Induction of Monocyte Proliferation and HIV Expression by IL-3 Does Not Interfere With Anti-Viral Activity of Zidovudine

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Myelosuppression is a major symptom in the acquired immunodeficiency syndrome (AIDS). Moreover zidovudine, an anti-retroviral drug used to treat AIDS patients has myelosuppressive side effects. Therefore treatment with IL-3, a multi-lineage hemopoietic growth factor may be beneficial for zidovudine-treated individuals. In this study we examined the effect of IL-3 on human immunodeficiency virus (HIV) expression. The proliferative response to rIL-3 and the effects on the replication of the monocytotropic HIV variant, HTLV-III Ba-L, in the absence or presence of the anti-retroviral drug zidovudine was studied in purified human peripheral blood monocytes. Zidovudine concentrations sufficient for complete inhibition of HIV replication did not affect rIL-3 induced monocyte proliferation. Although rIL-3, like rGM-CSF, was able to augment HIV expression in monocytes, it did not interfere with the anti-retroviral activity of zidovudine. These data indicate that rIL-3 is a potential candidate for use in myelosupportive therapy in AIDS patients treated with anti-retroviral drugs.

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MATERIALS AND METHODS

Preparation and culture of human peripheral blood monocytes. Human peripheral blood monocytes (>95% pure) were isolated from healthy HIV seronegative donors by Percoll density gradient separation followed by centrifugal elutriation as described previously. Monocytes were cultured in endotoxin-free Iscove's modified Dulbecco's medium (IMDM, GIBCO Laboratories, Grand Island, NY) supplemented with 7.5% (vol/vol) heat inactivated pooled human serum and antibiotics (penicillin, 100 IU/mL; streptomycin, 100 µg/mL) at a final concentration of 10^6 cells/mL and plated in 96-well plates, 100 µL per/well (Nunc).

Colony stimulating factors. Recombinant human IL-3 (10 ng/mL, Gust-broades, Delft, the Netherlands) was produced by Bacillus licheniformis using the cDNA as described. It is a 15 kD non-glycosylated protein and has a correct N terminus. The sample was endotoxin free as was determined from the Limulus assay. One unit of IL-3 activity, as defined by the half-maximal response of the IL-3 dependent cell-line AML 193 corresponds to 1 ng/mL. Recombinant human GM-CSF (100 U/mL) was obtained from Sandoz AG, Basel, Switzerland. Doses were recommended by suppliers. MDM were cultured in the presence of CSF during the first 5 days of culture.

Virus infection and detection. All studies were performed with the monocytotropic HIV variant HTLV-III Ba-L which was a kind gift of Dr M. Popovic (NCI, Bethesda, MD). Infection of cells was performed after 5 days of culture (dosis 5.10^6 cpm/mL RT activity). The cells were washed 24 hours after infection with 4 volumes of medium and further cultured under the conditions described above. The medium was changed every 5 days and tested for virus production in a p24-ag capture ELISA. In brief, inactivated culture supernatants were added to microtiter plates coated with purified human-α-HIV IgG. After washing, bound p24 was detected with horseradish peroxidase-labeled monoclonal antibody to p24, followed by substrate. Twenty minutes before infection of the
5-day precultured monocytes, the medium was changed with medium containing zidovudine (Wellcome) (1 nmol/L; 10 nmol/L; 100 nmol/L; 1 μmol/L; 10 μmol/L; and an untreated control). After infection the cells were washed and further cultured in medium with the indicated concentration of zidovudine.

**Proliferation assays.** CSF-induced proliferation was determined by ³H-thymidine incorporation. Purified monocytes (10⁵ per well) were cultured in 96-well flat bottom microtiter plates (Nunc) in a total volume of 100 μL medium containing CSF. On day 4, cultures were pulsed with 7.3 kBq ³H-thymidine and harvested after 18 hours.

To test the effect of zidovudine on CSF-induced proliferation, the anti-retroviral drug was added after 5 days of culture. Cultures were continued in the presence of the indicated doses of zidovudine. On day 13, cultures were pulsed with 7.3 kBq ³H-thymidine and harvested on day 14.

**RESULTS**

**rIL-3 induces proliferation of purified monocytes and enhances HIV expression.** rIL-3 induced a moderate proliferation in purified peripheral blood monocytes (Fig 1A). This proliferation was consistently higher than the proliferation observed upon treatment with rGM-CSF. The combination of both CSFs showed the strongest induction of monocyte proliferation (Figs 1A, 2A). In a preliminary set of experiments performed to determine the optimal treatment modality for enhancement of HIV expression by rIL-3 in freshly isolated monocytes, it appeared that immediate addition of rIL-3 at the start of culture had optimal effect (data not shown). A 5-day culture in the presence of 10 ng rIL-3/mL before inoculation showed an increase in virus production of 2 to 5 times compared to the unstimulated control (Fig 1B). Pre-incubation with 100 U rGM-CSF/mL showed a similar increase in virus production. Treatment with a combination of rIL-3 and rGM-CSF showed a less than additional effect on virus production compared to treatment with one of the CSFs alone (Fig 1B).

**Zidovudine inhibits HIV replication in IL-3 treated monocytes without impairment of monocyte proliferation.** Although rIL-3 was able to augment virus production in
monocytes, it did not interfere with the anti-retroviral effect of zidovudine (Fig 2B). In the presence of 10 ng/mL rIL-3 during the first 5 days of culture, a dose of 1 μmol/L zidovudine was found to be sufficient to establish complete inhibition of virus production compared to a 10 μmol/L dose zidovudine in the untreated control. Even when rIL-3 was continuously present, it did not negatively affect the anti-retroviral activity of zidovudine (data not shown). A similar potentiating effect was observed with rGM-CSF. The combination of both growth factors did not impair the activity of zidovudine. Knowing that zidovudine in vivo interferes with the outgrowth of hemopoietic precursor cells, we tested whether zidovudine, under conditions in which it completely inhibits HIV production, interferes with in vitro CSF-induced cell proliferation. However, under conditions of complete abolishment of HIV replication, rIL-3-induced monocyte replication was not affected by zidovudine treatment. This unaffected proliferation on day 14 of culture (Fig 2A), and the complete inhibition of virus expression by zidovudine at the same time indicate that down-regulation of virus replication by zidovudine in rIL-3 stimulated monocytes is not caused by inhibition of cell proliferation.

**DISCUSSION**

In this study we show that rIL-3, like rGM-CSF, is able to induce proliferation of purified peripheral blood monocytes and that it can augment HIV expression in this cell type. The anti-retroviral drug zidovudine was able to completely block HIV expression even in the presence of rGM-CSF or rIL-3.

The data on the enhancement of HIV expression by rIL-3 confirm previous findings by Koyanagi et al. The additional presence of rGM-CSF only slightly enhanced the rIL-3-induced effect. A similar phenomenon was recently described for rIL-3 and rGM-CSF-induced proliferation and was postulated to reflect the existence of both a common receptor and distinct receptors for these growth factors. We find that rIL-3, and to a lesser extent rGM-CSF, are able to induce proliferation in peripheral blood monocytes. However, the amount of 3H-thymidine incorporation is moderate, confirming earlier findings that only a very small fraction of the monocytes (3% to 5%) has the potential to proliferate in response to rIL-3 or rGM-CSF. Zidovudine, at therapeutic concentrations, did not affect CSF-induced proliferation. Even in the presence of the highest dose zidovudine, rIL-3-induced proliferation by monocytes was observed.

The anti-retroviral effect of zidovudine was not negatively affected by the presence of rIL-3 in the culture medium. In rIL-3 treated cultures, complete inhibition of virus production was established by a concentration of 1 μmol/L zidovudine, a concentration 10-fold lower than the concentration necessary to establish the same effect in cultures without rIL-3. The concentration sufficient to completely block virus production did not affect the growth factor induced monocyte proliferation.

It was reported by Perno et al. that GM-CSF potentiates the anti-retroviral effect of zidovudine by elevating the intracellular levels of the drug and by stimulating the phosphorylation of zidovudine to its active form. In our experiments we also found evidence for such potentiating effect by rGM-CSF, by rIL-3 and by the combination of both growth factors.

Proliferation of bone marrow cells is controlled by endogenous IL-3 produced by mature, CD28+ T cells. In previous studies, a selective loss of functionally and phenotypic mature T cells in early HIV infection was reported. The lack of endogenous IL-3 may contribute to the myelosuppression in HIV-infected individuals and may render them sensitive to the hematopoietic abnormalities induced by zidovudine treatment.

The enhancement of HIV replication in vitro by both rGM-CSF and rIL-3 observed by us and others seems to caution against treatment with these CSFs of HIV-infected individuals in the absence of anti-retroviral drugs, although adverse effects of mono-therapy with GM-CSF have not been reported. However, our results show that in combination with zidovudine, IL-3 is a potential candidate for the treatment of HIV- or drug-induced myelosuppression in AIDS patients.

**REFERENCES**


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