Effect of Zidovudine and Granulocyte-Macrophage Colony-Stimulating Factor on Human Immunodeficiency Virus Replication in Alveolar Macrophages

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The alveolar macrophage (AM), as a representative human tissue macrophage, was used in an in vitro system to examine the anti-human immunodeficiency virus type-1 (HIV-1) activity of zidovudine (AZT) and granulocyte-macrophage colony-stimulating factor (GM-CSF). AMs were infected with the IIIB strain of HIV-1 and exposed to AZT (1 μmol/L), GM-CSF (30 U/mL), a combination of AZT (1 μmol/L)/GM-CSF (30 U/mL), or medium control. At 10 or 20 days post-infection, phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear leukocytes (PBMLs) were added to the AM cultures as stimulated target cells. AZT effectively suppressed HIV replication and prevented transfer/amplification in target PBMLs as long as the drug was maintained in the medium. GM-CSF neither suppressed nor augmented HIV replication. The combination of AZT/GM-CSF was comparable with AZT alone in suppressing both the initial infection of AMs and the transfer to target PBMLs as long as the agents were maintained in the cultures. However, when the drugs were removed at the same time that PHA-stimulated PBMLs were added to the culture, the combination of AZT/GM-CSF was found to be more effective than AZT alone in preventing the transfer/amplification of HIV in the target lymphocytes. These results suggest that (1) AZT is effective in inhibiting HIV-1 infection in mononuclear phagocytes; (2) GM-CSF neither inhibits nor augments the replication of the IIIB strain of HIV in human AMs; and (3) the combination of AZT and GM-CSF may have an enhanced anti-HIV-1 activity compared with AZT alone. Clinical trials with the two agents in combination appear warranted.

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MATERIALS AND METHODS

Cells and media. Alveolar macrophages were obtained by bronchoalveolavation from HIV seronegative, normal human volunteers by standard techniques after written informed consent was obtained approved by our Institutional Review Board. The cells were washed in serum-free medium, placed in 35 mm, six-well plates (Costar, Cambridge, MA) at a concentration of 2 × 10⁶ cells per well, and, after a 2-hour incubation, nonadherent cells were removed. The remaining adherent cells were determined to be greater than 95% macrophages by phagocytosis of 1.1 μm latex beads. AMs were maintained in RPMI 1640 medium supplemented with 250 U/mL penicillin, 250 μg/mL streptomycin, 2 mmol/L L-glutamine, 10 mmol/L HEPES buffer, and 10% fetal calf serum (FCS; MA Bioproducts, Walkersville, MD).

Virus strain. The IIIB strain of HIV-1 (courtesy R.C. Gallo, National Cancer Institute) was propagated in H9 cells that, in turn, were maintained in RPMI 1640 medium supplemented with penicillin, streptomycin, L-glutamine, HEPES buffer, and 20% FCS.

Compounds. AZT (courtesy of Burroughs-Wellcome Co, Research Triangle Park, NC) was prepared as a 1 mmol/L stock solution and stored at −70°C. Recombinant GM-CSF (courtesy of

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the compounds used in these studies were based on our previous findings of their antiretroviral activity in the U937 cell line.15

HIV infection of AMs/drug treatment. AM monolayers were incubated in medium alone or medium containing 30 U/mL GM-CSF for 48 to 72 hours. After this incubation, the monolayers were washed, incubated with 25 μg/mL diethyl aminoethyl (DEAE) dextran for 30 minutes and infected with an inoculum of HIV consisting of 1 mL of a filtered, cell-free culture supernatant (mean reverse transcriptase activity [RT] of inocula = 5.6 log₂ cpd/mL) for 2 hours at 37°C. After the virus adsorption period, cultures that had been pretreated with GM-CSF were re-exposed to this compound alone or in combination with 1 μmol/L AZT. Cultures that had been incubated in medium alone pre-infection were left untreated or were exposed to 1 μmol/L AZT immediately post-infection. Controls included mock infected cultures maintained in medium alone or medium containing 30 U/mL GM-CSF and/or 1 μmol/L AZT. Drugs were replaced in the culture supernatants with each media change.

Ten days post-infection, phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear leukocytes (PBMLs) from HIV seronegative donors were added to some of the AM cultures as target cells (5 x 10⁵ PBMLs per well). Cultures were then followed for an additional 10 days, during which drug was maintained in the treated cultures.

To determine the effect of drug removal in this system, cultures that had been maintained for 20 days in the presence of AZT and/or GM-CSF, but to which PBMLs had not been previously added, were washed free of drug, and PHA-stimulated PBMLs were added at that point. These cultures were then monitored for an additional 15 days.

Monitoring cultures. Cultures were examined visually with an Olympus Model CK indirect microscope for evidence of cytopathic effects (CPE) or cytotoxicity. Evidence for HIV replication in the culture supernatants was evaluated by supernatant HIV antigen (Ag) determination by enzyme immunoassay (DuPont, Boston, MA) and by supernatant RT activity by standard techniques.18

Statistics. The Student's t test was used for all statistical comparisons.

RESULTS

In our experience, when normal AMs are infected in vitro with the IIIB strain of HIV-1, they exhibit no cytopathic effects and supernatant RT activity falls to low or undetectable levels by days 5 to 7 post-infection. Supernatant HIV antigen also predictably falls post-HIV exposure, but it remains detectable for up to 6 weeks if the cells are left in culture, usually in the range of 100 to 900 pg/mL. This indicates that a low-level, non-cytopathic infection is established in these cultures. If stimulated target cells, such as PHA-stimulated PBMLs are added at any point post-infection, a marked rise in RT activity and supernatant HIV antigen is seen within 4 days of their addition. This pattern of in vitro HIV infection has been observed consistently in over 30 AM cultures established from normal donors in our laboratory, and no differences have been observed with respect to the age, sex, or smoking status of the donor. We have specifically chosen the IIIB strain of HIV-1 for these studies because, in our laboratory, it consistently produces a low-level productive infection that permits the system to mimic the "reservoir" function presumed for macrophages.16 This system thus offers the opportunity to examine antiviral strategies designed to prevent primary infection of macrophages, as well as to inhibit transfer of infection from already infected macrophages to target lymphocytes.
EFFECTS OF AZT AND GM-CSF ON HIV REPLICATION IN AM

On days 6 and 10 post-infection, mean HIV antigen levels were reduced from 813 (±128) to 201 (±23) pg/mL (P = .002) and from 808 (±119) to 41 (±12) pg/mL (P < .001), respectively (Fig 2A and D). The combination was also effective in inhibiting transfer of infection to target PBMLs (Figs 1D and 2D). These results were comparable to those observed with 1 µmol/L AZT alone. Given the marked effectiveness of AZT alone, an additive effect of GM-CSF could not be elucidated. Notably, however, no antagonism of the effectiveness of AZT was demonstrated.

We next examined the pattern of HIV expression after drug removal and attempted to determine if virus expression could be observed in the cultures that had been completely suppressed by AZT or AZT + GM-CSF. To do this, infected AM monolayers that had been maintained in culture with or without drug exposure for 20 days post-infection were washed thoroughly, and PHA-stimulated PBMLs were added to the wells. The cultures were monitored for supernatant HIV antigen for 15 days, and representative results are shown in Fig 3. Supernatant antigen expression briskly rose in a comparable manner in infected control and GM-CSF-exposed cultures. In previously AZT-exposed cultures, virus expression was delayed after PBML addition compared with control cultures, but was still readily apparent by day 8 post-drug removal. In contrast, in four of six experiments, the combination of AZT + GM-CSF appeared to have a greater antiretroviral effect than AZT alone, in that virus expression was not seen after PBML addition and drug removal. In the other two experiments, the results with AZT + GM-CSF were comparable with AZT alone. Thus, although no differences in efficacy of viral inhibition could be seen between AZT and AZT + GM-CSF-treated cultures when drug was continuously present (Figs 1 and 2), differences became apparent after drug removal in the majority of studies (Fig 3).

No cytotoxic effects of AZT and/or GM-CSF were noted in AM cultures or in cultures treated with concentrations 10-fold higher than those used in these studies.

DISCUSSION

The increasingly recognized role of the monocyte/macrophage in the pathogenesis of HIV-related disorders has made it important to examine the efficacy of antiviral agents in cells of this lineage in addition to lymphoid cell systems. To this end, we have previously used the U937 monocytic cell line to examine the anti–HIV-1 activity of AZT, GM-CSF, and interferon-gamma.10,11 All showed inhibitory activity in this system, with the combination of AZT + GM-CSF demonstrating synergism. Despite the usefulness of continuous cell lines, it is important to examine the efficacy and potential toxicity of antiviral agents in normal human cells. We have chosen AMs obtained by bronchoalveolar lavage for this purpose, as they represent a differentiated tissue macrophage which can be placed in culture in a high degree of purity. The limitations of this system are the necessity for an invasive procedure to obtain the cells and the relatively small number of cells retrieved. In this study, we chose to try to extend our previous findings with AZT + GM-CSF in U937 cells to normal macrophages. The concentrations of 1 µmol/L and 30 U/mL were chosen based on the synergy previously demonstrated with these concentrations.13

Our results demonstrate that AZT at 1 µmol/L was effective in inhibiting the low-level, productive infection of AMs and in preventing transfer to stimulated target cells, as long as the drug was maintained in the culture system. This
confirms the reported efficacy of AZT in monocytic cell systems described by Perno et al.11

In contrast to the results in U937 cells, GM-CSF was not demonstrated to have antiviral activity in AMs. Importantly, however, potentiation of HIV replication was not demonstrated. This is relevant given the recent observation that this cytokine can upregulate the HIV genome in a nonproductive clone (designated U1) of U937 cells and in peripheral blood monocytes.15-17 However, in the latter report by Perno et al.,19 HIV replication in blood monocytes that were permitted to mature in vitro for 5 days was not affected by GM-CSF, a result consistent with our findings in a differentiated tissue macrophage. Whether the differential effect of GM-CSF on HIV replication in monocytes versus mature macrophages is related to the state of cellular differentiation or the number of cellular receptors for GM-CSF is not yet entirely clear.17

The ability of human AMs to respond to GM-CSF in other biologic systems18-20 implies the presence of GM-CSF receptors, and these cells have recently been directly shown to possess approximately 350 GM-CSF receptors per cell,20 a figure comparable with that reported for blood monocytes.21

In addition, our results indicate that the relatively lympho- cytotropic HIV strain, IIIB, was not converted to a more monocytotropic strain in AMs, as has been noted in peripheral blood monocytes.17 We specifically decided to use the IIIB strain in these studies in order to achieve the low-level, productive infection desired (see “Results”) and to examine the question of “conversion” of the tropic characteristics of an isolate by GM-CSF. It should also be noted that although the IIIB strain is generally termed lymphocytotropic, this is only a relative characteristic, as illustrated by the infected laboratory worker from whom the IIIB strain was isolated only from the blood monocyte fraction without a detectable viral genomic alteration.22 As GM-CSF has been, and is currently, in clinical trials in HIV-infected individuals, it is important to try to determine if this upregulation occurs in normal cells in vitro or in vivo. Although we did not examine these cells for viral mRNA or for the intracellular accumulation of virions in vacuoles, our data are encouraging with regard to supernatant parameters of HIV replication and the transfer to target lymphocytes. We have examined GM-CSF at concentrations up to 300 U/mL in the AM system and have confirmed that neither inhibitory nor stimulatory effects on HIVIIIB replication in AMs are seen (data not shown). Furthermore, in clinical trials in which GM-CSF has been given long-term to individuals with HIV infection, no consistent alterations in serum HIV antigen have been noted (J. Groopman, personal communication, October 1989).

The combination of AZT + GM-CSF demonstrated anti-HIV activity comparable with AZT alone at the concentrations examined. The degree of effectiveness of AZT precluded the ability to demonstrate an additive or synergistic effect of GM-CSF but, perhaps just as importantly, no antagonism was demonstrated. This is encouraging, as GM-CSF has demonstrated efficacy in reversing the cytopenias seen in HIV infected individuals14 and there is great interest in using GM-CSF to diminish the toxicity of AZT, irrespective of whether it possesses any antiviral activity of its own. The inability to induce virus replication in four of six AZT + GM-CSF--treated cultures after drug removal and PBML addition is of interest in that it suggests an additive antiviral effect of the combination. This may well be explained by the recent report of GM-CSF's ability to increase the anabolic phosphorylation of AZT in monocytes, resulting in a lowering of the AZT ED50.17

In summary, our results suggest that AZT is an effective anti--HIV-1 agent in cells of macrophage lineage and that its activity is not antagonized, and may be augmented, by GM-CSF. This study lends further support to the planned human trials using these agents in combination.

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