Vitamin C (ascorbic acid) is required for normal host defense and functions importantly in cellular redox systems. To define the interrelationship between human immunodeficiency virus (HIV) infection and vitamin C flux at the cellular level, we analyzed vitamin C uptake and its effects on virus production and cellular proliferation in HIV-infected and uninfected human lymphoid, myeloid, and mononuclear phagocyte cell lines. Chronic or acute infection of these cell lines by HIV-1 led to increased expression of glucose transporter 1, associated with increased transport and accumulation of vitamin C. Infected cells also showed increased transport of glucose analogs. Exposure to vitamin C had a complex effect on cell proliferation and viral production. Low concentrations of vitamin C increased or decreased cell proliferation depending on the cell line and either had no effect or caused increased viral production. Exposure to high concentrations of vitamin C preferentially decreased the proliferation and survival of the HIV-infected cells and caused decreased viral production. These findings indicate that HIV infection in lymphocytic, monocytic, and myeloid cell lines leads to increased expression of glucose transporter 1 and consequent increased cellular vitamin C uptake. High concentrations of vitamin C were preferentially toxic to HIV-infected host defense cell lines in vitro.

Infection with human immunodeficiency virus type 1 (HIV-1) ultimately leads to severe impairment in host defense cell function. Destruction of CD4 T lymphocytes and dysregulation of B lymphocyte and mononuclear phagocyte function underlie the global immunodeficiency seen in acquired immunodeficiency syndrome (AIDS) (1–5). The precise mechanisms by which HIV impairs host defense cell function are unknown but changes in cellular redox metabolism have been linked to HIV-induced cellular dysfunction (6–10). Vitamin C is an important intracellular redox reagent that can protect glutathione-depleted cells from oxidative damage (11), suppress HIV production (12–14), and enhance host defense function (15–18).

Vitamin C is vital to human physiology (19). Humans cannot synthesize vitamin C, and it must be provided exogenously and transported intracellularly (20, 21). We have shown that vitamin C enters cells via the glucose transporters in the form of dehydroascorbic acid and that the dehydroascorbic acid is reduced to ascorbic acid on entering the cell (22–24). This mechanism of transport-reduction allows for accumulation of the high concentrations of ascorbic acid found in human cells. Neoplastic transformation by oncoviruses and oncogenic viruses leads to increased expression of glucose transporters (25, 26), and we postulated that viral infection itself could have a similar effect. We therefore studied the uptake of vitamin C in host defense cell line models to ascertain the effect of HIV infection on vitamin C uptake and to determine the effect of vitamin C on cellular proliferation and HIV production. We found that HIV infection caused increased expression of glucose transporter 1 (GLUT1), with consequent increased transport and accumulation of glucose and vitamin C. Exposure to pharmacologic concentrations of vitamin C led to decreased HIV production and was preferentially toxic to the HIV-infected cells in vitro.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Virus Measurement**—Cell lines chronically infected with HIV-1, L-Mo-μ-HL-60 (J22), U1, ACH2, and 3BH9 (27–32) and uninfected parental cells, HL-60, U937, CEM, and H9, were maintained in culture in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Gemini Bio-Products, Inc., Calabasas, CA), 1% L-glutamine, and antibiotics. For cell assays, 1 × 10^6 cells/ml were cultured for 7 days in the absence or presence of increased concentrations of vitamin C (0, 0.1, 0.3, 1, and 3 mM) prepared and added daily in fresh medium. On days 1, 3, 5, and 7, the cells were harvested, and cell number was determined by counting the cells in a Neubauer chamber. Cell viability was assayed by trypan blue exclusion and was always found to be >95%. Culture supernatants were assayed for virus production by an enzyme-linked immunosorbent assay of the p24 antigen (Coatline HIV-1 p24 antigen; Coulter Corp., Hialeah, FL).

**Vitamin C and Hexose Uptake**—Uptake studies were performed as described (24). Briefly, 1 × 10^6 cells were incubated with 50 μM dehydroascorbic acid (DHA) in the presence of 2 units of ascorbate oxidase (Sigma) and 0.5 μCi of L-[14C]ascorbic acid (specific activity, 8.2 mCi/mmol; DuPont NEN) at room temperature for 0, 5, 10, 20, 30, 45, and 60 min. For accumulation studies, cells were incubated for 60 min in the presence of 0.01, 0.03, 0.1, 0.3, 1, and 10 mM dehydroascorbic acid. Cells were washed twice with phosphate-buffered saline without Ca^{2+} and Mg^{2+} at 4°C before lysis with 60% methanol containing 1 mM EDTA. The cell-associated radioactivity was determined by scintillation spectrometry. For uptake of 2-deoxy-D-glucose (deoxyglucose), 1 × 10^6 cells were incubated with 0.2 mM deoxyglucose and 1.5 μCi of 2-[14C]deoxyglucose (26.2 Ci/mmol, DuPont NEN) at room temperature for 0, 5, 10, 20, 30, 45, and 60 min. For uptake of α-D-methyl-D-glucose (methylglucose), 1 × 10^6 cells were incubated with 1 mM methylglucose and 5 μCi of 3-O-methyl-[14C]glucose for 0, 5, 10, 20, 30, 45, and 60 s. For inhibition studies of deoxyglucose on the uptake of dehydroascorbic acid, 1 × 10^6 cells were incubated with 50 μM dehydroascorbic acid, 2 units of ascorbate oxidase, and 0.5 μCi of α-D-methyl-[14C]glucose in the presence of
increasing concentrations of deoxyglucose (0, 0.1, 1, 3, 10, 30, and 100 mM) and incubated for 30 s. The cell-associated radioactivity was determined by scintillation spectrometry.

**Western Blotting and Immunolocalization**—For Western blot analysis, 5 × 10⁶ HIV-1-infected cells or their control uninfected cell lines were resuspended in cold 50 mM Tris-HCl, pH 7.4, containing 1 mM EDTA, 1 mM EGTA, and protease inhibitors (50 μg/ml aprotinin, 50 μg/ml leupeptin, 50 μg/ml soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride). After sonication, cell debris was removed by centrifugation at 10,000 × g for 10 min at 4°C, and the supernatant was then centrifuged at 100,000 × g at 1°C. After the supernatant was cleared at 100,000 × g for 1 h at 4°C, the pellet was resuspended in cold 50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 1 mM captoethanol, 100 mM dithiothreitol, 0.2% SDS, 0.5% Nonidet P-40, and protease inhibitors (50 μg/ml aprotinin, 50 μg/ml leupeptin, 50 μg/ml soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride). Samples were sonicated and resuspended with continuous shaking for 1 h at 4°C. After the supernatant was cleared at 100,000 × g for 60 min at 4°C, protein concentration was determined using a turbidimetric protein assay (33).

**RESULTS**

**Increased Transport and Accumulation of Vitamin C in HIV Infection**

**Increased HIV-1-infected Cell Lines—**Initial studies indicated that four cell lines chronically infected with HIV-1 (J22, U1, ACH2, and 3BH9) (27–32), as well as the parental control cell lines (HL-60, U937, CEM, and H9), were able to take up the oxidized form of vitamin C, dehydroascorbic acid (Fig. 1, A–D). In all of these cells, uptake of dehydroascorbic acid was linear for at least 10 min and approached a plateau at 60 min. On the other hand, uptake of reduced ascorbic acid proceeded in a linear fashion for the length of the 60-min incubation period but accounted for <5% of the uptake of dehydroascorbic acid under similar conditions (data not shown). In short period transport experiments, we observed that the cells failed to take up reduced ascorbic acid during the first 30 s of incubation, while the uptake of dehydroascorbic acid was very efficient and proceeded linearly for the duration of the incubation (data not shown). The uptake data are compatible with the oxidation of the ascorbic acid present in solution and the time-dependent generation of the transported substrate dehydroascorbic acid and are consistent with our previous findings that human host defense cells transport dehydroascorbic acid and do not transport ascorbic acid directly (22–24).

Four cell lines chronically infected with HIV-1 (J22, U1, ACH2, and 3BH9) showed an increased capacity to take up dehydroascorbic acid as compared with the uninfected parental cells HL-60, U937, CEM, and H9 (Fig. 1, A–D). As estimated from the slopes of the initial linear phase of uptake, infected cell lines (except U1) showed a 3–5-fold increase in their rate of uptake of dehydroascorbic acid. U1 cells showed a 2-fold increase in their uptake of dehydroascorbic acid. Furthermore, the infected cells showed an increased capacity to accumulate radioactive vitamin C at 60 min compared with the uninfected cells. The amount of radioactive material associated with the cells at the end of the 60-min incubation period greatly exceeded the amount expected for a transport mechanism of the facilitated type in which net uptake approaches zero when the intracellular concentration of the transported substrate equals its extracellular concentration. The data are consistent with the intracellular accumulation of the recently transported dehydroascorbic acid as ascorbic acid (data not shown; see also Refs. 22–24). The interpretation of uptake data in terms of the capacity of the cells to transport dehydroascorbic acid is confounded by the fact that long-term uptake experiments reflect both the transport of dehydroascorbic acid and its intracellular trapping as reduced ascorbic acid (22–24). We have shown with HL-60 cells that transport data can be obtained only from very short uptake experiments lasting 30 s or less (24).

We further explored the issue of vitamin C transport as distinct from accumulation. Short uptake experiments revealed that the initial rate of transport of dehydroascorbic acid was increased by 2–4-fold in the infected cell lines as compared to the respective uninfected controls, results that are consistent with the concept that the infected cells have a greater capacity than the uninfected controls to transport dehydroascorbic acid (Fig. 1, E–H). In addition, when the cells were incubated for 60 min in the presence of increasing concentrations of dehydroascorbic acid, the infected cells consistently accumulated 2–3 times more ascorbic acid than the respective uninfected controls (Fig. 1, I–L). These data also indicated that...
In cells incubated with concentrations of dehydroascorbic acid as high as 10 mM, >90% of the intracellularly accumulated radioactivity corresponded to ascorbic acid (data not shown), an observation consistent with the presence of mainly ascorbic acid in mammalian cells. Considering an intracellular exchange volume of 0.3–0.6 μl/10⁶ cells (data not shown; see also Refs. 22–24), the infected cells accumulated from 30 to 130 mM ascorbic acid intracellularly when incubated in the presence of 10 mM dehydroascorbic acid (Fig. 1, I–L). Under similar conditions, the uninfected cells accumulated 15 to 60 mM ascorbic acid. These data support the notion that the infected cells possess an extraordinary capacity to reduce the increased amount of dehydroascorbic acid transported to ascorbic acid.

**Involvement of Facilitative Glucose Transporters in the Uptake of Vitamin C in HIV-infected and Uninfected Cell Lines**—The above results indicate that the HIV-1-infected cells accumulated more ascorbic acid intracellularly than the control cells through a mechanism involving increased transport of dehydroascorbic acid coupled to its intracellular reduction to ascorbic acid. The identity of the intracellular mechanisms involved in the reduction of dehydroascorbic acid to ascorbic acid remains controversial and appears considerably more complex than a simple chemical reaction involving the direct participation of reduced glutathione (11, 35–41). Given our previous information regarding the transport of dehydroascorbic acid in leukocytes, we hypothesized that increased expression of the glucose transporters in the HIV-1-infected cells could explain their increased transport of vitamin C. Transport and accumulation studies using deoxyglucose indicated that the HIV-1-infected cells had a markedly increased capacity to take up this substrate which is specific for the glucose transporters (42, 43) as compared with uninfected cells (Fig. 2, A–D). The relative increase in deoxyglucose uptake paralleled that observed for the uptake of dehydroascorbic acid, an observation consistent with the concept that the increased transport of dehydroascorbic acid was related to the increased expression of glucose transporters in the infected cells. We analyzed the expression of glucose transporters in the infected cells by measuring the transport of methylglucose, an analog of glucose that is not metabolized and that enters the cells through the glucose transporters (42, 43). Methylglucose can be used as a reagent to isolate transport from accumulation and to relate variations in the rate of transport to changes in the number of glucose transporters. These studies showed increased transport of methylglucose in the HIV-infected cells compared with the uninfected controls (Fig. 2, E–H), confirming an increase in the number of functional glucose transporters in the HIV-1-infected cells. There was a close correlation between the increased expression of glucose transporters and increased ability of the HIV-1-infected cells to take up dehydroascorbic acid.

We directly tested the participation of glucose transporters in the transport of dehydroascorbic acid in the different cells by determining the effect of increasing concentrations of deoxyglucose on transport. Deoxyglucose inhibited the uptake of dehydroascorbic acid in both the HIV-infected and uninfected cell lines in a dose-dependent manner and with a similar concentration dependence (Fig. 2, I–L). Fifty percent inhibition of uptake (ID₅₀) was observed at ~4 mM deoxyglucose, a value consistent with the affinity of GLUT1 for deoxyglucose (22–24). Complete inhibition of transport was observed at 50 mM deoxyglucose. These data confirm the transport of dehydroascorbic acid by facilitative glucose transporters in both the control and the infected cells.

**Increased Expression of GLUT1 in HIV Chronically Infected Cells**—Six facilitative transporter isoforms have been described in mammalian cells (44) of which five, GLUT1–GLUT5, are expressed on the plasma membrane and participate in the cellular uptake of hexoses. Immunoblotting experiments allowed us to identify GLUT1 as the major glucose transporter present in both the infected and the uninfected cell lines (Fig. 3B). No bands immunoreactive with anti-GLUT2, -GLUT3, -GLUT4, or -GLUT5 antibodies were observed in these experiments under conditions that showed strong reactivity with anti-GLUT1 (data not shown). When blots were overexposed, immunoreactive bands were observed in samples obtained from the U937, U1, HL-60, and J22 cells probed with the anti-GLUT1 antibody. The anti-GLUT1 antibody recognized a broad immunoreactive band apparently composed of several overlapping bands ranging from ~40 to 80 Kd (Fig. 3B) in six of the eight cell lines studied. The T-lymphocytic cell lines ACH2 and H9 showed the presence of a broad GLUT1 immunoreactive band ranging from ~55 to 120 Kd. Control experiments revealed that the anti-GLUT1 antibody identified a broad but much less heterogeneous immunoreactive band of 45–50 Kd in membranes prepared from human erythrocytes (data not shown). The broad immunoreactive bands were absent in membranes incubated with anti-GLUT1 preabsorbed with the peptide used to generate the antibody, indicating the specificity of the reaction (Fig. 3C). The immunoblotting experiments also revealed that there was an increased amount of GLUT1-immunoreactive material in the HIV-infected cells compared with the uninfected controls. These results are consistent with the
transport experiments and indicate increased expression of GLUT1 in the HIV-infected cells. Immunolocalization studies using fluorescein-tagged antibodies also led to the identification of GLUT1 in the cell lines, with increased intensity of staining observed in the HIV-infected cells compared with the uninfected controls (Fig. 3A). As controls, cells were immunostained with anti-GLUT1 antibody previously preincubated with the peptide used to generate the antibody. No staining in either uninfected or infected cell lines was observed, confirming the specificity of the anti-GLUT1 immunoreactivity (data not shown).

Infection with HIV-1 Induces Increased Transport and Accumulation of Vitamin C and Hexoses and Augments GLUT1 Expression—The previous data were obtained using cells chronically infected with HIV that were maintained in culture for a long time. To directly relate changes in vitamin C transport and accumulation to HIV infection, we studied vitamin C and hexose uptake in a freshly infected cell line. CEM cells were infected with HTLV-IIIB and production of p24 was monitored for 6 days after infection (Fig. 4A). The infected cells showed an increased ability to transport dehydroascorbic acid and accumulate ascorbic acid as compared with the mock-infected cells (Fig. 4, B–D). They also showed an increased capacity to take up deoxyglucose (Fig. 4E) and to transport methylglucose (Fig. 4F). Deoxyglucose at 50 mM completely inhibited the uptake of dehydroascorbic acid in both cell types, with an ID_{50} of ~5 mM (Fig. 4G). These data indicate that the increased transport and accumulation of vitamin C observed in the HIV-infected cells are an early effect of the infection process that is maintained as a stable cell phenotype under conditions of chronic infection and long-term culture.

Vitamin C Affects Cell Survival and Viral Production—To study the effect of vitamin C on cell survival and viral production, HIV-1-infected and uninfected control cells were incubated with various concentrations of ascorbic acid. At tested concentrations of 0.1–3 mM, vitamin C decreased the proliferation and survival of the uninfected cells (Fig. 5A, E, I, and M). Only a minor effect on cell proliferation was observed at 0.1 and 0.3 mM vitamin C, with the greatest effect seen in HL-60 (Fig. 5I) and H9 cells (Fig. 5E). A clear cytotoxic effect was observed when the cells were incubated with 1 mM vitamin C, with only the H9 cells showing some degree of proliferation under those conditions (Fig. 5E). No living cells were recovered from samples treated for 24 h with 3 mM vitamin C.

Vitamin C had a complex effect on the proliferation and survival of the HIV-1-infected cells. The infected cell line 3BH9 showed a small (~50%) but consistent increase in proliferation when incubated in the presence of 0.1 and 0.3 mM vitamin C (Fig. 5F). On the other hand, 0.3 mM vitamin C preferentially decreased the proliferation of the HIV-1-infected lymphocytic cell line ACH2 (Fig. 5B) compared to the control cell line CEM (Fig. 5A). A similar effect was observed at 1 mM vitamin C for the lymphocytic cell line 3BH9 (Fig. 5F) and the monocytic cell line U1 (Fig. 5N). No cells other than the 3BH9 survived in the presence of 1 mM vitamin C for 7 days (Fig. 5F), and only dead cells were recovered from samples treated for 24 h or longer with 3 mM vitamin C (Fig. 5). With the exception of the cell line HL-60, which was similarly sensitive to high concentrations of vitamin C as the HIV-infected cell line J22, treating the cells for 1 week with pharmacological concentrations of vitamin C preferentially decreased the proliferation and survival of the HIV-infected cells compared with the uninfected control cells (Fig. 5, D, H, L, and P).

The effect of vitamin C on viral production was cell line dependent, with decreased viral production observed in all cells at high vitamin C concentrations. At 3 mM, vitamin C caused a major decrease in p24 production by all the cell lines (Fig. 5, C, G, K, and O). In infected monocyte U1 cells, vitamin C caused decreased p24 production at all concentrations tested (Fig. 5, O and P). This effect was most marked at concentrations of vitamin C ≥0.3 mM. Vitamin C at 3 mM caused a 2-fold decrease in p24, a result that is consistent with the cytotoxic effect of this high concentration of vitamin C on the cells. On the other hand, 0.1 and 0.3 mM vitamin C induced an increase in the production of p24 by the T-lymphocytic cell line 3BH9 (Fig. 5, G and H). Similarly, 0.1 mM vitamin C also induced increased p24 production by the T-lymphocytic cell line ACH2 (Fig. 5, C and D). The ACH2 cells, however, were very sensitive to higher con-

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GLUT1 Expression and Vitamin C Uptake in HIV Infection

Fig. 4. Infection of CEM cells with HIV-1 induces increased transport and accumulation of vitamin C and hexoses and augments GLUT1 expression. A, for infection, CEM cells were cultivated for 2 h with an infectious HIV-1 stock, obtained from the supernatant of the 3BH9 cells in the presence of 10 μg/ml Polybrene, washed, and cultured for several days to obtain the infected cell line CEM-HIV. Culture medium was changed daily, and the culture supernatants were harvested, centrifuged to remove cell debris, and monitored for the presence of the HIV p24 core antigen by enzyme-linked immunosorbent assay. B, uptake of DHA was measured for the time indicated in the figure in the chronically infected cell line ACH2 (E), the acutely infected cell line CEM-HIV (F), and the uninfected cell line CEM (G) as indicated in Fig. 1. C, for DHA transport studies, the acutely infected cell line CEM-HIV (●) (3 days after infection) and the uninfected cell line CEM (○) were incubated for 60 min with 30 μM DHA as indicated in Fig. 1. D, for inhibition studies of deoxyglucose on the uptake of DHA, the acutely infected cell line CEM-HIV (●) (3 days after infection) and the uninfected cell line CEM (○) were incubated for 60 min in the presence of increasing concentrations of deoxyglucose as indicated in Fig. 1. E, for deoxyglucose uptake studies, the acutely infected cell line CEM-HIV (●) (3 days after infection) and the uninfected cell line CEM (○) were incubated for 60 min in the presence of 0.2 mM deoxyglucose as indicated in Fig. 2. F, for OMG transport studies, the acutely infected cell line CEM-HIV (●) (3 days after infection) and the uninfected cell line CEM (○) were incubated in the presence of 1 mM OMG as indicated in Fig. 2. G, for inhibition studies of deoxyascorbic acid on the uptake of DHA, the acutely infected cell line CEM-HIV (●) (3 days after infection) and the uninfected cell line CEM (○) were incubated for 30 s with 50 μM OMG as indicated in Fig. 2. H, the uptake studies were performed with 0.3 mM vitamin C, which is not cytotoxic to the ACH2 cells. Vitamin C, at concentrations of 0.3 and 1 mM, caused a transient increase in p24 production by J22 cells that lasted for 4 days (Fig. 5K). After 7 days of exposure, however, vitamin C at 0.3 mM and higher caused decreased p24 production. Thus, only pharmacological concentrations of vitamin C consistently decreased p24 production in HIV-infected cells, whereas low vitamin C concentrations produced variable effects including increased p24 production.

DISCUSSION

HIV infection leads ultimately to the development of AIDS and severe immunodeficiency. The mechanisms involved in this process are poorly understood, but it has been suggested that changes in redox metabolism in cells infected with HIV-1 are closely linked to the decrease in their functional capacity. Changes in intracellular concentrations of glutathione have been reported to occur after HIV infection with a decrease in the fraction of cells with high content of glutathione (8). These findings led to the observation that high concentrations of N-acetylcysteine, a precursor of cellular glutathione, appeared to impair viral replication in some in vitro models, suggesting that manipulation of cellular redox metabolism could have potential clinical applications (10, 45).

Our findings indicate that HIV infection of lymphoid, mononuclear phagocytes, and myeloid human hematopoietic cell lines leads to increased capacity of these cells to transport and accumulate vitamin C and glucose analogs. Our data point to the participation of facilitative glucose transporters in the transport of vitamin C in the HIV-infected as well as the control uninfected cells. Six different facilitative glucose transporters have been identified in mammalian cells of which five (GLUT1–GLUT5) are expressed on the cell surface and participate in the cellular uptake of hexoses (44). We have shown that vitamin C is taken up by Xenopus oocytes expressing the mammalian glucose transporters GLUT1, GLUT2, GLUT3, GLUT4, and normal human neutrophils and HL-60 cells in the form of dehydroascorbic acid and trapped intracellularly by reduction to ascorbic acid (22–24). The evidence is consistent with the concept that HIV-infected and uninfected white blood cells do not transport reduced ascorbic acid and that the basal uptake observed in long uptake assays is due to the oxidation of ascorbic acid in solution with the concomitant generation of the
transported species dehydroascorbic acid (22–24). Our data point to increased expression and function of the glucose transporter GLUT1 as the mechanism leading to the increased transport of vitamin C by HIV-infected cells. The cells were able to transport deoxyglucose, a substrate that is specific for the facilitative glucose transporters, and cells chronically and acutely infected with HIV showed an increased capacity to take up this substrate compared with the uninfected cells. In addition, infected cells also showed an increased capacity to transport methylglucose, an analog of glucose that is useful in assessing transport as distinct from accumulation. Competition studies showed that the transport of dehydroascorbic acid was inhibited by deoxyglucose. Moreover, the expression of GLUT1 is augmented in HIV-1-infected cells as indicated by the immunodetection studies. Thus, our results strongly support the concept that GLUT1 mediates the transport of dehydroascorbic acid in these cells and that the changes in the capacity of the HIV-infected cells to transport dehydroascorbic acid and hexoses reflect increased expression of GLUT1 in the cells.

The increase in GLUT1 expression observed after infection with HIV, a nontransforming virus, is unprecedented. Although cell transformation after infection with oncogenic retroviruses leads to increased expression of GLUT1 (25, 26), no effect on glucose transporter expression or function has been reported in cells infected with nontransforming retroviruses. The increased expression of GLUT1 in HIV-infected cells may have important implications for the physiology of the infected cells. One of the primary characteristics of cancer cells is an increased uptake and anaerobic metabolism of glucose. The increased glucose uptake may give the malignant cells an advantage in terms of growth and survival because glucose provides most of the fuel necessary for cell function. Because glucose must be taken up from the surrounding media and not from intracellular stores, cancer cells require an increased ability to transport glucose, a process mediated by overexpression of the glucose transporter GLUT1. Thus, our data suggest that HIV infection of host defense cells is associated with changes in the metabolic state of the cells as reflected by the increased expression of GLUT1 and the concomitant increased transport and metabolism of glucose.

The observation that the HIV-infected cells possess an increased capacity to accumulate reduced ascorbic acid when incubated in the presence of dehydroascorbic acid may also have important physiological consequences for the infected cells. Vitamin C is fundamental to human physiology. Because humans cannot synthesize vitamin C, it must be provided exogenously in the diet and transported intracellularly. Prolonged absence of vitamin C in the diet leads to the development of scurvy with associated cellular dysfunction. Vitamin C is present in human cells at millimolar concentrations and has important roles in vascular and connective tissue integrity, normal hematopoiesis, leukocyte function, and defense against microorganisms. Our data are consistent with the concept that HIV-infected and uninfected white blood cells transport dehydroascorbic acid through the glucose transporter GLUT1. No definitive information is available regarding the mechanisms involved in the intracellular reduction of dehydroascorbic acid and trapping of ascorbic acid, or on the effect of increased intracellular concentrations of ascorbic acid on cellular physiology. In vitro animal studies have provided evidence for a coupling between the cellular levels of glutathione and vitamin C. In animals depleted of glutathione by treatment with buthionine sulfoximine, supplementation with vitamin C protected cells from oxidative damage (11). In addition, in scorbatic animals, supplementation with precursors of glutathione such as glutathione methyl ester protected the animals from early death (46). There is, however, no direct evidence indicating a relationship between the differential capacity of cells to accumulate ascorbic acid and their respective intracellular concentrations of glutathione. Evidence is also lacking regarding the relationship of intracellular concentrations of ascorbic acid to the enzymes glutaredoxin and protein disulfide isomerase, both of which appear to possess glutathione-dependent dehydroascorbate reductase activity.

Evidence is available suggesting that physiological concentrations of vitamin C may decrease HIV replication in vitro models (12). Our data indicate, however, a complex effect of different concentrations of vitamin C on cell proliferation on HIV-infected and uninfected cells and on viral production by HIV-infected cells. The effect of vitamin C was clearly dose dependent and strongly affected by the cellular model studied. At vitamin C concentrations of 100 μM, well within the physiological range, there was no correlation between the effects of the vitamin on cell proliferation (no effect or a small decrease or increase in both infected and uninfected cell lines) and on viral production (cell specific). We observed increased viral production by ACH2 cells in the presence of 100 μM vitamin C and by 3B9 cells in the presence of 0.1 and 0.3 mM vitamin C. On the other hand, a 1-week exposure to pharmacological concentrations of vitamin C generally decreased cell proliferation and survival of HIV-infected cells compared with the uninfected controls. Thus, the prominent decrease of viral production in cells exposed to millimolar concentrations of vitamin C was clearly related to the cytotoxic effect of vitamin C. Although the data provide a basis for understanding the interrelationship of vitamin C and HIV infection, the complexity of our observations and the lack of information of the molecular components that are part of the cellular mechanisms linking cellular vitamin C and viral production leave interpretation of the data incomplete in mechanistic terms. Overall, our results are compatible with evidence indicating that vitamin C and compounds that increase the cellular content of glutathione or affect the cellular redox potential are able to modulate viral production in short-term experiments in vitro, although their mechanisms of action remain a matter of controversy (6, 9, 10, 12–14).

Our findings indicate that HIV infection increases vitamin C uptake in human lymphocytic, myeloid, and monocyctic cell lines because of increased expression of glucose transporters and show that pharmacological concentrations of vitamin C are preferentially toxic to host defense cell lines infected with HIV. The data provide a foundation on which to begin to understand the interrelationship among vitamin C, HIV infection, and cell metabolism but do not address the therapeutic implications of vitamin C in AIDS.

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