Correlation between Oligo-2', 5'-adenylate Synthetase and Expression of Human T-Lymphotropic Virus Type-I Specific *gag* Protein

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ABSTRACT

The effect of human interferon-α (IFN-α) on the production of virus specific *gag* protein was investigated in four human T cell lines persistently infected with human T-lymphotropic virus type-I (HTLV-I). These four cell lines (MT-2, SMT-1, HUT 102, and OKM-2) differed in sensitivity to the functions (antivirus activity, antiproliferative activity, and oligo-2', 5'-adenylate synthetase induction) of IFN. The expression of HTLV-I *gag*-protein, p 53, p 33, p 28, p 24, and p 19, was found in each IFN-α or non-treated cell lines by Western blotting analysis. However, production of p 33, p 28 and p 24 was different among these cell lines. Protein bands of p 53, p 28, p 24, and p 19 were detected in MT-2 cell lines, and p 33 was found in SMT-1 and OKM-2 cell lines instead of p 28. Those of p 28 and p 24 were undetectable in HUT 102 cell line. Furthermore, the expression of these virus antigens was hardly affected by exogenously added IFN-α in spite of the induction of oligo-2', 5'-adenylate synthetase (2-5AS) activity.

Key words: HTLV-I, *gag* protein, Interferon, Oligo-2', 5'-adenylate synthetase

INTRODUCTION

Human T-lymphotropic virus type-I (HTLV-I) is the causative agent of adult T cell leukemia (ATL) (6, 15). Human T lymphoid cell lines persistently

Table 1 *Characterization of cells persistently infected with HTLV-I*

Cell lines	Spontaneous IFN-γ production[a]	2-5AS Induction[b]	Cell growth inhibition[a]	Antivirus state by IFN-α[a]	gag protein
MT-2	+	Suppression	−	−	p53, p28, p24, p19
SMT-1	+	Suppression	+	−	p53, p33, p24, p19
HUT 102	+	+	−	+/−	p53, p33, p19
OKM-2	−	+	+	+	p53, p33, p24, p19

[a] Data are from reference 3.
[b] Data are from reference 4.

infected with HTLV-I have been established in various laboratories. More than half of the cell lines could produce several lymphokines spontaneously (3, 7, 8, 12, 13). These cell lines with HTLV-I also differed in sensitivity to the functions of IFN. In previous paper (3), we showed that antivirus state was not induced in IFN-γ producing cell lines (MT-1, MT-2, and SMT-1), but in IFN-γ non-producing cell line (OKM-2). Anti-proliferative effect of IFN on these cell lines was also different (Table 1). It is still unknown what mechanism(s) control these responses to IFN. However, virus proteins or antigens might be associated with these phenomena.

Moore *et al.* reported that inversely correlation between HTLV-I expression and IFN-γ production was recognized in cultured T lymphocytes (9). However, Sugamura *et al.* and Oka *et al.* noted that IFN-γ producing cell lines were strongly positive for the HTLV-I antigens (11, 14). It is well known that 2-5AS inhibits virus relication through the suppression of virus protein synthesis (1). It has been reported that the activity of 2-5AS is scarcely induced in IFN-γ producing cell lines (4). As shown in Table 1, induction of 2-5AS was recognized in OKM-2 and HUT 102 cell lines, but hardly ever seen in MT-2 and SMT-1 cell lines. Therefore, it is important to investigate the effect of IFN or 2-5AS activity on the expression of virus specific antigens in these four cell lines.

MATERIALS AND METHODS

Cell culture.

Four human cultured cell lines of T cell origin carrying HTLV-I (MT-2, SMT-2, HUT 102, and OKM-2) were used in this experiment. They were cultured with RPMI 1640 medium supplemented with 10% heat inactivated foetal bovine serum (Gibco) and 100 U/m*l* of penicillin G at 37 C in a humidified 5% CO_2 incubator.

Enzyme assay.

After the treatment of cells with various concentrations of IFN-α (3×10^6

IU/vial, Lot 02-31, Japan Red Cross Society) for several days, the activity of 2-5AS was measured according to the procedure previously described (2).

Western blotting analysis.

Western blotting analysis was performed as previously described (5). Cellular protein lysates (40 μg) from each cultures were electrophoresed on 10% polyacrylamide gel, after which proteins on gel were electrophoretically transferred to a nitrocellulose membrane and treated with anti-HTLV-I antibody (EPITOPE Inc. Lot : 2040302) recognizing gag protein. HTLV-I antigens were visualized after the incubation with alkaline phosphatase labelled anti-mouse IgG antibody (Promega Corp.), following the reaction with the substrate of nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP).

RESULTS AND DISCUSSION

The expression of HTLV-I specific *gag* protein was investigated in four T lymphoid cell lines persistently infected with HTLV-I. The activity of 2-5AS increased by IFN in two (OKM-2 and HUT 102) of these cell lines, but the

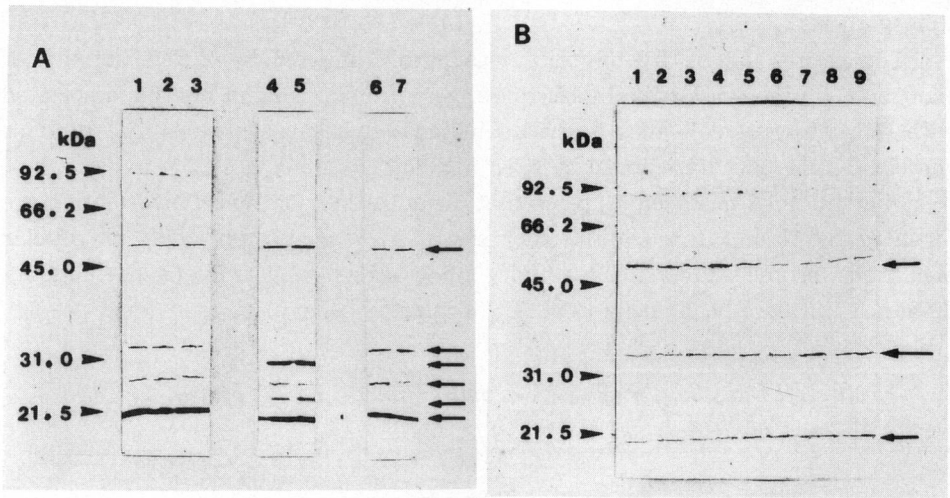

Fig. 1 *Western blot analysis of gag*-protein.
(A) SMT-1 cells (lanes 1 to 3), MT-2 cells (lanes 4 and 5) and OKM-2 cells (lanes 6 and 7) were treated with 1,000 IU/m*l* of IFN for 1 day (lane 2) or 10 days (lanes 3, 5, and 6). The cells treated in the same way but with no IFN added were used as a control (lanes 1, 4, and 7). (B) HUT 102 cells were treated with 0 (lane 1), 10^2 (lane 2), 10^3 (lane 3) and 10^4 IU/m*l* (lane 4) of IFN for 1 day.
The cells were also cultured with 10^3 IU/m*l* of IFN for 5 (lane 5), 10 (lane 6), 15 (lane 7), 20 (lane 8) and 30 days (lane 9). After treatment, the cells were lyzed by NP-40 lysis buffer, and *gag*-proteins were analyzed by western blot analysis.

remaining two cell lines (MT-2 and SMT-1) were not (4). A significant level of 2-5AS was detected in IFN-γ non-producing cell line, OKM-2 without IFN. MT-2, SMT-1, and HUT 102 cells were IFN-γ producing cell lines (3). As shown in Fig. 1, the expression of *gag* proteins, p 53, p 33, p 28, p 24, and p 19, was recognized in these four cell lines by Western blotting analysis. Protein bands of p 53, p 28, p 24, and p 19 were detected in MT-2 cell lines, and those of p 53, p 33, p 24, and p 19 were found in SMT-1 and OKM-2 cell lines (Fig. 1, A). In these three cell lines, the production of *gag* protein was hardly affected even after treatment with 1,000 IU/m*l* of IFN-α for 10 days, though 2-5AS activity was induced slightly (MT-2 and SMT-1) or significantly (OKM-2) by IFN-α on 10 th day (Table 2). As for OKM-2 cell line, it is clear that even a significant activity of 2-5AS could not suppresse the expression of *gag* protein. These results were consistent with the finding for MT-2 cell line reported OKa *et al.* (11).

In contrary, a slight decrease of p 53 density was shown in HUT 102 cell line after treatment with 1,000 IU/m*l* of IFN-α for 10 to 30 days, while the density of p 33 and p 19 was gradually increased by IFN-α (Fig. 1, B). The result might be caused by the modification of processing of *gag* protein or the supression of p 53 synthesis.

The expression of *gag* proteins was hardly affected by 2-5AS activity or endogenous IFN-γ. Furthermore, these *gag* proteins might not be associated with the nature of cell lines described in table 1, because none of *gag* proteins contributed the activity induced by IFN (Table 1).

In addition, it has been reported that virion release or syncytia formation are inhibited by IFN-α through the suppression of virus glycoproteins or cellular membrane proteins (10). The activity of 2-5AS, however, might not interact directly with these phenomena in HTLV-I infected cells.

Table 2 *Induction of 2-5AS activity (nmole/mg of protein·hr) by IFN-α.*

Cell lines[a)]	IFN-α (IU/m*l*)	
	0	1,000
MT-2	5.0	58.2
SMT-1	4.5	42.6
HUT 102	14.3	316.3
OKM-2	39.7	894.4

[a)] Cells were treated with 1,000 IU/ml of IFN-α for 10 days. After treatment, 2-5AS activity induced in these cells was measured by the method described in Materials and Methods.

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