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# Inhibitory Effect of 3′-Azido-3′-Deoxythymidine on Proliferative Responsiveness of $CD8^+$ Lymphocytes to Interleukin-2

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**Although several studies have shown that 3′-azido-3′-deoxythymidine (AZT) is not toxic for $CD4^+$ lymphocytes, its effect on $CD8^+$ cells has never been studied in a systematic way. We purified $CD8^+$ cells from the peripheral blood mononuclear cells of both human immunodeficiency virus (HIV)-seronegative and HIV-infected individuals by means of magnetic beads that had been coated with monoclonal antibodies. We report that AZT, but not two other nucleosides tested, inhibited the interleukin-2-dependent proliferation of $CD8^+$ lymphocytes in a dose-dependent manner. No such effect was observed with regard to $CD4^+$-enriched populations. The AZT-mediated antiproliferative effect did not appear to be related to either the $CD4^+$ count or to prior treatment with this drug in the case of HIV-seropositive subjects.**

The use of 3′-azido-3′-deoxythymidine (AZT) in the treatment of human immunodeficiency virus type 1 (HIV-1)-associated disease is associated with both the emergence of viral drug resistance (6, 15) and a variety of toxic complications (5, 14). The latter may be due, in part, to the effects of this drug on the immune system, although little evidence in this regard exists. However, it has been reported that AZT can be incorporated into the chromosomal DNA of bone marrow-derived human cells (17).

$CD8^+$ lymphopenia is a relatively late event in HIV-1-infected subjects, usually occurring after the significant loss of $CD4^+$ lymphocytes (14). This phenomenon is still unexplained, although it has been reported that the $CD8^+$ cells of patients with AIDS possess diminished clonogenic potential (13). We studied the effects of three antiviral nucleosides, AZT, 2′-deoxy-3′-thiacytidine (3TC), and 2′,3′-dideoxyinosine (ddI), on the responsiveness of $CD8^+$ lymphocytes to the lymphokine interleukin-2 (IL-2).

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**Isolation of peripheral $CD8^+$ lymphocytes.** Peripheral blood mononuclear cells were obtained from both healthy HIV-seronegative and HIV-infected donors by Ficoll-Hypaque centrifugation (Pharmacia, Uppsala, Sweden) as described previously (15) and were stimulated with phytohemagglutinin (PHA-PI) (5 μg/ml; Difco Laboratories, Detroit, Mich.) for 3 days. After this time, nonadherent cells ($\approx 10^7$) were washed, and the cells were incubated on ice with 5 μg of the anti-CD8 monoclonal antibody (MAb) OKT8 for 30 min as described previously (10). Cells were then washed twice and were incubated at 37°C for 1 h with magnetic particles coated with goat anti-mouse immunoglobulin G. The latter were separated by using a magnetic field as described previously (10) and were maintained at 37°C in RPMI 1640 medium (Gibco Corp., Toronto, Ontario, Canada) supplemented with 10% fetal calf serum–10 U of IL-2 (Boehringer-Mannheim, Montreal, Quebec, Canada) per ml–2 mM L-glutamine–250 U of penicillin per ml–250 μg of streptomycin per ml. After 48 h, the magnetic particles were separated from the cells by means of a magnet and the cells were maintained at a density of $5 \times 10^5$ to $1 \times 10^6$ cells per ml in the same medium (10).

Flow cytometry was performed on enriched $CD8^+$ populations by using both anti-CD4 (Leu3a-fluorescein isothiocyanate [FITC]; Becton-Dickinson, Mountain View, Calif.) and anti-CD8 (Leu2a-phycoerythrin [PE]; Becton-Dickinson) MAbs with a FACScan flow cytometer (Becton-Dickinson) (10). When cultures of cells from healthy, HIV-seronegative donors were established, between 93 and 97% of cells were $CD8^+$, while most contaminating cells (2 to 5%) were $CD4^+$ (Fig. 1). In the case of HIV-infected individuals, the same degree of $CD8^+$ purity was generally not achieved (85 to 91% $CD8^+$ cells).

**Proliferative responsiveness of $CD8^+$ lymphocytes.** $CD8^+$ cells were plated in triplicate ($10^5$ cells per well) in 96-well plates in the presence or absence of different drug concentrations and 10 U of IL-2 per ml. After 72 h, cultures were pulsed with 1 μCi of [$^3$H]thymidine ([$^3$H]TdR; New England Nuclear, Boston, Mass.) for a further 22 h, after which the cells were harvested on glass fiber filters and the levels of incorporated radioactivity were determined as described previously (18). AZT, ddI, and 3TC were gifts of Burroughs-Wellcome, Inc., Durham, N.C.; Bristol-Myers Inc., Wallingford, Conn.; and Glaxo Ltd., Greenford, United Kingdom, respectively.

**Effects of AZT, 3TC, and ddI on $CD8^+$ lymphocyte proliferative responsiveness.** Table 1 shows that the levels of incorporation of [$^3$H]TdR by IL-2-maintained $CD8^+$ lymphocytes were reduced by about 55 and 30% in the presence of AZT concentrations of 4.0 and 0.4 μM, respectively. In several cases (e.g., subject 3), we used cells from asymptomatic HIV-seropositive donors and obtained findings similar to those obtained when seronegative subjects were studied. In contrast, a variety of concentrations of each of 3TC and ddI had little or no effect in this regard. Cell counts of lymphocyte populations showed that fewer lymphocytes were present after exposure to 4.0 or 0.4 μM AZT in our protocol. Similar results were

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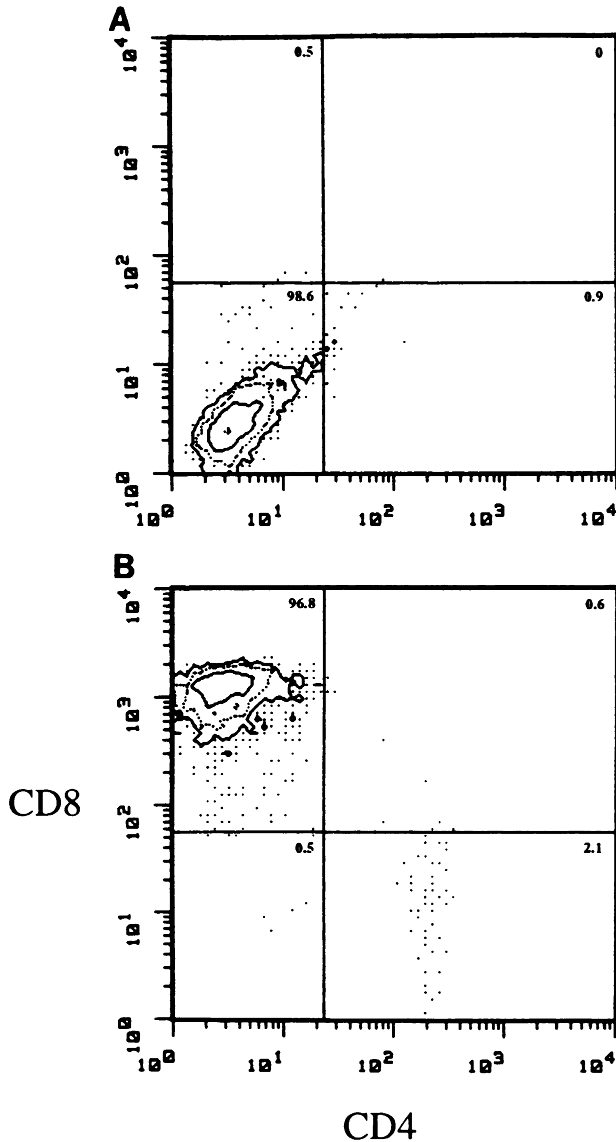


FIG. 1. Two-color flow cytometry analysis of enriched CD8+ cells labeled with Leu3a-FITC and Leu2a-PE MAbs (B). The culture studied was derived from a healthy HIV-seronegative donor. Controls were CD8+ cells labeled with mouse MAbs directed against actin (A).

obtained with cells from each of 11 individuals studied, 5 of whom were not infected with HIV. Of the six HIV-seropositive individuals, two were asymptomatic, while the others possessed various numbers of CD4 cells and ranged from being mildly symptomatic to having advanced disease. No significant differences between drug-treated and untreated cultures were observed in regard to the percentages of viable cells, as determined by trypan blue exclusion.

Summary results are shown in Table 2 and indicate an impairment of CD8+ proliferative responsiveness to IL-2 in the presence of AZT in each instance. No increased effect was observed when CD8+ cells from HIV-seropositive individuals were studied, regardless of whether such individuals had been treated with AZT. The doses of AZT used were between 400 and 500 mg/day for each of these individuals (the duration of therapy was 16 to 32 months). Nor did the CD4 count or degree of symptomatology apparently influence the AZT-mediated antiproliferative effect. The two mildly symptomatic subjects studied presented with slight lymphadenopathy, but neither had suffered opportunistic infections at the time of the

TABLE 1. Inhibition of IL-2-driven proliferation of CD8+ cells by AZT

| Subject | HIV sero-status | Drug | Concn (μM) | % Inhibition of incorporation of [3H]TdR | No. of viable cells ($10^5$) | % Cell viability |
|---|---|---|---|---|---|---|
| 1 | − | AZT | —[d] | 0 | 2.8 ± 0.4 | 98 |
| | | | 0.04 | 12.5 ± 2.1[a] | 2.1 ± 0.2 | 98 |
| | | | 0.4 | 31.8 ± 5.0 | 1.6 ± 0.2 | 97 |
| | | | 4.0 | 55.0 ± 4.8 | 1.2 ± 0.2 | 98 |
| | | 3TC | — | 0 | 2.8 ± 0.4 | 96 |
| | | | 0.1 | 3.4 ± 0.5 | 2.6 ± 0.2 | 99 |
| | | | 1.0 | 0[b] | 3.5 ± 0.4 | 93 |
| | | | 10.0 | 0 | 3.0 ± 0.4 | 99 |
| | | | 100.0 | 6.5 ± 0.8 | 2.5 ± 0.5 | 98 |
| | | ddI | — | 0 | 2.8 ± 0.4 | 97 |
| | | | 0.1 | 0 | 3.1 ± 0.2 | 98 |
| | | | 1.0 | 5.6 ± 0.9 | 2.6 ± 0.4 | 95 |
| | | | 10.0 | 7.5 ± 0.6 | 3.2 ± 0.3 | 96 |
| | | | 100.0 | 3.1 ± 0.5 | 2.4 ± 0.3 | 98 |
| 2 | − | AZT | — | 0 | 3.0 ± 0.4 | 97 |
| | | | 0.04 | 6.2 ± 0.9 | 2.4 ± 0.3 | 98 |
| | | | 0.4 | 27.5 ± 4.7 | 1.7 ± 0.3 | 96 |
| | | | 4.0 | 58.4 ± 6.8 | 1.4 ± 0.2 | 95 |
| | | 3TC | — | 0 | 3.0 ± 0.4 | 97 |
| | | | 0.1 | 3.5 ± 0.5 | 3.2 ± 0.4 | 98 |
| | | | 1.0 | 0 | 2.8 ± 0.4 | 98 |
| | | | 10.0 | 5.7 ± 0.4 | 2.6 ± 0.4 | 98 |
| | | | 100.0 | 0 | 2.9 ± 0.5 | 97 |
| | | ddI | — | 0 | 3.0 ± 0.4 | 97 |
| | | | 0.1 | 0 | 2.9 ± 0.5 | 98 |
| | | | 1.0 | 4.7 ± 0.7 | 3.3 ± 0.9 | 99 |
| | | | 10.0 | 0 | 2.4 ± 0.6 | 98 |
| | | | 100.0 | 6.3 ± 0.8 | 2.6 ± 0.5 | 97 |
| 3 | +[c] | AZT | — | 0 | 2.4 ± 0.5 | 96 |
| | | | 0.04 | 10.3 ± 1.9 | 1.9 ± 0.4 | 98 |
| | | | 0.4 | 42.7 ± 5.3 | 1.5 ± 0.2 | 95 |
| | | | 4.0 | 61.6 ± 8.3 | 1.2 ± 0.3 | 94 |
| | | 3TC | — | 0 | 2.4 ± 0.5 | 99 |
| | | | 0.1 | 0 | 2.7 ± 0.6 | 97 |
| | | | 1.0 | 4.7 ± 0.6 | 2.3 ± 0.3 | 96 |
| | | | 10.0 | 0 | 2.9 ± 0.5 | 98 |
| | | | 100 | 2.8 ± 0.4 | 2.6 ± 0.2 | 98 |
| | | ddI | — | 0 | 2.4 ± 0.5 | 97 |
| | | | 0.1 | 1.3 ± 0.1 | 2.0 ± 0.3 | 97 |
| | | | 1.0 | 0 | 3.2 ± 0.8 | 96 |
| | | | 10.0 | 4.6 ± 0.8 | 2.5 ± 0.6 | 98 |
| | | | 100 | 7.5 ± 1.2 | 1.9 ± 0.5 | 97 |

[a] Values are means ± standard errors.
[b] The number of counts per minute incorporated was greater than that in the absence of drug. For subjects 1, 2, and 3, the numbers of counts per minute incorporated in the absence of drug were 223,076, 186,958, and 157,814, respectively.
[c] The subject was asymptomatic.
[d] —, no drug.

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TABLE 2. Summary of results obtained for 11 subjects studied

| HIV serostatus | Health status | No. of CD4 cells/mm$^3$ | History of anti-HIV therapy | % Impairment of CD8$^+$ response to IL-2 in presence of AZT[a] | Impairment of CD4$^+$ response to IL-2 in presence of AZT |
|---|---|---|---|---|---|
| –[b] | Healthy | 925[c] | – | 56.0[c] | –[d] |
| + | Asymptomatic | 610 | – | 61.6 | – |
| + | Asymptomatic | 520 | – | 52.7 | – |
| + | Mildly symptomatic | 275 | – | 65.4 | ND[e] |
| + | Mildly symptomatic | 180 | AZT[f](16mo) | 42.9 | ND |
| + | Symptomatic | 120 | AZT(32mo) | 58.0 | ND |
| + | Symptomatic | 160 | AZT(23mo) | 54.3 | – |

[a] Concentration of 4 μM AZT.
[b] Five subjects.
[c] Values are the averages for the five subjects.
[d] Negative for two of two subjects studied.
[e] ND, not done.
[f] AZT had been administered at doses that varied between 300 and 400 mg/day.

study. The two symptomatic subjects had each experienced one episode of *Pneumocystis carinii* pneumonia.

In several cases, we recovered populations of CD4$^+$-enriched cells from our separations and examined them for their proliferative responsiveness to IL-2, as described above for CD8$^+$ cells. No impairment of incorporation of [$^3$H]TdR was detected in the presence of AZT (Table 2).

The use of AZT in HIV-infected individuals was first authorized on the basis of the drug's antiviral effect and lack of toxicity, including an absence of adverse effects on immunological responses in vitro, such as mitogen-induced cell proliferation (14). However, AZT can be toxic for human and mouse hematopoietic progenitors, as tested in vitro, and can exert antiproliferative effects on such cells (2–4, 16).

We have shown that AZT can also inhibit the ability of CD8$^+$ cells to proliferate in the presence of IL-2 without affecting cellular viability. In contrast, two other antiviral nucleosides, 3TC and ddI, had no such effect. CD8$^+$ cells are thought to be important in limiting viral spread by inhibiting HIV-1 replication in CD4$^+$ cells through the secretion of soluble factors (7) and cytotoxic CD8$^+$ T-lymphocyte lysis of HIV-infected targets (8, 12). Loss of these activities is associated with clinical deterioration (8). Our findings suggest that AZT may adversely affect CD8$^+$ cell proliferative responsiveness. Conceivably, this may be a factor in explaining the limited clinical benefit of AZT in certain populations of treated subjects (1).

Possible mechanisms that may govern the reduced proliferative responsiveness of CD8$^+$ cells to IL-2 are under investigation. It is known, however, that AZT can prevent the establishment of HIV infection in CD8$^+$ cells, suggesting that this drug is taken up and metabolized to its active triphosphate form in such cells (9). We are trying to determine whether the AZT-mediated antiproliferative effect described here is attributable to the phosphorylated or native form of this compound. In this regard, other groups have reported that AZT may inhibit DNA repair in human lymphocytes (11) and affect hematopoietic precursor cells in both animals and humans (2–4).

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