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Effect of beta-interferon on HTLV-I infection of fractionated mononuclear cells of human cord blood

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INTRODUCTION

HTLV-I is a human T lymphotropic retrovirus associated *in vivo* with malignant leukemic transformation of infected cells. Actually it has been linked to cases of adult T-cell leukemia in Japan and of cutaneous T-cell lymphoma in USA [1].

HTLV-I infection causes a profound immune depression involving either natural immunity and antigen-dependent immune responses [2,3].

Preferential targets of HTLV-I in adults are T cells bearing CD4 phenotype. However, *in vitro* studies have shown that also mononuclear cells from human cord blood (CBMC),belonging to different T cell subsets,and bone marrow cells are highly susceptible to HTLV-I infection.Moreover these cells are more susceptible to retroviral infection than peripheral blood mononuclear cells [4,5]. In addition further studies performed on subpopulations separated through density gradient, indicate that HTLV-I preferentially replicates in T-cell fractions. In contrast the fraction enriched in large granular lymphocytes is highly resistant to the infection [5].

This *in vitro* model offers the possibility to investigate the protective effect of antiviral and immunomodulant agents on mononuclear cells, including populations of immune effector cells.

The present communication illustrates the effect of recombinant β-interferon (rβIFN) on the susceptibility to HTLV-I of human T cell subpopulations expressing CD4 or CD8 phenotype.

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MATERIALS AND METHODS

Cell preparation

CBMC were separated by Ficoll-Hypaque density gradients (Pharmacia, Uppsala Sweden), washed twice in RPMI-1640 (Gibco, Grand Island, USA) and separated into CD4+/CD8+ population through immunomagnetic beads conjugated with specific anti-CD4 or anti-CD8 (Oxoid Norway),monoclonal antibodies (MAb) according to a standard a standard procedure as described by Gaudernack et al. [6].

Briefly, CBMC were mixed with immunomagnetic beads at a final concentration of 20-40 × 10^6 CBMC/ml and anti-CD4-MAb-conjugated magnetic particles at a ratio of 2-3 beads

per cell expressing the specific membrane marker. Magnetic beads were previously washed in cold Phosphate buffered saline (PBS),containing 0.02% bovine serum albumin (Sigma, St.Louis, MO, USA), at 4°C. The mixture was incubated at 4°C for 30′ on a rotating wheel. Then the cell suspension was diluted 1:5 times in PBS containing 2% fetal calf serum (FCS, Gibco) and the cells were separated from the beads by application of a magnet (Oxoid). The positively selected CD4 subset was incubated overnigth at 37°C in order to detach the magnetic beads.The negatively selected cells were incubated with anti-CD8 conjugated particles, adopting the same procedure described above. The day after, the CD4 and CD8 subsets were washed in PBS/FCS by using the magnet to eliminate the beads detached from the cells.

The purity of the cell populations was evaluated by flow cytometry analysis (data not shown).

In vitro infection with HTLV-I

MT-2, an HTLV-I producing cell line derived from virus infected cord blood[7], was grown in RPMI-1640 medium supplemented with 20% FCS, glutamine, penicillin-streptomycin, (hereafter referred to as complete medium (CM)), in absence of IL-2 and passaged twice a week.

HTLV-I transmission was performed by coculturing CBMC, in toto or isolated CD4 or CD8 subsets, with lethally irradiated (12,000 Rad, 120 Gy, Cesium Gamma Cell 1000, Canada Atomic Energy LTD, Canada) MT-2 cells at ratio of 5:1. Cocultures were maintained by addition of 20IU/ml of recombinant IL-2 (Hoffman La Roche, Basel, Switzerland), to CM. Recombinant Interferon beta was provided by Serono Laboratories, Rome, Italy).

Evaluation of infection

Infection was evaluated by indirect immunofluorescence assay for the p19 virus core protein on methanol/acetone (1:3) fixed cells. The amount of integrated provirus in the genome of infected cells was determined using a sensitive «dot blot» hybridization assay.

Briefly, cells diluted at 1.5×10^6/ml were spotted on nitrocellulose membrane filters (Schleicher and Schull, Dassel, FRG), previously saturated with 0.5 N NaOH containing 1.5 M NaCl. The filters were then neutralized in 0.2 M Tris-HCL and 2x SSC (Sodium citrate 0.3M, NaCl, 3M pH 7.0) dried and baked at 80°C, for 2 hours.

Viral transcripts were evaluated by spotting 1.5×10^6 cells on the filters. The filters were then fixed in 3% NaCl, 10 mM $NaHPO_4$ 40 mM -1% glutaraldehyde and rinsed with proteolytic buffer, according to a procedure previously described[8].

DNA and RNA were hybridized with the Sst I-Sst I fragment of HTLV-I (8.5 Kilobases) digested from pMT-2 plasmid (kindly provided by R.C. Gallo). The probe was radiolabelled with ^{32}P-ATP by nick translation procedure. After hybridization, filters were exposed for autoradiography (Kodak XAR-5 films) for 72 hours.

RESULTS

T cell subsets, obtained through a positive selection, were found to be highly pure, being the percentage of CD4+ or CD8+ cells of 95 to 98% respectively (data not shown). CD4+ or CD8+ cells were exposed to HTLV-I by coculturing with MT-2 donor cell line and were subjected to one single treatment with 1000 IU/ml of beta-rβIFN at the onset of the coculture. The percentage of infected cells was evaluated weekly. The cocultures showed a doubling time of 7 days.

Both virus-exposed and control CD8+ cultures, showed lower number of viable cells in presence of rßIFN in the first week of culture,if compared with both CD4+ subset and *in toto* CBMC, grown at the same culture conditions (data not shown).

In a number of experiments, the extent of HTLV-I infection in CD4 subsets ranged from 5 to 12% and in CBMC from 1 to 5% p-19 positive cells after 1 week of culture. Cotreatment with rβIFN resulted in 70% and 50% inhibition of virus core protein expression in CD4/MT-2 and in CBMC/MT-2 cocultures re-

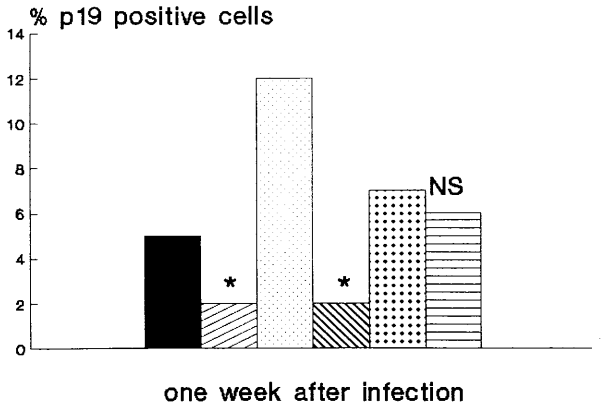

Figure 1 - Expression of p-19 in CBMC exposed to HTLV-I one week post infection.

■ CBMC/MT-2; □ CBMC/MT-2+ rβIFN 1000IU/ml; □ CD4/MT-2; □ CD4/MT-2+ rβIFN 1000IU/ml; □ CD8/MT-2; ≡ CD8/MT-2+ rβIFN 1000IU/ml.

spectively (*Figure 1*). On the other hand, CD8/MT-2 cocultures did not seem to be protected by rβIFN, since no significative decrease of the percentage of p19 positive cells occurred 1-2 weeks after infection (*Figure 1*).

In order to investigate whether the decrease of p19 expression following rβIFN treatment was correlated with a parallel changes of virus integration in the genome of infected cells, DNA dot blot analysis was performed. It was found that the amount of integrated provirus decreased in both CD4/MT-2 or CD8/MT-2 cocultures treated with rβIFN, two weeks post infection (*Figure 2*). Densitometric analysis quantified these data, confirming a range of 30 to 60% inhibition of virus integration in T cell subsets, comparable to that detected in *in toto* CBMC/MT-2 cocultures.

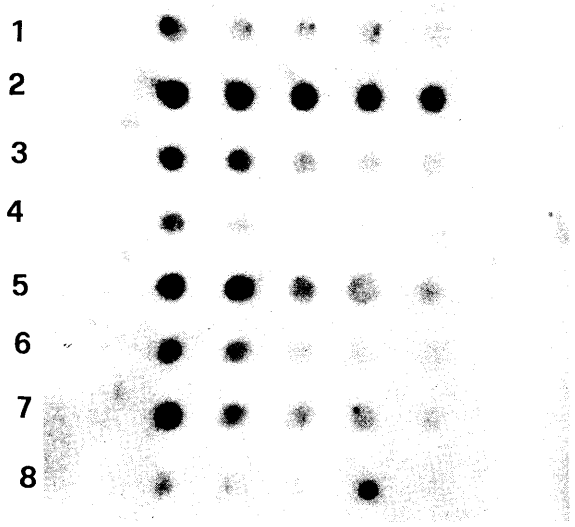

Figure 2 - Dot blot analysis of genomic DNA in CBMC exposed to HTLV-I, two weeks post infection.

1 HL-60, uninfected promyelocytic cell line; 2 MT-2; 3 CBMC/MT-2; 4 CBMC/MT-2+rβIFN 1000IU/ml; 5 CD4/MT-2; 6 CD4/MT-2+rβIFN 1000/ml; 7 CD8/MT-2; 8 CD8/MT-2+rβIFN 1000IU/ml.

DISCUSSION

Previous studies have pointed out that one single treatment with rβIFN is able to prime CBMC or PBMC to a long lasting antiviral response [9-11].

One of the mechanisms underlying rßIFN activity in this model could be related to interaction of the cytokine with effector cells of the immune system,thus counteracting HTLV-I transmission either by a direct antiviral effect and/or by enhancing immunological responses against virus- infected cells.

Either in toto CBMC and CD4+ populations were found to be partially protected by a single IFN treatment at the onset of the coculture. This was demonstrated by the extent of p19 expression that was greately reduced in CBMC/MT-2 and CD4/MT-2 cocultures exposed to rβIFN. Virus integration (*Figure 2*) and transcription (data not shown) were inhibited as well.

Similar results were found in CD8/MT-2 cocultures, although the p19 expression was only slightly affected by IFN treatment in the first two weeks of culture. It is possible that the total number of CD8+/p19+ cells was not affected by IFN treatment, whereas the number of provirus copies and RNA transcripts per cell was reduced by exposure to this cytokine, with respect to untreated controls. In any case the protective effect of IFN on CD8+ cells was evident in a later phase of infection, causing a degree of inhibition of the percentage of p19 positive cells with respect to that of untreated controls, similar to that observed in the CD4+ population (data not shown).

Interpretation of the present results is complicated by the finding that the CD4+ subpopulation is more susceptible to HTLV-I infection than the CD8+ subset in the first 2-3 weeks of exposure to HTLV-I. Conversely the extent of HTLV-I expression in CD8/MT-2 cocultures increased later. In this case the inhibitory effect of IFN appeared to be more pronounced than that observed during the first two weeks post infection (data not shown).

In conclusion, the present report points out that rßIFN is capable of reducing HTLV-I infection in both CD4+ and CD8+ populations. Further studies are needed to understand the mechanism of rββIFN impairment of virus transmission, in order to esthablish whether immunological and/or direct biochemical events are involved.

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