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RECOMBINANT INTERFERON-ALPHA 2 INHIBITS HIV REPLICATION IN CHRONICALLY INFECTED PROMONOCYTIC CELLS

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Summary. - Promonocytic cells U937 with previously established HIV-1 persistent infection, were treated with increasing doses of the recombinant INF-alpha 2. This resulted in a significant decrease of virion-associated reverse transcriptase levels in medium of the cultures studied, most pronounced by the highest interferon doses, but depending on this cytokine presence. In spite of the marked restrictive effect of the interferon on the infectious virus production the synthesis, of viral structural proteins by the U937 cells, as detected by immunofluorescence, was not affected. The therapeutic index of interferon was considerably high.

Key words: rIFN-alpha 2; promonocytic cells; human immunodeficiency virus; viral multiplication; restriction

Human immunodeficiency virus (HIV) can infect various cell types bearing CD4 molecules at their surface, among them cells of monocyte/macrophage lineage. Infection of these cells results in a persistent production of HIV without obvious cytopathogenicity or target cell lysis. Thus, cells of mononuclear phagocyte lineage might serve as circulating tissue reservoir of HIV in the human host. Moreover, persistently infected macrophages are less susceptible to the antiretroviral effect of dideoxynucleosides, e. g. 3'-azido-3'-deoxythymidine (AZT) (reviewed by Crowe *et al.*, 1989). However, endogenous interferon showed greater efficacy to inhibit HIV replication in chronically infected monocytes and macrophages than AZT (Poli *et al.*, 1989). We studied the prolonged effect of purified recombinant interferon-alpha 2 (rIFN-alpha 2) in a promonocytic cell line (U937) with previously established HIV infection. U937 cells share several properties of immature monocytes and are susceptible to infection with HIV (Asjo *et al.*, 1987).

To asses the effect of rIFN-alpha 2 on the cell growth, uninfected cell cultures were exposed to various concentrations of this cytokine (10 U/ml, 100 U/ml, 1000 U/ml). After 3 days of treatment, the viable cell count was checked by trypan blue dye exclusion method and the percentage reduction of the

Table 1. The effects 3'-azido-3'-deoxythymidine (AZT) and of the recombinant interferon-alpha 2 on the HIV replication in chronically infected promonocytic U937 cells

Antiviral	CyD_{50}	ED_{50}	Therapeutic index
rIFN-alpha 2	1493.3 U/ml	3.4 U/ml	496.7
AZT	104.2 μm	78.5 μm	1.3

CyD_{50}: the drug/cytokine concentration reducing the viable uninfected cell count by 50 percent

ED_{50}: the drug/cytokine concentration reducing the HIV-1 reverse transciptase activity in the nutrient medium of the infected U937 cell cultures by 50 per cent

viable cells was calculated. Further, the drug concentration required to reduce the viable cell count by 50 % (CyD_{50}) was determined from exponential dose-response curve (Hu *et al.*, 1989). The results shown in Table 1 represent mean values of three independent experiments.

The inhibitory effect of rIFN-alpha 2 on HIV-replication in persistently infected promonocytic cells was investigated. Persistence of virus production in infected U937 cells was shown as stable over 2 months (reverse transcriptase-RT activity in supernatants from infected cultures checked once a week). U937 cells were cultivated 14 days in the presence of 1000 U/ml, 100 U/ml or 10 U/ml of rIFN-alpha 2, respectively. The antiviral effect was determined according to HIV-associated RT amounts released in medium and according to viral antigen expression in infected cells. RT activity of cell-free supernatants from rIFN-alpha 2 treated cultures started to decrease not later than 4 days after first treatment. This effect appeared as dose-dependent. When compared to untreated HIV-infected U937-cells, RT activity at the end of experiment was reduced in the presence of 1000 U/ml to 5.3 %, at 100 U/ml to 14.6 % and at 10 U/ml to 37.6 %. The percentages were calculated as mean values of RT levels on day 7 and 14 of IFN-α2 continuous presence in two experiments. The concentration of the cytokine, which reduces RT activity by 50 % was determined (ED_{50}) (Hu *et al.*, 1989). Indirect immunofluorescent assay to check the effect of IFN on viral antigen synthesis in infected U937 cells was performed by using polyclonal serum from a HIV-seropositive patient. The range of cells expressing HIV-antigens was 81–89 % in infected controls and 83–91 % in cultures treated by 1000 U/ml of IFN-α2. At studied concentrations rIFN-alpha 2 did not reduce the percentage of cells expressing HIV antigens, what suggests strongly, that in chronically infected promonocytic cells rIFN-alpha 2 exerts its anti-HIV effect in the late stages of retrovirus life cycle. The therapeutic indexes (CyD_{50}/ED_{50} ratio) of rIFN-alpha 2 and of AZT were determined. CyD_{50} and ED_{50} for AZT were calculated, from our previous experiments with HIV-infected U937 cells (Uherová *et al.*, 1991).

The therapeutic index of rINF-alpha 2 in the cell-culture system used is rather high. However, a complete inhibition of HIV replication was not observed even at the final concentration of 1000 U/ml of rIFN-alpha 2. Moreover, removal of this cytokine from previosly treated HIV-infected cultures resulted in a rapid increase of RT levels within 7 days (data not shown). Further study is needed to define the mechanismus involved in the control of HIV replication in chronically infected promonocytic cells by INF-alpha 2, which seems to be different from that one reported in acute HIV infection (Gendelman *et al.*, 1990).

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