, 55–63.
19. Schwartz, O., Henin, Y., Marechal, V. & Montagnier, L. (1988) *AIDS Res. Hum. Retroviruses* **4**, 441–448.
20. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) *Nature (London)* **312**, 763–767.
21. Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J. C. & Montagnier, L. (1984) *Nature (London)* **312**, 767–768.
22. Murata, A. & Kitagawa, K. (1973) *Agric. Biol. Chem.* **37**, 1145–1151.
23. Murata, A. & Uike, M. (1976) *J. Nutr. Sci. Vitaminol.* **22**, 347–354.
24. Wong, K., Morgan, A. R. & Paranchych, W. (1974) *Can. J. Biochem.* **52**, 950–958.
25. Kazakov, S. A., Atashkina, T. G., Mamaev, S. V. & Vlassov, V. V. (1988) *Nature (London)* **335**, 186–188.
26. Chiou, S.-H. (1983) *J. Biochem. (Tokyo)* **94**, 1259–1267.
27. Evans, R., Currie, L. & Campbell, A. (1982) *Br. J. Nutr.* **47**, 473–482.
28. Varma, S. D. (1987) *Ann. N.Y. Acad. Sci.* **498**, 280–291.
29. Dabrowski, K. & Köck, G. (1989) *Int. J. Vit. Nutr. Res.* **59**, 157–160.
30. Nakanishi, Y., Isokashi, F., Matsunaga, T. & Sahamoto, Y. (1985) *Eur. J. Biochem.* **152**, 337–342.
31. Garland, D., Zigler, S. J., Jr., & Kinoshita, J. (1986) *Arch. Biochem. Biophys.* **251**, 771–776.
32. Ortwerth, J., Feather, M. S. & Olesen, P. R. (1988) *Exp. Eye Res.* **47**, 155–168.
33. Yarchoan, R., Brouwers, P., Spitzer, A. R., Grafman, J., Safai, B., Perno, C. F., Larson, S. M., Berg, G., Fischl, M. A., Wichman, A., Thomas, R. V., Brunetti, A., Schmidt, P. J., Myers, C. E. & Broder, S. (1987) *Lancet* **i**, 132–135.
34. Poli, G., Orenstein, J. M., Kintner, A., Folks, T. M. & Fauci, A. S. (1989) *Science* **244**, 575–577.
35. Roederer, M., Staal, F. J. T., Raju, P. A., Ela, S. W., Herzenberg, L. A. & Herzenberg, L. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4884–4888.