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Effect of Interferon on Cells Persistently Infected with Human T Cell Leukemia Virus (HTLV-I)

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ABSTRACT

Spontaneous production of interferon-gamma (IFN-γ) was shown in four (MT-1, MT-2, SMT-1 and HUT 102) of five human T-lymphoblastoid cell lines persistently infected with human T cell leukemea virus type I (MT-1, MT-2, SMT-1, HUT 102 and OKM-2). These four cell lines were not susceptible to the antivirus effect of IFN. In contrast, the multiplication of vesicular stomatitis virus (VSV) was restricted in the nonproducer cell line of IFN, OKM-2 cells by treatment of IFN-α or IFN-γ. Anti-proliferation effect of IFN was investigated in producer cell line of IFN (MT-2 and SMT-1) and non-producer cell line of IFN (OKM-2). The growth of MT-2 cells was not affected by IFN-α or by IFN-γ. However, SMT-1 and OKM-2 cells were affected by treatment with externally added IFN-α.

Key words: Interferon, Cell growth, Antivirus state, HTLV-I

INTRODUCTION

Cells acutely or persistently infected with viruses became to produce and secret interferons (IFNs). IFNs are well known to play an important role in the defense mechanism against virus infection. Some viruses including human retro virus, HTLV-1, appear to produce a persistent infection in animals and cultured cells. The antivirus state induced by IFN may be closely associated with persistent infection due to the inhibitory or suppressive effect of IFN on virus replication.

The production of IFN-γ and several other lymphokins has been reported in

human T cell lines persistently infected with HTLV-I (10, 12, 13). Matsuyama *et al.*, reported that high producer cell lines of IFN-γ (TCL-Fuj) are not susceptible to the antivirus or anti-proliferative effect of IFN(11). It is, therefore, suggested that IFN-γ produced spontaneously may not be associated with persistent infection. We investigate whether spontaneous production of IFN-γ is correlated with antivirus state and cell proliferation in other cell lines persistently infected with HTLV-I than TCL-Fuj cells.

MATERIALS AND METHODS

Cells

Cultured cell lines of T cell origin (MT-1, MT-2, SMT-1, HUT 102, OKM-2, TALL-1, CCRF-CEM and Molt-4) were used in this experiment. All of the cultured cell lines of T cell origin were CD4 antigen positive. MT-1, MT-2, SMT-1, HUT 102 and OKM-2 cells were carried with HTLV-1, but the others were not. They were cultured with RPMI 1640 medium supplemented with 10% foetal bovine serum (GIBCO) and 100 U/m*l* of penicillin G at 37 C in a humidified 5% CO_2 incubator.

Assay of interferon

IFN assay was performed by dye-binding methods using human FL cells and VSV as previously described(2, 4). After the cultivation of cells for 5 days, IFN samples were harvested by centrifugation at 1,600×g for 5 min of culture medium. Samples were dialyzed against 0.2 M KCl-HCl buffer, pH 2.0 at 4 C for 48 h and were further dialyzed against RPMI 1640 medium at 4 C for 24 h. Control samples were dialyzed against PBS (Phosphate buffer saline, pH 7.2) instead of pH 2.0. Samples were also neutralized by rabbit polyclonal antibody to human IFN-α (Hayashibara Biochemical Laboratories Inc., Lot No PIF1-91101) as previously described(2).

Susceptibility to virus infection

One m*l* of OKM-2 or HUT 102 cells (5×10^6 cells) treated with or without 10^3IU/m*l* of IFN-α (3×10^6IU/vial, Lot 02-31, Japan Red Cross Society, $>10^7$ IU/mg protein) or human recombinant IFN-γ (3×10^6U/vial, Lot GTH-001, Toray) was inoculated with 0.2 m*l* of VSV at an input multiplicity of 0.1. The mixture of cells and virus were incubated for 1 h at the room temperature and then washed twice with medium by centrifugation at 1,600×g for 5 min. The cell pellets suspended in 5 m*l* of fresh medium and cultured in a plastic flask. After the cultivation of cells for 24 h, the culture medium containing infectious virus was harvested by centrifugation at 1,600×g for 5 min. Subconfluent monolayer

of MT-2 and SMT-1 cells treated with or without 10^3IU/m*l* of IFN-α was also inoculated with VSV, washed and cultured with fresh medium for 24 h in 60 mm plastic dish.

Assay of infections virus

Titer of infectious virus in the culture medium was measured by plaque assay on monolayers of Vero cells in a 6 well plastic dish with 0.1 m*l* of virus inoculum per well at an appropriate dilution. After virus adsorption for 30 min, the monolayers were overlaid with 3 m*l* of RPMI 1640 medium containing 1% carboxymetyl cellulose, 5% foetal bovine serum, and cultured. Thereafter, the monolayers were washed with a 0.85% NaCl solution, stained with gentian violet solution for 2 or 3 min, washed thoroughly with water, and dried at room temperature. Plaques were couted on the 3d day after the inoculation of the virus.

Effect of IFN on cell growth

OKM-2 and HUT 102 cells (2×10^6 cells) were cultured with 5 m*l* of RPMI 1640 medium containing 10% foetal bovine serum, 10^3IU/m*l* of IFN-α in a plastic culture flask. The number of live cells were counted on the 6 th day after treatment of IFN. SMT-1 and MT-2 cells (4×10^5 cells) were cultured in a 60 mm plastic dish for 6 days. After which the anti-proliferation effect of IFN was estimated by the methods using gentian violet solution as previously described(7, 8).

RESULTS

Production of IFN

Table 1. *Acid-stability and antigenicity of IFN obtained from HTLV-1 infected cells.*

Cell lines	ATLA (%)[a]	IFN titer (IU/m*l*) after treatment with		
		pH 7.4	pH 2.0	Anti-IFN-α
MT-1	1-10	16	< 4	12
MT-2	100	32	< 4	32
SMT-1	100	64	< 4	64
HUT 102	100	8	< 4	4
OKM-2	100	< 4		
CCRF-CEM	0	< 4		
Molt-4	0	< 4		
TALL-1	0	< 4		

[a] The percentage of ATLA was determined as previously described(9).

The culture fluids from each cell lines of T cell origin were assayed for the activity of IFN. TALL-1, CCRF-CEM and Molt-4 cells did not produce IFN, however four out of five cell lines persistently infected with HTLV-I produced IFN spontaneously. The activity of this IFN was inactivated by pH 2.0, but not by anti-IFN-α serum (Table 1). It is therefore proposed that IFN produced by these persistently infected cells is the gamma type of IFN. In a biological assay, IFN-γ was undetectable in culture medium of OKM-2 cells.

Though production of IFN-γ is demonstrated, IFN titer was much lower than that of TCL-Fuj cells reported by Matsuyama *et al.*(11).

Susceptibilities of persistently infected cells to the antivirus effect of IFN

The multiplication of VSV in eight cell lines of T cell origin was examined. In both producer and non-producer cell lines of IFN-γ, VSV multiplied significantly with a cytopathic effect and infectious virus titer of 10 pfu/m*l* was obtained 24 h after infection (Table 2). It is suggested that IFN-γ produced spontaneously had no effect on antivirus functions of these producer cell lines. Next we examined the effect of externally added IFN-α or IFN-γ on VSV multiplication in five persistently infected cell lines.

In non-producer, OKM-2 cells, pretreatment of the cells with 10^3IU/m*l* of IFN-α or IFN-γ decreased the yield of infectious virus. On the contrary, virus titer in culture medium of producer cell lines was not reduced by externally added IFM-α or IFN-γ although a slight reduction of VSV yield was shown in HUT 102 cells (Table 2).

Table 2. *VSV multiplication in HTLV-1 infected cells.*

Cell lines	VSV titer (pfu/m*l*) in the presence of		
	None	IFN-α	IFN-γ
MT-1	4.4×10^7	3.0×10^7	3.8×10^7
MT-2	5.2×10^7	4.8×10^7	4.8×10^7
SMT-1	6.2×10^7	3.4×10^7	3.6×10^7
HUT 102	1.6×10^7	8.2×10^6	1.0×10^6
OKM-2	7.0×10^7	7.7×10^5	6.8×10^5
CCRF-CEM	1.6×10^5		
Molt-4	5.6×10^6		
TALL-1	6.6×10^7		

Susceptibility of cells to the anti-proliferative effect of IFN

Anti-proliferative effect of IFN was examined in three producer cell lines HUT 102, MT-2 and SMT-1, and one non-producer cell line OKM-2. Both MT

Table 3. *Effect of IFN on cell growth.*

Cell lines	IFN-α (IU/ml)	
	0	1000
MT-2	0.63[a]	0.56
SMT-1	0.55[b]	0.23
HUT 102	1.8×10^6/ml[c]	1.6×10^6/ml
OKM-2	1.6×10^6/ml[d]	5.3×10^5/ml

[a] Absorbance (A_{550})
[b] Absorbance (A_{550})
[c] The number of live cells
[d] The number of live cells

-2 and SMT-1 cells passaged and grew for several months in the presence of autogenous IFN-γ. The growth of HUT 120 and MT-2 cells was not affected by externally added IFN-α. However, the proliferative ability of SMT-1 was reduced by treatment with externally added IFN-α. Cell growth of non-producer cell line OKM-2 was also affected by exogenous IFN-α (Table 3).

DISCUSSION

The result of IFN-γ production indicated that there might be two types of producer and non-producer cell lines. They also differed in response to IFN. A marked difference was found in their susceptibility to the antivirus and anti-proliferative effects of IFN. Non-producer cell line was susceptible to both effects of IFN, but producer cell line was resistent to IFN. The results obtained from this experiment are consistent in a large part with the reports by Matsuyama et al with the exception of SMT-1 cells(11). SMT-1 cells was susceptible to the anti-proliferative effect of IFN in spite of producer cell line of IFN-γ. A dissociation of the antivirus and anti-proliferative effects of IFN was shown in this cell line. It is well known that antivirus and anti-proliferative effects of IFN are closely associated with the induction of oligo-2', 5'-adenylate synthetase (2-5AS)(1). Therefore, a change of the activity of this enzyme seems to be casual factor of the difference between producer and non-producer cell lines. In addition to this, suppression of 2-5AS induction has been reported in various persistent infection, in which the antivirus effect of IFN was reduced markedly(3, 5, 6, 8). It is important to study induction of 2-5AS activity in cells persistently infected with HTLV-I.

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