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# GLQ223: An inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage

(AIDS/macrophages/T lymphocytes/antiviral compound)

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ABSTRACT **GLQ223 is a highly purified, formulated preparation of trichosanthin, a 26-kDa plant-derived ribosome-inactivating protein with potent inhibitory activity against human immunodeficiency virus (HIV) *in vitro*. The compound produced concentration-dependent inhibition of HIV replication in acutely infected cultures of T-lymphoblastoid cells (VB cell line). Treatment with GLQ223 selectively reduced levels of detectable viral proteins compared to total cellular protein synthesis and produced a selective decrease in levels of viral RNA relative to total cellular RNA in acutely infected cells. Substantial inhibition of viral replication was observed at concentrations of GLQ223 that showed little inhibition of parallel uninfected cultures. Selective anti-HIV activity was also observed in cultures of primary monocyte/macrophages chronically infected with HIV *in vitro*. When freshly drawn blood samples from HIV-infected patients were treated with a single 3-hr exposure to GLQ223, HIV replication was blocked for at least 5 days in subsequently cultured monocyte/macrophages, without further treatment. The anti-HIV activity of GLQ223 in both acutely and chronically infected cells and its activity in cells of both lymphoid and mononuclear phagocytic lineage make it an interesting candidate as a potential therapeutic agent in HIV infection and AIDS.**

GLQ223 is a highly purified, formulated preparation of trichosanthin, a 26-kDa basic protein isolated from root tubers of *Trichosanthes kirilowii* (1, 20). Based on structural and functional properties, the protein belongs to the family of single-chain ribosome-inactivating proteins, which inhibit *in vitro* translation in cell-free systems (2). Trichosanthin has been reported to show a 56% similarity at the amino acid level with the A chain of ricin toxin when both identical and conservative residues are considered (2) and shows substantial amino acid similarity with several other single-chain ribosome-inactivating proteins (Michael Piatak, personal communication). Partially purified preparations of trichosanthin have been administered in China, in single doses from 5 to 12.5 mg, as a midtrimester abortifacient (3) and, in multiple dose regimens involving 5–12 mg per dose, for treatment of trophoblastic tumors (4). In general, these doses have been reported to be both effective for the intended clinical indication and well tolerated (3, 4).

In this communication, we report that GLQ223 selectively inhibits replication of human immunodeficiency virus (HIV), the etiologic agent for AIDS, in cells of both lymphoid and mononuclear phagocyte cell lineages *in vitro*.

## MATERIALS AND METHODS

**Source and Purification of GLQ223.** Root tubers of *T. kirilowii* were obtained from southern China. GLQ223 was purified from an aqueous extract of homogenized tuber material by using modifications of a previously described procedure (1). Purity of the material obtained was determined to be >98% by laser densitometric scanning of Coomassie blue-stained gels ($NaDodSO_4$/PAGE) and by size-exclusion HPLC analysis (data not shown). Authenticity of the material obtained was confirmed by Western blot analysis with rabbit antiserum raised against a reference preparation and by N-terminal amino acid sequence analysis (data not shown).

**Cell Lines and Preparation of Primary Cell Populations.** The VB cell line (5) was used for acute infectivity assays; cells were maintained in RPMI-1640 supplemented with 10% (vol/vol) heat inactivated fetal calf serum. Primary peripheral blood-derived monocyte/macrophages were prepared and cultured from either HIV-seronegative healthy donors or from donors known to be infected with HIV by using procedures described in detail elsewhere (6). The research protocol was approved by the Human Subjects Committee, University of California, San Francisco.

**Bioassays for Anti-HIV Activity.** *T-cell assays: Acutely infected cells.* The VB cell line was used to assess the effects of GLQ223 on primary HIV infection of T-lymphoblastoid cells. Briefly, cells were inoculated with a titered cryopreserved virus stock [isolate HIV-$1_{DV}$ (6)] at a multiplicity of infection of ≈0.005. Cells were incubated at a density of 1–5 × $10^7$ cells per ml with the inoculum for 60 min at 37°C to permit adsorption of viral particles and then washed to remove unbound virus. Cells were then resuspended at 1.0 × $10^5$ cells per ml in RPMI-1640 supplemented with 10% fetal calf serum and cultured in 24-well culture plates (1 ml per well) with or without GLQ223 added to the desired concentration for the duration of culture. Cells were cultured for 4 days, when supernatants were harvested for quantitation of HIV p24 antigen content by using a commercially available capture immunoassay (Coulter). In this assay system, at the multiplicity of infection used, virally mediated cytopathic effects and HIV p24 levels peak at day 4; anti-HIV activity was thus evaluated at the time of maximum virus production.

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Abbreviations: HIV, human immunodeficiency virus; AZT, 3′-azido-3′-deoxythymidine.

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Treated and untreated, infected and uninfected cultures (i.e., four different types of cultures) were also plated in 96-well plates for determination of incorporation of [$^3$H]leucine and [$^3$H]thymidine into trichloroacetic acid-precipitable protein and cellular DNA, respectively, as determined by scintillation counting of harvested cells. As the extent of viral cytopathology by day 4 was too great to allow reliable quantitation of [$^3$H]leucine and [$^3$H]thymidine incorporation or meaningful comparison to treated cultures, cells for incorporation studies were harvested on day 3.

For RNA studies, cells were inoculated at a multiplicity of infection of 0.03 and then cultured in 75-cm$^2$ culture flasks in the continuous presence of the desired concentration of GLQ223. Aliquots of these cultures were harvested at 48 hr, the cells were lysed, and RNA was extracted (7) for analysis of viral and cellular RNA by Northern blot (8). Cell-free culture supernatants were harvested for determination of HIV p24 content at the time RNA was prepared. [$^3$H]Leucine and [$^3$H]thymidine incorporation were determined for aliquots of the cultures, plated as above in 96-well plates, pulsed with [$^3$H]leucine or [$^3$H]thymidine, and harvested at 48 hr.

*Macrophage assays: Cells chronically infected* in vitro *with exogenous virus.* Monocyte/macrophages were isolated from peripheral blood of HIV-seronegative healthy donors, infected *in vitro* with HIV (HIV-$1_{DV}$ isolate; multiplicity of infection of 1.0), and cultured as described (6). HIV antigen expression was assessed by flow cytometric analysis. Cells were stained with a p24-specific murine monoclonal antibody after using an isotype-matched monoclonal antibody of irrelevant specificity to set the threshold for background fluorescence. The percentage of cells with HIV-specific (above background—fluorescence channel 150) fluorescence was then determined. Cultures were used for studies described below only if the percentage of HIV antigen-expressing cells, as determined by flow cytometric analysis, was ≥30%. After establishing the extent of infection, cells were treated with a 3-hr pulsed exposure to GLQ223. Alternatively, cells received continuous exposure to 3′-azido-3′-deoxythymidine (AZT, 40 μM; Burroughs Wellcome). Treated cells were maintained in suspension culture in Teflon jars (PTFE; Savillex, Minnetonka, MN) as described. After 4 days of culture, HIV antigen expression was assessed in GLQ223-treated cultures and buffer-treated control cultures by flow cytometric analysis (6).

*Macrophage assays: Cells infected* in vivo. Experiments were also conducted with monocyte/macrophages infected *in vivo* with HIV. Freshly drawn EDTA anti-coagulated whole blood from HIV-seropositive patients was treated for 3 hr at 37°C with 500 ng of GLQ223 per ml or sham-treated with buffer. Mononuclear cells were then isolated over Ficoll/Hypaque, with extensive washing, and placed in culture in Teflon jars. Five days later, HIV antigen expression was quantitated by flow cytometric analysis (6).

## RESULTS

GLQ223 was tested for inhibition of HIV replication in a variety of assays using both primary and transformed target cells and cells representing both lymphoid and monocytoid lineages. The compound was tested in assays of both acute and chronic HIV infection and in experiments using primary cells from several distinct *in vivo*-infected donors, each presumably infected with a different strain of HIV.

Cultures of the highly susceptible T-lymphoblastoid cell line VB were inoculated with HIV and then treated with GLQ223. Infection and viral replication were monitored in the cultures by following the appearance of characteristic HIV-induced cytopathic changes, by measuring HIV p24 in culture supernatants, and by assessing production of viral antigens by immunofluorescence analysis. As shown in Table 1, in multiple experiments, GLQ223 treatment reproducibly resulted in concentration-dependent inhibition of HIV replication as measured in this acute primary infection assay system. Inhibition of HIV replication was observed by measurement of supernatant p24 content (Table 1) and by decreased viral cytopathic effects (data not shown). The majority of HIV p24 production was abolished at relatively low concentrations of GLQ223 (16–63 ng/ml), with essentially complete inhibition seen at higher concentrations (1–2 μg/ml). Greater than 75% inhibition was observed at concentrations that produced little inhibition (≤20%) of total cellular protein synthesis or cellular DNA synthesis in infected cells or parallel cultures of uninfected VB cells, but inhibition of these parameters was seen at higher concentrations with 3 days of continuous exposure to GLQ223 (Tables 1 and 2).

A similar acute infection experiment was used to assess effects of GLQ223 on HIV replication at the RNA level. Cells were harvested for RNA extraction at day 2 of culture to prevent the extensive RNA degradation observed at later time points in untreated cultures, presumably as a consequence of advanced viral cytopathic effects and cell lysis. Concentration-dependent inhibition of HIV replication was again observed, as measured by supernatant HIV p24 levels (Fig. 1) and decreased virally induced cytopathology (data not shown). Essentially complete inhibition of HIV p24 production was observed at concentrations of GLQ223 that did not measurably inhibit total cellular protein synthesis or cellular DNA synthesis in infected cells or parallel cultures of uninfected VB cells in this 2-day assay (Fig. 1*C*). GLQ223 treatment also resulted in a selective proportional decrease in the amount of viral RNA present in treated cells relative to total RNA extracted from the cells (Fig. 1 *A* and *B*). RNA from infected untreated cells probed with a $^{32}$P-labeled nick-translated full-length HIV DNA probe derived from pHXB-2 (9) showed a strong signal with characteristically sized hybridizing bands corresponding to full-length (≈9.5 kilobases) and spliced forms [4.3 and ≈2.0 kilobases (10)] of HIV RNA (Fig. 1*A*, lane 1), with the hybridizing species of less than 2 kilobases probably representing degraded viral message. No hybridization of the HIV probe to RNA from uninfected cells was seen, regardless of GLQ223 treatment (Fig. 1*A*, lanes 3 and 4). GLQ223 treatment resulted in a decrease in the amount of viral RNA detectable by hybridization (Fig. 1*A*, compare lanes 1 and 2), despite equivalent amounts of total RNA visible in each lane in the ethidium bromide-stained gel prior to transfer (data not shown). In contrast, GLQ223 treatment did not affect levels of message detectable by hybridization with a full-length γ-actin cDNA probe from pHF A-1 (11) in either HIV-infected treated cells (Fig. 1*B*, lanes 1 and 2) or uninfected treated cells (Fig. 1*B*, lanes 3 and 4).

Table 1. Inhibition of viral replication by GLQ223 in T-lymphoblastoid cells: 96-hr continuous exposure

| GLQ223 treatment, μg/ml | p24, ng/ml × $10^{-2}$ | p24, % control |
|---|---|---|
| 0 | 18.5 ± 1.4 | 100 ± 8 |
| 0.016 | 4.9 ± 1.6 | 27 ± 9 |
| 0.031 | 3.7 ± 1.4 | 20 ± 8 |
| 0.063 | 2.3 ± 1.2 | 13 ± 6 |
| 0.126 | 1.4 ± 0.7 | 6 ± 4 |
| 0.251 | 0.7 ± 1.4 | 4 ± 2 |
| 0.502 | 0.3 ± 1.2 | 2 ± 1 |
| 1.005 | <0.3 | 0 |
| 2.010 | <0.3 | 0 |

HIV p24 production is shown relative to untreated infected control cultures as a function of GLQ223 concentration for acutely infected cells of the T-cell line VB. Cells were continuously exposed to GLQ223 for 96 hr. p24 values are means ± SD for eight experiments at each concentration.

Table 2. Effect of GLQ223 on [$^3$H]thymidine and [$^3$H]leucine incorporation in uninfected and acutely HIV-infected VB cultures: 72-hr continuous exposure

| GLQ223 treatment, μg/ml | [$^3$H]Thymidine, % control | [$^3$H]Leucine, % control |
|---|---|---|
| | Uninfected cells | |
| 0 | 100 ± 4 | 100 ± 9 |
| 0.012 | 87 ± 3 | 85 ± 8 |
| 0.025 | 91 ± 3 | 80 ± 8 |
| 0.049 | 86 ± 4 | 83 ± 2 |
| 0.098 | 84 ± 3 | 83 ± 4 |
| 0.196 | 84 ± 2 | 90 ± 13 |
| 0.393 | 80 ± 5 | 71 ± 4 |
| 0.785 | 73 ± 2 | 61 ± 5 |
| 1.570 | 60 ± 0 | 48 ± 4 |
| 3.140 | 46 ± 2 | 33 ± 3 |
| | HIV-infected cells | |
| 0 | 78 ± 5 | 89 ± 6 |
| 0.012 | 90 ± 3 | 104 ± 10 |
| 0.025 | 89 ± 3 | 98 ± 7 |
| 0.049 | 91 ± 4 | 99 ± 5 |
| 0.098 | 89 ± 2 | 98 ± 5 |
| 0.196 | 88 ± 3 | 93 ± 9 |
| 0.393 | 87 ± 3 | 95 ± 5 |
| 0.785 | 75 ± 4 | 62 ± 4 |
| 1.570 | 69 ± 3 | 50 ± 3 |
| 3.140 | 54 ± 2 | 45 ± 4 |

Cells ($2 \times 10^4$ per well) were seeded in 96-well plates and then pulsed either with 1 μCi (1 Ci = 37 GBq) of [$^3$H]leucine or [$^3$H]thymidine 8 hr prior to harvesting cells at 72 hr of culture. Labeled precursor incorporated into trichloroacetic acid-precipitable cell-associated macromolecules was determined by scintillation counting. Values shown are the means ± SD for six experiments. Results are normalized to counts obtained for untreated uninfected control cultures ([$^3$H]thymidine control value = 136,500 ± 5007; [$^3$H]leucine control value = 7897 ± 691).

GLQ223 treatment of monocyte/macrophages chronically infected with HIV *in vitro* also inhibited HIV replication, as assessed by flow cytometric analysis 4 days after a short exposure of infected cells to GLQ223. Cells were infected with HIV-$1_{DV}$ and then cultured for 20 days prior to either a pulsed 3-hr exposure to GLQ223 or initiation of continuous exposure to AZT (40 μM). After a subsequent 4 days of culture, flow cytometric analysis was performed. A representative experiment is shown in Fig. 2, where untreated cultures (Fig. 2*A*) showed 34% of the cells displaying HIV-specific fluorescence (solid lines) above the background fluorescence threshold set with irrelevant control antibody (dashed lines; background threshold set at fluorescence channel 150). No HIV-specific fluorescence was detectable in GLQ223-treated cells (Fig. 2*B*). In contrast to the complete abrogation of HIV antigen expression seen in GLQ223-treated cultures, AZT-treated cultures (Fig. 2*C*) showed 36% of cells displaying HIV-specific fluorescence. No change in viability was seen in GLQ223-treated cultures at the time of analysis, 4 days after treatment. However, 2 weeks following treatment, an ≈40% loss of cellular viability was noted by flow cytometry in GLQ223-treated, *in vitro*-infected cultures when HIV antigen expression remained undetectable (data not shown). Identically treated uninfected cultures studied in parallel showed no change in cellular viability.

Additional studies were conducted using monocyte/macrophages isolated from individuals infected with HIV *in vivo*. In an *ex vivo* experimental system designed to simulate as closely as possible the potential *in vivo* administration of GLQ223, freshly drawn EDTA anticoagulated whole blood from HIV-infected patients was treated with GLQ223 (500 ng/ml) or sham-treated with buffer for 3 hr at 37°C. After isolation of mononuclear cells by density centrifugation with

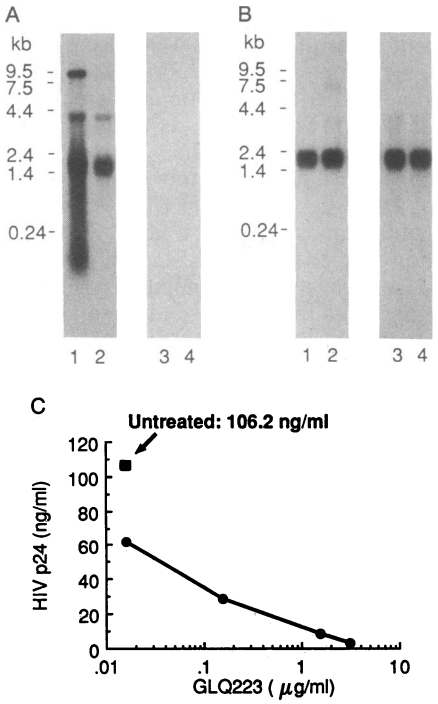


FIG. 1. GLQ223 treatment selectively decreases relative levels of HIV RNA: 48-hr continuous exposure. (*A*) HIV probe. No hybridization of the HIV probe to RNA from uninfected cells that were untreated (lane 3) or treated with GLQ223 at 3.14 μg/ml (lane 4) was seen, whereas a strong signal with characteristically sized bands corresponding to full length and spliced forms of HIV RNA was seen in untreated infected cells (lane 1). GLQ223 treatment at 3.14 μg/ml (lane 2) resulted in a decrease in the amount of viral RNA detectable by hybridization. (*B*) γ-Actin probe. GLQ223 treatment did not affect levels of γ-actin message detectable by hybridization in untreated infected cells (lane 1), infected cells treated with GLQ223 at 3.14 μg/ml (lane 2), untreated uninfected cells (lane 3), and uninfected cells treated with GLQ223 at 3.14 μg/ml (lane 4). The similarity in size of the hybridizing bands for γ-actin and spliced HIV message is coincidental and does not represent cross-hybridization; despite hybridization of the γ-actin probe to RNA from uninfected cells (lanes 3 and 4), there is no hybridization of the HIV probe to the same RNA preparations (lanes 3 and 4 in *A*). (*C*) Aliquots of the cultures used for RNA studies were also tested for supernatant p24 content at 48 hr. p24 values are single determinations at each concentration; variability of the assay employed is <10% for replicate samples. [$^3$H]Leucine incorporation values were as follows: uninfected untreated cells, 19,673 ± 3137; uninfected cells (3.14 μg/ml GLQ223), 20,285 ± 2019; infected untreated cells, 15,325 ± 1116; infected cells (3.14 μg/ml GLQ223), 15,220 ± 482. [$^3$H]Thymidine incorporation values were as follows: uninfected untreated cells, 54,840 ± 5763; uninfected cells (3.14 μg/ml GLQ223), 60,600 ± 2589; infected untreated cells 32,889 ± 2995; infected cells (3.14 μg/ml GLQ223), 55,021 ± 2905. (Mean values ± SD in cpm; $n = 4$.)

extensive washing, cells were cultured with no further exposure to GLQ223. HIV antigen expression was quantitated on day 5 of culture by flow cytometric analysis. As shown in Table 3, this single pulsed treatment with GLQ223 completely abrogated HIV antigen expression by monocyte/macrophages from five of eight evaluable patients (quantifiable HIV-specific immunofluorescence detectable in parallel untreated cultures) and substantially inhibited expression in the other three cultures. The treatment did not affect cellular viability at day 5 (data not shown).

## DISCUSSION

HIV is the etiologic agent for AIDS and a spectrum of related disorders. Although a variety of potentially vulnerable steps in the viral life cycle could theoretically serve as targets for

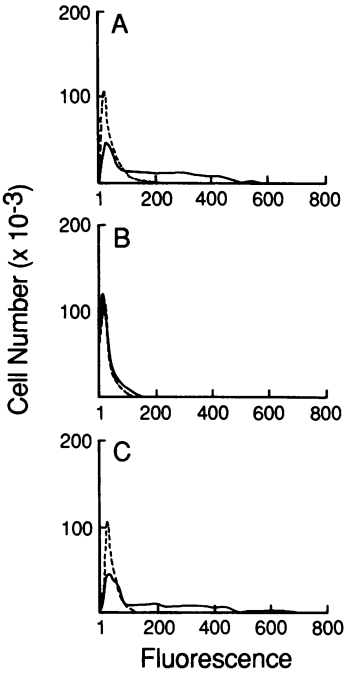


FIG. 2. Selective inhibition of HIV replication in chronically infected monocyte/macrophage cultures. Flow cytometric analysis (linear fluorescence scale) for HIV p24 expression in cytoplasm of infected cells is shown for GLQ223 and AZT-treated monocyte/macrophage cultures chronically infected, *in vitro*, with HIV. Cells were either untreated (*A*), pulse treated with GLQ223 (500 ng/ml for 3 hr followed by washing, without further GLQ223 exposure) (*B*), or treated continuously with 40 μM AZT (*C*).

therapeutic intervention, the *in vivo* pathobiology of HIV infection will probably determine the ultimate efficacy of potential therapies. CD4-expressing lymphoid cells are susceptible to HIV infection, and depletion of CD4-bearing T lymphocytes is both the hallmark of HIV-associated disease and the apparent cause of many of the clinical consequences of HIV infection. However, cells of the mononuclear phagocyte lineage actually appear to be a major *in vivo* reservoir for HIV (6, 12), as is the case for most other retrolentiviruses (13, 14). Therefore, agents that are not active in mononuclear phagocytes are unlikely to exert significant clinical effects, no matter how promising the activity observed *in vitro* in T-cell-based assay systems. Furthermore, monocyte/macrophages appear to be relatively more resistant to the cytopathic effects of HIV infection than T cells, more readily allowing establishment of chronic infection with potential for persistence of an *in vivo* viral reservoir. The importance of chronic infection of cells, especially cells of the mononuclear phagocytic lineage, in the *in vivo* pathobiology of HIV infection may represent an intrinsic limitation to the ultimate clinical efficacy of nucleoside analogs such as AZT. As AZT appears to act by inhibiting reverse transcription, an early event in the HIV life cycle, it would be expected to have little or no effect on chronically infected cells presumably producing viral proteins from integrated proviral DNA. *In vitro* studies show this to be the case in both macrophages and T cells (Fig. 2; M.S.M., unpublished observations; ref. 15).

Table 3. Effect of pulsed GLQ223 treatment of whole blood on HIV expression in subsequently cultured monocyte/macrophages from infected donors

| | % p24-expressing cells* | | Integrated total HIV fluorescence† | | |
|---|---|---|---|---|---|
| Patient | Control | Treated | Control | Treated | % inhibition |
| 1 | 6.3 | 0 | 3502 | 0 | 100 |
| 2 | 2.1 | 0.1 | 1158 | 71 | 95 |
| 3 | 5.7 | 0 | 3156 | 0 | 100 |
| 4 | 3.1 | 0 | 1813 | 0 | 100 |
| 5 | 3.0 | 0 | 1797 | 0 | 100 |
| 6 | 5.3 | 0.9 | 3332 | 613 | 82 |
| 7 | 7.1 | 0 | 4371 | 0 | 100 |
| 8 | 4.4 | 0.8 | 3158 | 584 | 83 |

The results shown are for representative experiments involving eight different donors. Under the culture conditions employed (no exposure of cells to mitogens or other activation stimuli), p24-expressing cells were observed only among cells in the macrophage, but not lymphocyte, gating region.

*Percent p24-expressing cells indicates the percentage of cells in cultures expressing p24-specific (above background—fluorescence channel 150) fluorescence on day 5 of culture.

†Integrated total HIV fluorescence refers to the integrated area of the fluorescence histogram obtained with the p24-specific antibody, above fluorescence channel 150, and reflects both the percentage of p24-expressing cells and the intensity of fluorescence seen for these cells.

GLQ223 inhibits HIV replication in both T cells and monocyte/macrophages and shows activity in *in vitro* assays of both acute and chronic infection. Inhibition of HIV replication is manifest as a selective inhibition of levels of viral antigen and viral RNA relative to host cell protein synthesis and total cellular RNA levels in treated cells. GLQ223 shows anti-HIV activity in *in vitro* assays against both cells infected *in vitro* with the virus and against cells infected with HIV *in vivo*. Activity of GLQ223 against *in vivo*-infected cells from different patients (Table 3) demonstrates the *in vitro* activity of the compound against a variety of presumptively distinct virus strains. Typically, monocytes freshly isolated from HIV-infected patients do not express detectable levels of HIV antigens. Brief *in vitro* cultivation of these cells allows HIV expression in a subpopulation of monocyte-derived cells from such donors; expressed viral antigens are readily detectable by flow cytometric analysis (M.S.M., unpublished observations). A pulsed exposure of whole peripheral blood from HIV-infected patients to GLQ223 resulted in essentially complete suppression of viral antigen expression by subsequently cultured cells from a majority of donors. No viral antigens were detectable 5 days after a single brief exposure to the compound. In preliminary studies, a single 3-hr pulsed exposure to GLQ223 inhibits expression of HIV antigens in subsequently cultured treated infected cells for up to 4 weeks. As a decrease in cellular viability is noted 2–3 weeks after treatment, when viral antigen expression remains undetectable, but not at earlier time points (4–5 days after treatment) or in identically treated uninfected cultures, GLQ223 appears to exert a selective inhibitory activity that initially suppresses viral replication and ultimately selectively kills HIV-infected monocyte/macrophages. Despite the fact that freshly isolated peripheral blood monocytes from HIV-infected subjects do not typically express viral antigens, treatment of freshly drawn whole blood from such subjects with GLQ223 prevented expression of HIV antigens by subsequently isolated and cultured cells, suggesting that the compound may be capable of acting on at least some infected cell populations that do not express detectable viral antigens. Based on its potent anti-HIV activity in both T cells and monocyte/macrophages, as well as its activity in assays of both acute and chronic infection, GLQ223 is an interesting agent for evaluation for anti-HIV activity *in vivo*. Animal testing will permit assessment of any toxicities associated with the compound, including any toxic effects related to immunogenicity. Although essentially complete inhibition of viral antigen expression was observed, *in vitro*, at concentrations of GLQ223 that did not appear to inhibit incorporation of [$^3$H]leucine or [$^3$H]thymidine, higher concentrations of the compound did inhibit [$^3$H]leucine and [$^3$H]thymidine incorporation, especially when present continuously in culture for

several days. However, relatively brief, pulsed exposure to the compound appears to be sufficient for anti-HIV effects and seems to minimize any inhibitory effects on noninfected cells, *in vitro*, a finding of potential relevance to minimizing any observed *in vivo* toxicities.

The mechanism of action whereby GLQ223 inhibits HIV replication is currently unknown. Although the compound belongs to the family of single-chain ribosome-inactivating proteins, it has not been established whether the anti-HIV activity is directly related to this property. Two general classes of mechanisms can be envisioned. In the first, the selective antiviral activity of the compound may be due to selective binding or uptake by virally infected cells (16, 17). Once inside the infected cells, the compound may exert nonspecific effects through catalytic inactivation of ribosomes (18, 19). In the second class of mechanism, selective binding or uptake by infected cells may or may not occur, but the anti-HIV activity of the compound may be attributed to differential effects on viral as opposed to host cell nucleic acid or protein synthesis, processing, or stability. Treatment of acutely infected T-lymphoblastoid cells with GLQ223 resulted in decreased levels of viral proteins at concentrations of GLQ223 that did not affect cellular protein synthesis (Fig. 1*C*), at a time when immunofluorescence analysis showed ≥75% of untreated infected cells expressing viral antigens (48 hr after infection; immunofluorescence data not shown). As selective binding or uptake by infected cells followed by nonspecific ribosomal inactivation might be expected to result in a measurable inhibition of total cellular protein synthesis under these conditions, this observation argues against a mechanism based on nonspecific inhibition of protein synthesis through ribosomal inactivation. Treated cells showed a selective proportional decrease in the amount of viral RNA relative to total cellular RNA at 48 hr of culture (when ≥75% of untreated cells were productively infected; data not shown), with no effect on levels of RNA encoding the cellular gene for γ-actin, suggesting a possible selective effect of GLQ223 on viral nucleic acid synthesis, processing, or stability. Additional studies are needed to clarify the mechanism that underlies the observed selective anti-HIV activity of GLQ223 and other related compounds that show similar activity *in vitro*.

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1. Yeung, H. W., Wong, D. M., Ng, T. B. & Li, W. W. (1985) *Int. J. Pept. Protein Res.* **27**, 325–333.
2. Zhang, X. & Wang, J. (1986) *Nature (London)* **321**, 477–478.
3. Cheng, K. F. (1982) *Obstet. Gynecol.* **59**, 494–498.
4. Chan, Y., Tong, M. K. & Lau, M. J. (1982) *Shanghai J. Chin. Med.* **3**, 30–31 (in Chinese).
5. Lifson, J. D., Reyes, G. R., McGrath, M. S., Stein, B. S. & Engleman, E. G. (1986) *Science* **232**, 1123–1127.
6. Crowe, S., Mills, J. & McGrath, M. (1987) *AIDS Res. Hum. Retrov.* **3**, 135–145.
7. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
8. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 202–203.
9. Fisher, A. G., Collati, E., Ratner, L., Gallo, R. C. & Wong-Staal, F. (1985) *Nature (London)* **316**, 262–265.
10. Muesing, M. A., Smith, D. H., Cabridilla, C. D., Benton, C. V., Lasky, L. A. & Capon, D. J. (1985) *Nature (London)* **313**, 450–457.
11. Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H. & Kedes, L. (1983) *Mol. Cell. Biol.* **3**, 787–795.
12. Gartner, S., Markovits, P., Markovits, D. M., Kaplan, M. H., Gallo, R. C. & Popovic, M. (1986) *Science* **233**, 215–219.
13. Haase, A. T. (1986) *Nature.* **322**, 130–136.
14. Narayan, O., Kennedy-Stoskopf, S. & Zink, M. C. (1988) *Ann. Neurol.* (Suppl.) **23**, S95–S100.
15. Perno, C.-F., Yarchoan, R., Cooney, D. A., Hartman, N. R., Gartner, S., Popovic, M., Hao, Z., Gerrard, T. L., Wilson, Y. A., Johns, D. G. & Broder, S. (1988) *J. Exp. Med.* **168**, 1111–1125.
16. Fernandez-Puentes, C. & Carrasco, L. (1980) *Cell* **20**, 769–775.
17. Foa-Tomasi, L., Campadelli-Fiume, G., Barbieri, L. & Stirpe, F. (1982) *Arch. Virol.* **74**, 322–332.
18. Endo, Y. & Tsurugi, K. (1987) *J. Biol. Chem.* **262**, 8128–8130.
19. Stirpe, F., Bailey, S., Miller, S. P. & Bodley, J. W. (1988) *Nucleic Acids Res.* **16**, 1349–1357.
20. Wang, Y., Qian, R. Q., Gu, Z. W., Jin, S. W., Zhang, L. Q., Xia, Z. X., Tian, G. Y. & Ni, C. Z. (1986) *Pure Appl. Chem.* **58**, 789–798.