Suppressive effects of interferons on the production and release of human T-lymphotropic virus type-I (HTLV-I)

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Summary. The effects of human α-, β-, or γ-interferon (IFN) on the replication and production of human T-lymphotrophic virus type-I (HTLV-I) were investigated in a human T-cell line, MT-2. Virus transmission and production estimated by syncytium formation and HTLV-I-associated reverse transcriptase (RT) activity were strongly suppressed in the presence of α- and β-IFN, but not γ-IFN. However, the expression of virus specific proteins gp46 but not p19, p24, p28, p36, and gp68 was affected with IFNs as revealed by Western blotting analysis. Electron microscopic observations showed that some of the HTLV-I particles were trapped in the intracellular vacuoles in the presence of high doses of α- or β-IFN. Continuous supply of IFNs appeared to be essential for the constant suppression of RT activity. These results suggest that α- and β-IFN do not inhibit HTLV-I gene expression strikingly but suppress processing or assembly of virus proteins and/or releasing of virions in the late phase of maturation.

Introduction

Interferons (IFNs) comprise a family of gene products which were first identified by their abilities to prevent the virus infections [1]. The gene structures of IFNs and the amino acid sequences of the encoded proteins have been characterized in detail [2, 5]. IFNs are classified into three types, alpha, beta, and gamma, according to their antigenicities. In human there are 20 species of α-IFN but only one of β-IFN and of γ-IFN [5]. IFNs have been reported to have various biological activities including not only antiviral actions but also inhibition of cell growth and proliferation[3], regulation of the expression of specific genes [4], modulation of cell differentiation and activation of various types in the immune systems [5].

Human T-lymphotropic virus type-I (HTLV-I), which is a type C retrovirus isolated from adult T-cell leukemia/lymphoma (ATLL), can immortalize T lymphocytes and fuse the fibroblasts or epithelial cells to produce large syn-
The same virus has been related to certain chronic myelopathies in the tropics and Japan, called HTLV-I-associated tropical spastic paraparesis (HTLV-I/TSP) or HTLV-I-associated myelopathy (HAM) [7]. Several immunological abnormalities have been observed in ATLL or HAM patients, such as alterations of peripheral blood lymphocyte (PBL) subpopulations, IL-2 independent proliferation of T-lymphocytes or autologous proliferative response (APR) in vitro [6], and indiscriminant helper functions [8]. Furthermore, the expression of HTLV-I-related viral proteins as well as virus production is tightly restricted in fresh lymphocytes of ATLL patients and virus carriers in vivo [9]. This suggests that the expression of viral-specific proteins or the virus production are negatively feedbacked in response to cytokines or lymphokines to evade from immunological surveillance in vivo. Therefore, it is reasonable to hypothesize that immunological surveillance mechanisms play an important role to control HTLV-I infection and transmission in the early stage and progression into leukemic stage. The purpose of the present study is to examine the role of human $\alpha$-, $\beta$-, and $\gamma$-IFN on the replication, production, and transmission of HTLV-I in vitro.

**Materials and methods**

**Cell culture**

MT-2 [10], a human cord T cell line established by co-culture with fresh ATL cells; Rab-3 [11], a rabbit-lymphoid cell line, established by co-culture with lethally irradiated MT-2 cells, and TALL-1 [12], a human leukemic T cell line, were cultured in RPMI-1640 medium supplemented with 15% heat-inactivated fetal calf serum (FCS).

The monolayer culture line used as an indicator of syncytium formation was $S^+L^-$ CCC [13], a clonal cell line derived from feline kidney fibroblasts transformed by Moloney murine sarcoma virus (Mo-MSV) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS. All these cells were cultured in a humidified 7.0% CO$_2$ atmosphere at 37°C.

IFNs used were recombinant type human $\alpha$-IFN (rIFN) 2a (Roferon-A, specific activity: $2 \times 10^8$ I.U./mg protein) kindly provided from Japan-Roche Co. Ltd., affinity purified natural type human $\beta$-IFN (nIFN) (BM532, specific activity: more than $2 \times 10^7$ u/mg protein), and recombinant type human $\gamma$-IFN (rIFN) (GI-3, specific activity: more than $1 \times 10^7$ u/mg protein) from Toray Co. Ltd. Japan, and natural type human $\alpha$-IFN (n-IFN) (Sumiferon, specific activity: 1 to $2 \times 10^8$ u/mg protein) from Sumitomo-Pharmaceutical Co. Ltd. Japan.

In order to estimate the suppressive effects of each IFN treatment on the growth of MT-2 cells, $2 \times 10^5$ cells/ml were cultured for 48 or 72 h in the presence of various doses of IFNs and viable cells were counted by trypan blue exclusion.

**Syncytia formation assay**

$5 \times 10^4$ $S^+L^-$ CCC cells/well were cultured overnight in 24-well tissue culture plate, followed by polybrene treatment (2 $\mu$g/ml, Sigma) for 30 min. Then, $5-50 \times 10^4$ MT-2 cells along with different doses of each IFN were inoculated in each well. After 3-day culture, cells were fixed with methanol and stained with Giemsa. Numbers of syncytia containing more than ten nuclei were counted under an inverted microscope. $S^+L^-$ CCC cells rarely formed syncytium spontaneously and usually possessed less than five nuclei [6].