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Protective effect of interferon β on human T cell leukaemia virus type I infection of CD4+ T cells isolated from human cord blood

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Abstract. The present study shows the effect of human interferon β (IFNβ) on the susceptibility of highly purified cord blood CD4+ T cells to infection with the human T cell leukaemia virus type I (HTLV-I). Unfractionated cord blood mononuclear cells (CBMC), or a separated CD4+ T cell subpopulation (CBCD4) were exposed to HTLV-I by cocultivation with a chronically infected virus-donor cell line. The results show that presence of proviral DNA as well as virus transcription was markedly reduced by IFNβ in both populations, indicating that this cytokine protects not only unfractionated CBMC but also purified CBCD4 cells from virus infection. Moreover IFNβ treatment caused 60%–80% inhibition of virus expression in CBCD4, assayed as the presence of virus core protein p19. This study demonstrates that IFNβ is able to inhibit HTLV-I infection of CBMC through a mechanism that does not necessarily involve cell-mediated natural or antigen-dependent immunity afforded by CBMC subpopulations distinct from targets of HTLV-I infection. Therefore it is reasonable to conclude that IFNβ has a direct protective effect on CBCD4, through induction of antiviral resistance/activity in target cells.

Key words: HTLV-I – Interferon – Cord blood – 2′,5′-oligo(A) synthetase

Introduction

Human retrovirus infections are frequently associated with lymphoproliferative disorders, immunodeficiency and neurological diseases [9, 29]. Human T cell leukaemia virus type I (HTLV-I) has been aetiologically linked to adult T cell leukaemia [32], to cutaneous T cell lymphoma [26] and recently to tropical spastic paraparesis [20]. The predominant targets of HTLV-I infection in vivo are CD4+ T cells, which undergo clonal expansion following virus infection [12, 27]. However, the mechanisms responsible for this selective effect in vivo are still unclear. In vitro studies have shown that HTLV-I is able to infect a broad range of either lymphoid human cells of different phenotype and origin [16, 19] or non-lymphoid human cells such as endothelial cells [13], osteosarcoma cell lines [3], and cells of neuroectodermal origin [18]. However, it is suggested that only T cells are immortalized by HTLV-I in vitro. It follows that a number of mechanisms must be specifically involved in the virus-mediated activation of the target T cells [11] and successively in the selection of HTLV-I permanently proliferating clones [14].

Immortalization of T lymphocytes by HTLV-I in vitro, is not restricted to mature peripheral T cells but includes also bone marrow, thymus and cord blood cells [1, 16, 19]. The most suitable model of HTLV-I transmission to normal recipient cells in vitro is represented by virus exposure to mononuclear cells isolated from human cord blood (CBMC) instead of mononuclear cells from adult peripheral blood (PBMC). In fact virus core protein is expressed in an earlier stage of HTLV-I infection in CBMC as compared to PBMC [17]. This facilitates a short-term screening of drug effects on virus infection.

It is known that interferons (IFN) play a pivotal role in the early response against virus infections [31]. Previous studies have shown that IFN and especially IFNβ are able to prevent virus production and release from a cell line chronically infected with HTLV-I, (i.e. the MT-2 line) [23], and transmission of the virus to mononuclear cells in toto [8, 17, 24]. In fact, one single treatment at the onset of the donor/recipient cell coculture allows a relatively long-lasting protection of CBMC or PBMC exposed to virus infection [4, 5, 17]. Other classes of biological response modifiers, such as prostaglandins A, were found to prevent expansion of the transformed clone in a late phase of infection [6]. However, the exact mechanism underlying the impairment of CBMC or PBMC infection in vitro by IFNβ

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is still under investigation. In fact two possible and not mutually exclusive mechanisms could be involved: (a) a direct antiviral effect on donor and/or recipient cells and (b) an indirect effect mediated by enhancement of the functional activity of immune effector cells, capable of controlling HTLV-I infection. The present study was designed to distinguish the direct activity of IFNβ from immunomediated effects, possibly afforded by immune cells other than CD4 lymphocytes. Therefore CD4+ T cells were isolated from whole CBMC and tested in the presence of IFNβ in an HTLV-I transmission model in vitro.

Materials and methods

Preparation of CD4+ T cells from CBMC. Pure CD4+ T cells (hereafter called CBCD4) were obtained as follows: whole CBMC were separated by Ficoll/Hypaque density gradients (Pharmacia, Uppsala, Sweden). The cells were then washed twice in RPMI-1640 medium (Gibco, Paisley, UK) and positively separated through immunomagnetic beads (Dynabeads, Oslo, Norway) conjugated with specific monoclonal antibodies (mAb) anti-CD4 (Dynabeads) according to a standard procedure described by Gaudernack et al. [10]. Briefly, CBMC, at a final concentration of 20×10^6–40×10^6 cells/ml were mixed with anti-CD4-conjugated immunomagnetic beads at a ratio of two or three 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.) at 4 °C. The mixture was incubated at 4 °C for 30 min on a rotating wheel. The cell suspension was then diluted 1:5 in PBS containing 2% fetal calf serum (Gibco, PBS/FCS) and the cells adherent to the beads were separated from the other cells by application of a magnet (Dynabeads). The positively selected CBCD4 were incubated overnight at 37 °C in order to detach the cells from the magnetic beads. The purity of the cell populations was evaluated by flow cytometry analysis using a FACscan (Becton Dickinson, Mountain View, Calif.).

In vitro infection with HTLV-I and IFNβ treatment. MT-2, an HTLV-I-producing cell line derived from virus-infected cord blood [22], was grown in RPMI-1640 medium supplemented with 20% FCS, glutamine, penicillin/streptomycin (Flow Lab., Irvine, UK) (complete medium), in the absence of interleukin-2 (IL-2) and passaged twice a week. HTLV-I transmission was performed by coculturing CBMC in toto or CBCD4 with lethally irradiated (120 Gy, from a cesium gamma cell irradiator 1000, Canada Atomic Energy Ltd., Canada) MT-2 cells at a 5:1 ratio. Cocultures were maintained in the presence of 20 U/ml recombinant interleukin-2 (IL-2; Hoffman La Roche, Basel, Switzerland), and split weekly. Randomly chosen cultures contained 1000 U/ml natural human IFNβ (kindly provided by Sclavo SpA, Siena, Italy), added at the onset of the coculture. In some experiments recombinant IFNβ (Serono SpA, Rome, Italy) instead of the natural cytokine was used at the same concentration.

Time-controlled exposure of CBCD4 cells to HTLV-I. In order to obtain a time-controlled exposure of recipient cells to the virus, plastic-adherent monolayers of MT-2 cells were prepared using 1 ml poly-L-lysine (90 kDa, Sigma) at 500 μg/ml in RPMI-1640 medium supplemented with 10 mM HEPES buffer. Polylysine was added to 35×10-mm petri dishes (Nunc, Naperville, Ill.) for 1 h at room temperature. The plates were then washed three times in RPMI-1640 medium to remove unbound polylysine. Previously irradiated (120 Gy) MT-2 cells were washed three times, suspended in 1 ml at the desired concentration and placed on polylysine-coated dishes at room temperature for 45 min. Non-adherent cells were then poured off and the petri dishes were washed three times with RPMI-1640 medium. Separated CBCD4, suspended in RPMI-1640 medium containing 20% FCS and 25 mM HEPES (Flow), were added to the monolayer of MT-2, in the presence or not of 1000 U/ml IFNβ, at a recipient/donor ratio of 5:1. The plates were then incubated at 37 °C for 3 h. At the end of the incubation period, the non-adherent CBCD4 cells were removed by careful pipetting and 1 ml warm RPMI-1640 medium with 20% FCS was added once to wash the plate. The cells were tested in a cytotoxicity assay and for (2′-5′)oligo(A) synthetase activity (see below).

Cell-mediated cytotoxicity. The cytotoxicity assay was performed by mixing 0.1 ml target cells (5×10^5/ml), previously labelled for 1 h at 37 °C with 0.1 mCi ^{51}Cr (New England Nuclear, Boston, Mass.), and 0.1 ml effector cells at effector/target ratios ranging from 50:1 to 12.5:1 in 96-well round-bottomed microtitre plates. The plates were incubated in 5% CO_2 at 37 °C for 4 h; 0.1 ml was then collected from each well and radioactivity was counted with a gamma scintillation counter (model 5142 Packard Instrument Co., Downers Grove, Ill.). The specific percentage lysis was calculated as follows:

$$Lysis(\%) = \frac{S - C}{I} \times 100$$

where S represents the radioactivity (cpm) of the sample, A the radioactivity of the autologous control (i.e. of target incubated with 5×10^5 non-labelled target cells/well), and T (i.e. total count) the radioactivity of 0.05 ml labelled target cells.

Preparation of cell extracts and assay for (2′-5′)oligo(A) synthetase. The post-mitochondrial supernatant fraction (S-10) was prepared at 0–4 °C from 10^7 packed cells, either untreated or treated with IFNβ, lysed into 1.5 volumes of homogenization buffer (10 mM TRIS/HCl, pH 7, 7 mM EtSH, 10 mM KCl, 1.5 mM $Mg(OAc)_2$, 0.5% NP-40, Sigma). The homogenate was centrifuged at 10 000 *g* for 10 min. The (S-10) supernatant was either assayed immediately or stored in aliquots at −80 °C. Protein concentration was determined by using the Bio-Rad protein assay. Proteins (120 μg) from the S-10 cell extract were then incubated for 1.5 h at 30 °C in 20 μl 20 mM HEPES pH 7.4, 15 mM KCl, 25 mM $Mg(OAc)_2$, 1 mM dithiothreitol, 5 mM ATP, 4 mM fructose 1,6-bisphosphate, 20 μg/ml poly(rI) · poly(rC). All reagents were purchased from Sigma, and [2, 8-^{3}H]ATP from Amersham (UK). The synthesized radioactive $(2'\text{-}5')A_n$ oligomers were isolated by chromatography on DEAE-cellulose, as described [21].

Evaluation of the infection. HTLV-I infection was evaluated by indirect immunofluorescence assay for the p19 virus core protein on methanol/acetone(1:3)-fixed samples, as previously described [28]. The amount of provirus present in the infected cells was determined using a sensitive "dot-blot" hybridization assay [15]. Briefly, cells were adjusted at 1.5×10^6/ml and serial dilutions from 200 μl to 12.5 μl were spotted on nitrocellulose membrane filters (Schleicher & Schüll, Dassel, Germany) previously saturated in 0.5 M NaOH and 1.5 M NaCl. The filters were then neutralized in 0.2 M TRIS/HCl and 2 × SSC (0.3 M sodium citrate; 3 M NaCl, pH 7.0), dried and baked at 80 °C for 2 h. The amount of viral transcript was evaluated by spotting 1.5×10^6 cells on the filters as above. The filters were then fixed in 3% NaCl, 10 mM NaH_2PO_4, 40 mM Na_2HPO_4, 1% glutaraldehyde (Merck, Darmstadt, Germany) and rinsed with proteolytic buffer (50 mM EDTA, 0.1 M TRIS pH 8), according to a procedure previously described [25]. DNA and RNA were hybridized with the *SstI-SstI* fragment of HTLV-I (8.5×10^3 bases; 8.5 kb) digested from the pMT-2 plasmid, kindly provided by R. C. Gallo, NIH Bethesda USA. Nitrocellulose filters were hybridized for about 20 h in 10 × Denhardt's solution, 4 × SET (0.1 M NaCl, 10 mM TRIS · Cl pH 8, 1 mM EDTA pH 8) and 0.1% sodium dodecyl sulphate (SDS; Sigma). Unspecific background was removed by washing and decreasing the salt concentration up to 1 × SET/0.1% SDS. The probe was radiolabelled with [^{32}P]ATP by the nick-translation procedure. After hybridization, filters were exposed for autoradiography to Kodak XAR-5 films (Kodak Company, Rochester, N.Y.) for 72 h.

Immunofluorescence staining and flow cytometry analysis. Ficoll/Hypaque-separated fresh cells or cells from cultures were washed with RPMI-1640 medium and resuspended in PBS at 1×10^6/ml. Immunofluorescence staining and flow cytometry analysis were then performed. The following mouse anti-(human Ig) mAb were utilized: fluoresce-

in(FITC)-conjugated CD4 plus phycoerythrin(PE)-conjugated CD8, and PE-conjugated CD3 plus FITC-conjugated TCRαβ for a two-colour analysis; FITC-conjugated HLA-DR and FITC-conjugated CD25 for a single-colour analysis. PE- and FITC-conjugated mouse IgG were used as unrelated isotype controls for background determination. All mAb were purchased from Becton Dickinson. Staining was performed at 4 °C for 30 min. After treatment, the cells were washed twice in PBS, containing 0.02% sodium azide and used immediately. Flow cytometry analysis was performed using a FACscan (Becton Dickinson). In the two-colour analysis, markers were set to indicate quadrant boundaries limiting 98.0%–99.0% of the background events in the lower-left quadrant, in the respective control samples. In single-colour analysis, markers were set to indicate the boundary limiting 99.0% of the background events in the respective control sample. The background positivity (%) was subtracted from the results of the respective experimental samples. Live lymphoid cells were gated by foward- and single-side-angle scatter, to exclude dead cells, debris, very large cells and cell aggregates.

Results

Effect of IFNβ on CBMC or on CBCD4 cell growth, after HTLV-I infection

Purified CBCD4 (95%–98% pure as determined by flow cytometry analysis (Fig. 3A) were exposed to HTLV-I infection by cocultivation with irradiated MT-2 donor cell line. In order to perform a study over a period of several weeks, all the cultures were grown by adding IL-2 to complete medium. The mixed donor/recipient cell population was divided into two culture conditions, i.e. untreated or treated with 1000 U/ml IFNβ at the onset of the coculture. The growth rate of infected CBCD4 (hereafter called CBCD4/MT) untreated controls was similar to that of IFNβ-treated CBCD4/MT, the doubling time of both cell cultures being close to 1 week throughout the entire period of culture (Fig. 1B). Similar results were obtained in CBMC exposed to HTLV-I (hereafter called CBMC/MT) in the presence or not of IFNβ as shown in Fig. 1A. In this case, however, IFNβ showed antiproliferative effects after 5 weeks of culture. Control experiments performed starting from CBCD4 or CBMC not exposed to HTLV-I and cultured for 2–3 weeks, showed that IFNβ treatment did not result in antiproliferative effects (data not shown). Both CBMC/MT and CBCD4/MT were protected also by recombinant IFNβ treatment, showing a 50%-30% decrease of p19 expression after 3–5 weeks in culture. At this time neither CBMC/MT nor CBCD4/MT were expressing chromosomal markers associated with the donor cell line, indicating that the cultures were free of contaminating MT-2 cells (data not shown). Moreover, previous studies showed that irradiated MT-2 cells added to a CBMC suspension were not detectable after 4 days of coculture; this was demonstrated by flow cytometry analysis of DNA, taking into account that MT-2 cells are hyperploid [8].

Expression of p19 virus core protein in CBMC/MT or in CBCD4/MT cell cultures treated with IFNβ, following HTLV-I infection

The expression of the HTLV-I core protein p19 was evaluated in CBCD4/MT, throughout 5 weeks of culture.

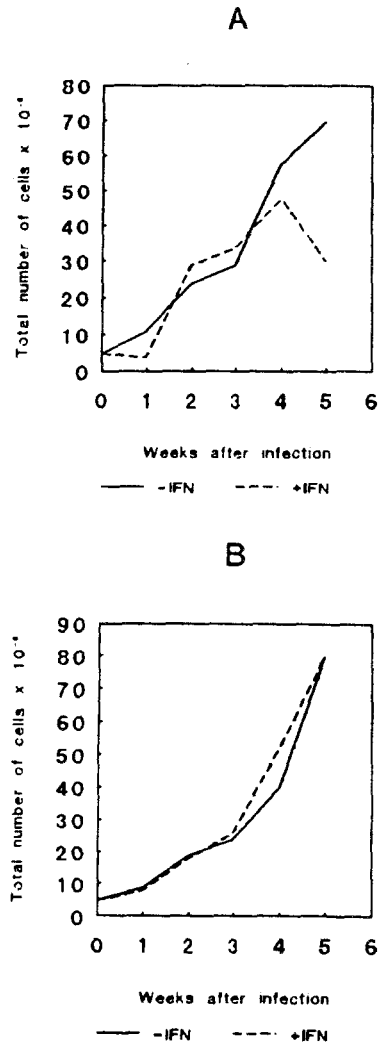

Fig. 1. Results of one representative experiment out of five showing growth curves, expressed as total number of viable cells $\times 10^{-6}$ of (**A**) cord blood mononuclear cells (CBMC)/MT (——), CBMC/MT+interferon β (IFNβ) 1000 U/ml (· – · – ·); (**B**) CBMC CD4+ subpopulation (CBCD4)/MT (——), CBCD4/MT+IFNβ 1000 U/ml (· – · – ·), after different lengths of time in culture. MT represents the HTLV-I-donor cells, MT-2

Treatment with IFNβ significantly reduced the percentage of p19-positive cells compared to that of untreated controls (Fig. 2B). This effect was particularly pronounced up to 4 weeks after exposure to HTLV-I. However, inhibition afforded by IFNβ treatment had decreased progressively down to 10% 5 weeks after infection (Fig. 2B). Similar results were obtained in the unseparated CBMC exposed to the virus, 1–4 weeks after infection (Fig. 2A). After 4 weeks in culture, the inhibitory effects of IFNβ on CBMC infection declined remarkably and did not reach statistically significant values (Fig. 2A). Similar results were obtained when CD4+ cells, isolated from PBMC, were treated with IFNβ. However, a detectable expression of p19 in infected PBMC (hereafter called PBMC/MT) appeared in a later phase of infection, with respect to that in CBMC/MT. In the case of PBMC/MT, IFNβ produced significant inhibition of the p19-positive CD4 cells, up to 7–8 weeks after infection (data not shown). Between 8 and

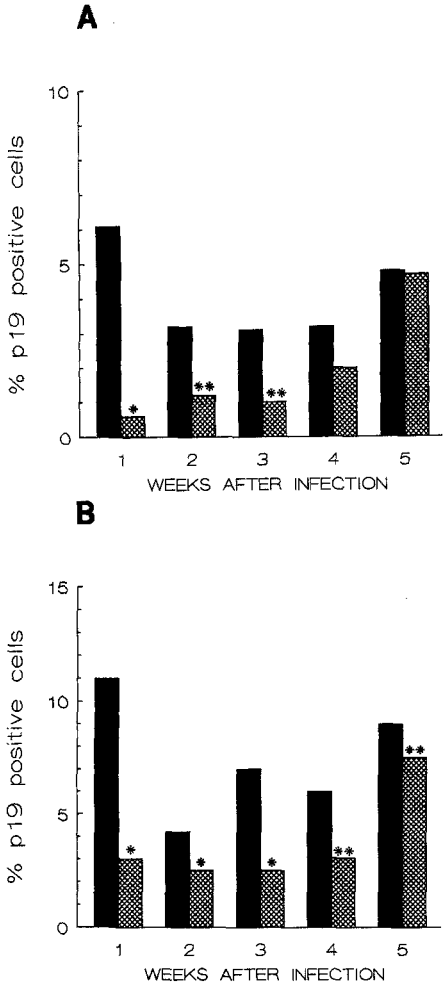

Fig. 2. Percentage of p19-positive cells in CBMC/MT (**A**) or CBCD4/MT (**B**) cultures untreated (*dark bars*) and treated with interferon β (IFNβ; *light bars*). The *columns* indicate the mean of five experiments. The percentage of p19-positive cells is significantly decreased in CBMC/MT and in CBCD4/MT (*$P<0.05$, **$P<0.01$), as determined by Student's *t*-test

10 weeks after infection, rapidly growing immortalized clones could arise from an infected CD4+ subpopulation, of either CBMC or PBMC origin, whether or not it had been subjected to IFNβ treatment.

Phenotypic analysis of IFNβ-treated CBCD4/MT cell culture

The phenotypic analysis of either untreated or IFNβ-treated CBCD4/MT during HTLV-I infection was accomplished by flow cytometry analysis. A low percentage of double-positive, CD4+ CD8+ cells was found in CBCD4/MT, untreated or treated with IFNβ, 1 week after infection (Fig. 3B, C). In all cases these cells retained the CD4 phenotype essentially unchanged up to 5 weeks after infection (Fig. 3D, E). The TCRαβ determinant was partially down-regulated in CBCD4/MT, untreated or treated with IFN, 1 week after infection (Fig. 3G, H). This marker returned to be fully expressed 5 weeks after infection (Fig. 3I, J). On the other hand, CD3 expression remained essentially unchanged during the entire period of observation. Moreover IFNβ treatment of CBCD4/MT did not alter the percentage of cells expressing HLA-DR and CD25, i.e. the main cell markers that are known to be related to an activation state, including that produced by HTLV-I infection (Fig. 3). In fact CBCD4/MT cell cultures, untreated or treated with IFNβ, did not show any variation in the quantitative expression of HLA-DR after 1–5 weeks in culture (Fig. 3L, M, N, O) as detected by fluorescence intensity. On the other hand the fluorescence intensity of anti-CD25 was differently distributed in untreated or IFNβ-treated CBCD4/MT, being higher in the former with respect to the latter 1 week after infection (Fig. 3Q, R). However, in the late phase of infection CBCD4/MT, whether treated or not with IFN, expressed the CD25 marker to the same extent. Similar results have been obtained in unseparated CBMC exposed to HTLV-I, treated or not with IFNβ (data not shown).

Effect of IFNβ on proviral DNA and on viral RNA transcription in CBCD4 exposed to HTLV-I infection

In order to investigate the effect of IFNβ on proviral DNA and on virus transcription in infected cells, DNA and RNA dot-blot analysis was performed. In the first 3 weeks, recipient cells cocultivated with HTLV-I-donor MT-2 cells in the presence of IFNβ showed a decreased amount of proviral DNA (Fig. 4) and viral RNA transcription (Fig. 5) with respect to that found in the controls. Both inhibitions were remarkably evident 2 weeks after infection either in CBCD4/MT or in unfractionated CBMC/MT cultures. Densitometric analysis of the autoradiographs, showed an inhibition range of either proviral DNA and viral RNA transcription of 50%–70% in IFNβ-treated CBCD4/MT or total CBMC/MT in the first 3 weeks of culture. Southern blot analysis of CBMC/MT or CBCD4/MT indicated that both the entire provirus (8.5 kb) and two defective components (6.1 kb and 4.5 kb) were present in untreated or IFNβ-treated recipient cells. This confirms that IFNβ treatment mainly affects the extent of virus integration for all the three HTLV-I genomes (data not shown).

Cytotoxic activity of CD4+ cells following exposure to HTLV-I and treatment with IFNβ

To study a possible immune mechanism underlying the effect of IFNβ towards separated CD4+ T cells, the cytotoxic activity against K562 or MT-2 target cells of HTLV-I-exposed CBCD4 was assayed. CBCD4 were subjected to short-term exposure to HTLV-I by cocultivation with an irradiated adherent (see Materials and methods) monolayer of MT-2 cells for 3 h in the presence or not of 1000 U/ml IFNβ. This was done in order to avoid gross contamination with irradiated dead MT-2 cells, following brief exposure of CBCD4 to the HTLV-I donor cell line. Successively, recipient CBCD4, recovered from the MT-2 monolayer, were washed and incubated at 37 °C overnight. CBCD4 that had been treated with IFNβ during cocultivation with MT-2 cells were re-exposed to the same concentration of

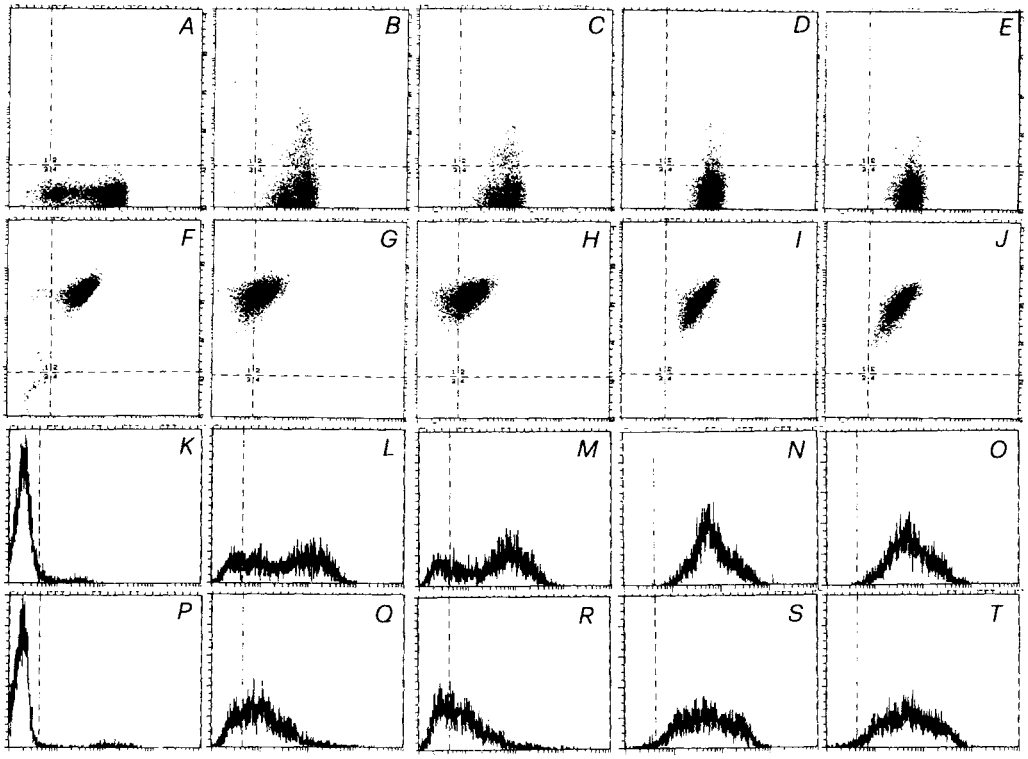

Fig. 3 A-T. Flow cytometry analysis of CBCD4/MT at various times after infection. Cells were stained with fluorescein-isothiocyanate(FITC)-labelled mAb as follows: **A–E** FITC-CD4 (*horizontal axis*) and PE-CD8 (*vertical axis*); **F–J** FITC-TCR αβ (*horizontal axis*) and PE-CD3 (*vertical axis*); **K–O** FITC-HLA-DR; **P–T** FITC-CD25. **A, F, K, P** Uninfected untreated control before infection; **B, G, L, Q** HTLV-I-infected untreated cells 1 week after infection. **C, H, M, R** HTLV-I-infected cells treated with IFNβ 1 week after infection. **D, I, N, S** HTLVI-infected untreated cells 5 weeks after infection. **E, J, O, T** HTLV-I-infected cells treated with IFNβ 5 weeks after infection

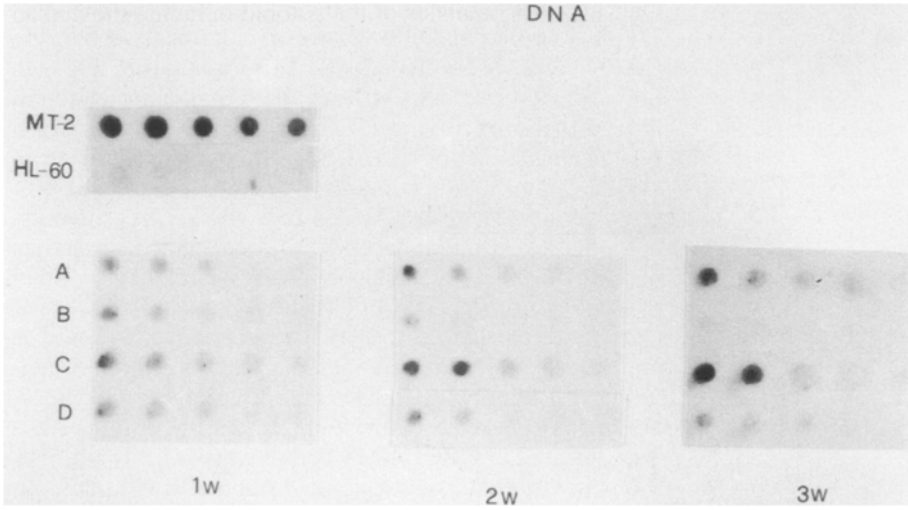

Fig. 4. Dot-blot analysis of proviral HTLV-I DNA in unfractionated CBMC/MT or separated CBCD4/MT from the same donor. Cells at 1.5×10^6 were spotted on nitrocellulose membrane filters. DNA was hybridized with the *SstI-SstI* fragment of HTLV-I (8.5 kb). *A*, CBMC/MT-2; *B*, CBMC/MT+IFNβ 1000 U/ml; *C*, CBCD4/MT; *D*, CBCD4/MT+IFNb 1000 U/ml. The chronically infected MT-2 cell line was used as the HTLVI-positive control, HL-60, a promyelocitic leukaemia, as a negative one

IFNβ during overnight incubation. Thereafter the cytotoxic activity of the CBCD4 cells was assayed against K562 or MT-2 targets. Table 1 shows that control or IFNβ-treated CBCD4 were not able to develop natural cytotoxic activity either against K562 or MT-2 targets. Conversely CBMC were able to lyse K562 and, to a lesser extent, MT-2 target cells. In this case IFNβ treatment markedly boosted the cytotoxic activity against MT-2 cells.

Analysis of (2′–5′)oligo(A) synthetase activity in cell extract from CBCD4 following exposure to HTLV-I

To explore whether the sensitivity to IFNβ treatment was correlated with the induction of IFN-inducible enzymes, the (2′-5′)oligo(A) synthetase activity was determined in cell extracts of CBCD4 exposed to irradiated MT-2 donor cells, alone or in the presence of IFNβ (1000 U/ml) (Table 1). The enzymatic activity of (2′-5′)oligo(A) syn-

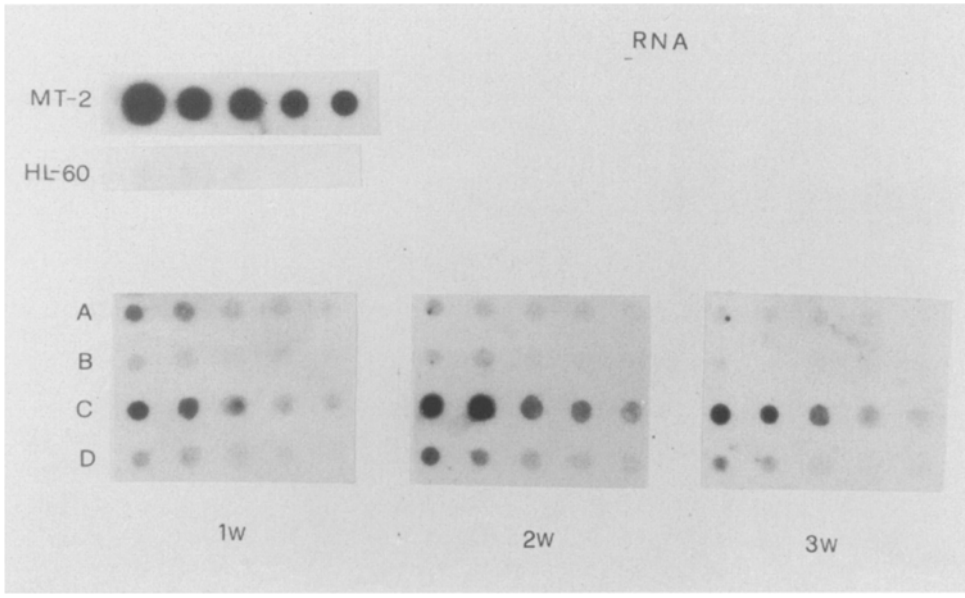

Fig. 5. Dot-blot analysis of HTLV-I transcription. For the different culture conditions see legend of Fig. 4

Table 1. (2′-5′)oligo(A) synthetase activity and cytotoxicity of cord blood CD4+ cells (CBCD4) exposed to HTLV-I[a]

Expt.[b]	Effector cell type	(2′-5′)oligo(A) synthetase activity (cpn)	Cytotoxicity %[c] against targets:	
			K562	MT-2
1	Unseparated	NT[d]	20±1	4±1
2	Unseparated/+IFN	NT	55±2	13±1
3	CD4+/MT-2	895	3±1	<1
4	CD4+/MT-2/+IFN	6210	<1	<1
5	Irradiated MT-2	570	<1	<1
6	Irradiated MT-2+IFN	605	<1	<1

[a] (2′-5′)oligo(A) synthetase activity was determined in S-10 extracts in the presence or not of interferon IFNβ (1000 U/ml). Data are from one representative experiment performed in duplicate

[b] Experimental conditions: Cord blood mononuclear cells (CBMC) isolated from human cord blood (1), CBMC treated overnight with 1000 U/ml IFNβ (2), CBCD4 exposed to irradiated MT-2 in a short-term assay (3), CBCD4 exposed to irradiated MT-2 cells (HTLV-I donors) and treated with 1000 U/ml IFNβ in a short-term assay (4), 5×10^6 irradiated MT-2 (5), 5×10^6 irradiated MT-2 treated with 1000 U/ml IFNβ

[c] Cytotoxic activity is expressed as percentage of ^{51}Cr released. E:T ratio 5:1

[d] Not tested

thetase appeared to be induced in CBCD4 after 3 h of incubation with irradiated MT-2 only in the case of treatment with IFNβ (Table 1). On the other hand no increase of (2′-5′)oligo(A) synthetase activity occurred in irradiated MT-2 cells alone that had been exposed to IFNβ, thus indicating that there was no contribution of the virus-donor cells to enzyme activity in CBCD4 exposed to MT-2 and treated with IFNβ.

Discussion

The present study shows that IFNβ was able to protect isolated CBMC CD4+ T cells from HTLV-I infection. The presence of proviral DNA was found to be remarkably lower with respect to that of untreated controls, especially 2 weeks after infection. This indicates that IFNβ could interfere with the integration of the HTLV-I provirus and/or with the synthesis of proviral DNA or with virus entry. The analysis at the molecular level reveals that HTLV-I preferentially replicated in cultures from purified CBCD4 with respect to CBMC. This finding is in line with previous observations showing that cell-mediated immunity, possibly involving NK cells present in the unseparated cell population (i.e. CBMC), could play a role in HTLV-I in vitro infection [16]. It follows that the susceptibility of cultures from CBCD4 cells to HTLV-I infection could be essentially independent of mechanisms based on natural or antigen-dependent immunity relative to other cell populations present in CBMC. Thus the model using CD4+ pure T cells (i.e. CBCD4) as recipient cells could be considered more reproducible and less subjected to individual variation with respect to that involving CBMC. A substantial decrease of the total amount of viral mRNA was detected in CBCD4/MT cells treated with IFNβ. Previous investigations have pointed out that IFNβ could interfere with the early steps of virus penetration and virus uncoating as shown in the murine system [2] or that it can inhibit viral mRNA transcription in chronically infected MT-2 cells [7]. Therefore it could be suggested that reduction of the total amount of HTLV-I mRNA afforded by IFNβ was the result of (a) a diminished amount of proviral DNA with respect to untreated CBCD4/MT, either because of a low integration or because of a low virus penetration, or (b) impairment of HTLV-I transcription induced by IFNβ.

The resistance to HTLV-I infection induced by IFNβ in CBCD4/MT was not apparently due to the antiproliferative

effect of this cytokine, the growth curve of IFNβ-treated CBCD4/MT being similar to that of the untreated controls. In addition IFNβ treatment only slightly affected the expression of activation markers such as HLA-DR and CD25 present on virus-exposed CD4+ cells, without interfering with the number of positive cells. Moreover the CD4 phenotype was not influenced by IFNβ treatment and appeared to be stable in culture. Only a limited amount of CD4+ CD8+ double-positive cells was detected 1 week after infection, and it was not influenced by pre-exposure to IFNβ. These results suggest that the protection afforded by IFNβ does not necessarily require the presence of a third-party effector cell of non-CD4 phenotype. However it can be hypothesized that two mechanisms, not mutually exclusive, could be involved in the protective effect of IFNβ towards HTLV-I infection of unfractionated CBMC. One mechanism could be due to IFNβ-induced modulation of antiviral cell-mediated immunity afforded by spontaneously cytotoxic cells, since unfractionated CBMC are protected by HTLV-I infection. The other mechanism could be ascribed to a direct influence of IFNβ on CD4+ target cells. Regarding the second hypothesis, it seems that IFNβ, following overnight treatment, does not induce natural cytotoxic activity against HTLV-I virus-donor MT-2 cells in CBCD4. Therefore the protective effect afforded by IFNβ was not apparently due to the killing of the virus-donor cells by the recipient CD4+ cells. Moreover the results illustrated in Table 1 show that IFNβ treatment was able to activate a (2′-5′)oligo(A) synthetase pathway in CBCD4 exposed to MT-2 cells for a short incubation time. In fact, IFN-inducible enzymes have been postulated to play a role in similar experimental conditions such as in the HIV infection [30]. Therefore IFNβ could trigger biochemical events in CD4 cells that became partly resistant to HTLV-I infection. This is confirmed by the observation that the percentage of cells expressing the virus core protein p19 was more efficiently reduced by IFNβ in the early (i.e. 0–4 weeks postinfection) phase of virus exposure than after 5 weeks in culture. It is conceivable that these biochemical events decline during the time after infection and that, in the late phase, immortalized IFNβ resistant clones could outgrow and progress toward a transformation stage. Other authors have, in fact, recently demonstrated that repeated treatments with low doses of recombinant IFNβ are able to inhibit the HTLV-I immortalization of human PBMC [24].

In conclusion the present study indicates that IFNβ protection in the early phase of CBCD4 exposure to HTLV-I is due to a direct antiviral activity on target cells. Moreover the model of virus transmission to the isolated CD4+ subset seems to represent a good approach for the study of the direct effects of different biological response modifiers on the target cell of virus infection, in order to prevent allogenic HTLV-I transmission.

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