Immunological functions of adult T cell leukemia cells of a patient complicated with synchronous double primary gynecologic cancer

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Abstract : A patient with triple malignancies is reported, who presented cervical cancer, vulvar cancer and adult T cell leukemia (ATL). ATL was diagnosed as a smouldering type, because antibody to human T cell leukemia virus associated antigen (ATLA) was positive with a titer of 1 : 160. Although her malignant cells had an OKT $4^+8^-3^+Tac^+$ phenotype, the cells did not display helper T cell functions. Namely they showed no response to Phytohemagglutinin (PHA) and Interleukin 2 (IL-2) and suppressed the PWM driven IgG synthesis of B cells obtained from healthy donor. They did not produce IL-2 by stimulation with PHA and phorbol myristate acetate (PMA). Furthermore, these ATL cells were producing IL-2 inhibitor like factors. As synchronous triple malignancies are extremely rare, two gynecologic cancers seem to ascribe to the suppressing state of the immunosurveillance mechanism by viral infection. J. Med. Invest. 44 : 99-102, 1997

Key Words : *ATL ; triple malignancies ; IL-2 ; IL-2 inhibitor*

INTRODUCTION

Adult T cell leukemia (ATL) has been thought to be caused by infection of human T cell leukemia virus (HTLV) type I to T cells (1-2). In patients with ATL, complication with second primary cancer of the lungs, vagina, stomach and so on have been reported (3-4), and occurrence of these clinical cancers are attributed to an underlying immunodeficiency state of the hosts due to dysfunction of the helper T cells by the viral infection.

This is the first report of a ATL case complicated with synchronous primary double cancer of the uterine cervix and vulva. Leukemic cells of this patient were examined in detail.

A 70-year-old gravida 4, para 3, Japanese housewife was referred to the Tokushima University hospital for further examination of the carcinoma of the vulva. She complained of vulval itching for 3 years, but there was no other previous history. Histological examination revealed the presence of double primary squamous cell carcinomas of the vulva (clinical stage 0) and the uterine cervix (stage 1 b). The region located was isolated and the intraepithelial region was not supposed as a metastasis of the cervical cancer. There were no skin regions, lymphadenopathy or hepatosplenomegaly. On admission, hematological examination revealed leukocytosis up to 383,000/mm 3 with 3.0% polymorphonuclear cells, 1.5% bands, 0% monocytes and 85.0% abnormal lymphoid cells. Hemoglobin was 9.0 g/dl and platelet count was 77,000/mm^3. Serum IgG was 1,314 mg/dl, IgM 122 mg/dl, and IgA 87 mg/dl. Bone marrow aspiration revealed a diffuse infiltration of lymphoid cells. Surface marker analysis of the peripheral blood lymphocytes (PBL) was as follows : OKT3, 32.4% ; OKT4, 94.1% ; OKT6, 0.4% ; OKT8, 7.1% ; OKT9, 10.3% ; OKT10, 6.9% ; OKT11, 93.5% ; OKTM1, 2.7% ; Leu7, 1.6% ; B1, 3.3%. Tac antigen was expressed in 31.3% of peripheral mononuclear cells. Serologic test for antibody against HTLV associated antigen (ATLA) was positive at a titer of 1 : 160. From these data, she was diagnosed as ATL complicated with double primary cancer.

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MATERIALS AND METHODS

Responsiveness to mitogen and interleukin 2 (IL-2)

The mononuclear cells (MNCs) separated by Ficol-Conray density gradient centrifugation were cultured in a 200 μl aliquot in a flat bottomed 96-well microtiter plate (A/S Nunc Kamstrup DK-4000 Roskilde Denmark) with mitogen or IL-2 in RPMI 1640 (Nissui Pharmaceutical Co., Tokyo Jaapan) containing 10% fetal calf serum (FCS) (GIBCO, Wheaton USA), 2 mM L-glutamine at 37℃ in 5% CO_2 in air. Namely the MNCs were cultured at 1×10^6 cells/ml with 2 phytohemagglutinin (PHA) (DIFCO Laboratories, Detroit, MI) and graded concentrations of

IL-2 (Boehringer Mannheim GmbH, W-Germany) for 24 hours. The cells cultured were pulsed for 4 hours with 0.25 μCi ^{3}H-thymidine, harvested onto paper filters by Labomash (Labo Science Co., Tokyo, Japan) and the incorporated radioactivity was determined by a liquid scintillation counter.

IL-2 production

Ability to produce IL-2 by the leukemic cells was evaluated according to the method described by Andrew et al. Peripheral blood mononuclear cells of the patient were cultured at 4×10^6 cells /ml in RPMI-1640 containing 5% FCS, 1% PHA and 10 ng/ml PMA (SIGMA, St Louis, USA) for 48 hours at 37℃ and IL-2 activity in the supernatant was assessed. Lymphokine activated killer cells (LAK cells) induced from MNCs from a healthy donor by culturing for 15 days with 10% IL-2 and IL-2 dependent T cell clone provided by Dr. Hirai (Otsuka Pharmaceutical Co.) were used as IL-2 dependent cells. Standard IL-2 were diluted in serial two-fold dilutions with RPMI-1640 medium. Fifty microtiters of samples and each dilution was poured into a 96-well microtiter plate in triplicate. Fifty microliters of the cell suspension at 2×10^5 cells/ml in RPMI-1640 containing 10% FCS was added to each well and the mixture was cultured for 18 hours. DNA synthesis of IL-2 dependent cells was calculated by incorporation of ^{3}H-thymidine.

Effect on PWM driven lgG synthesis (6)

Peripheral blood mononuclear cells of the patient and healthy donors were separated into T and non-T fractions by the nylon wool column methods (7). OKT3$^+$ cells were more than 97% in the T cell fraction and 21-25% in the non-T cell fraction, in which the B cell population was more than 51%. Each fraction of T cells was added at 2×10^4 and 1×10^5 cells/well to 1×10^5 cells/well healthy donor B cells in 200 μl RPMI-1640 medium containing 10% FCS in a 96-well microtiter plate. The mixtures were cultured with 20 μl of 1 : 10 diluted PWM (DIFCO Laboratories, Detroit, Michigan USA) in RPMI-1640 medium for 7 days. On day 4, the medium was totally exchanged with new medium. IgG production in the cultur supernatant was measured by a double antibody sandwich enzyme immunoassay using immunoplate (Microwell plate, Nunc, Denmark) coated with goat anti human lgG (TAGO. Inc. Burlingame CA) at a concentration of 50 μg/ml. After adding 100 μl standard human lgG or samples, the plate was incubated for 1 hour at 37℃ and washed 3 times with 0.01 M phosphate buffered saline (PBS) (pH 7.4) containing 0.1% Teeen 20 (PBST). Futher serial incubation with 100 μl of biotinylated goat anti human lgG antibody (VECTOR Laboratories Inc. Burlingame, CA) at 10 μg/ml for 30 min, 100 μl of horseradish peroxidase avidin D (VECTOR Laboratories, Inc. CA) at 1 μg/ml for 10 min and 100 μl of substrate solution (o-phenylene diamine dihydrochrolide ; 0.4 mg/ml in 0.05 M citrate buffer pH 4.8 containing 0.007% H_2O_2) per well for 10 min were carried out at 37℃, and the reaction was stopped with 6 NH_2SO_4. Absorbance at 490 nm was measured on a SLT 310 ELISA reader (SLT-Labinstrument, Austria). The samples were assayed in triplicate. The assay range was from 10 to 500 ng/ml.

RESULTS

Table 1 summarizes the results on responsiveness to mitogen and IL-2. PBL obtained from the patient did not respond to PHA or IL-2. On IL-2 assay using LAK cells as IL-2 dependent cells, no IL-2 activity was detected in the culture supernatant of patient cells (PC). Furthermore, the supernatant suppressed the proliferation of LAK cells in a dose dependent manner (Table 2). No suppression was detected on the proliferation of PBLs. Similar results were obtained in the 2 nd experiment using IL-2 dependent cell line (Table 2). Effects of PC and T cell fraction obtained from a normal healthy donor on PWM-driven lgG production are shown in Fig 1. T cell fraction of the healthy donor showed helper activity, while PC suppressed lgG production. Cytotoxic effect of the supernatant was excluded by trypan blue dye exclusion test (data not shown) and an absence of the suppressing effect on the proliferation of normal lymphocytes (Table 2).

DISCUSSION

The diagnosis of ATL for the present patient was based on the findings of positive serologic test for ATLA and characteristic cell surface marker phenotype of PBL (8), OKT1$^+$3$^+$4$^+$8$^-$ and Tac$^+$ (9), though direct evidence of the infection of HTLV to leukemic cells was not obtained. She seemed to have suffered from smouldering type of ATL for a long time, because only a few leukemic cells showed abnormal shaped nuclei and no clinical findings

Table 1. Responsiveness of peripheral blood mononuclear cells (PBL) to PHA and IL-2

Experimental number	Mediuml) containing	PBL from the patient	PBL from a health donor
1	PHA 2 μg/ml	306±19 cpm 2)	13220±207
	control	494±33	2328±174
2	IL- 2 1 : 8	64±13	1059±718
	1 : 32	80±21	10463±523
	1 : 128	204±68	10311±1948
	1 : 512	101±30	9150±1840
	control	306± 4	7624±791

1) All cultures were carried out in RPMI-1640 medium containing 10% FCS and 2 mM L-glutamine
2) ^{3}H-thymidine uptake (count per minutes, mean±standard deviation)
PHA : Phytohemagglutinin
IL-2 : interleukin 2
PBL : peripheral blood mononuclear cells

Table 2. Effect of culture supernatant of the leukemic cells on proliferation of IL-2 dependent cells

Experiment	1		2
	LAK	PBL	CTLL
medium	11564±3814cpm*	181691±1061	5271±1294
IL-2 (10%)	20499±2175	15513±4593	19221±325
supernatant			
1 : 32	10770±3074	ND	ND
1 : 16	10915±3186	ND	4596±352
1 : 8	630±291	ND	2972±256
1 : 4	278±114	2183±5911	1734±284
1 : 2	ND	ND	1012±152

Exp.1 culture supernatant of PBL not stimulated
Exp.2 culture supernatant of PBL stimulated with PHA and PMA. All cultures were carried out in RPMI-1640 medium containing 10%. FCS and 2 mM (L-glutamine*
^{3}H-thymidine uptake (count per minutes , mean±standard deviation)
IL-2 : Interleukin-2
LAK : Iymphokine activated killer cells

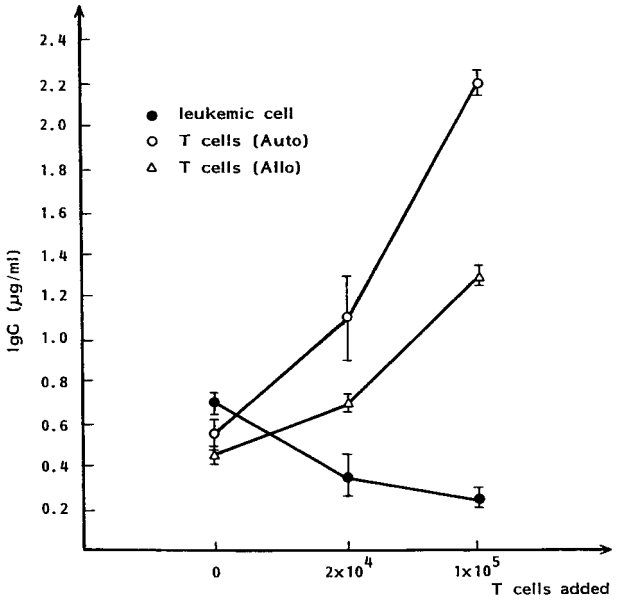

Fig. 1. Effect of Leukemic Cells lgG Production

that are often found in patients with ATL such as cutaneous lesions, hepatosplenomegaly, Iymphadenopathy or hypercalcemia, which are thought to be due to infiltration of malignant cells (10). Immunological investigation revealed the dysfunction of the lymphoid cells in the patient. The leukemic cells did not respond to PHA (11) or IL-2, although 31.3% of the cells had Tac^{+} phenotype, which is known to be the site of IL-2 receptor. The leukemic cells had a phenotype of helper T cell, OKT3^{+}4^{+} and OKT8^{-}, but the function was more like suppressor T cells than helper T cells. Similar results were reported previously by several investigators (12-13). Furthermore, in the IL-2 production assay, supernatant of the leukemic cells cultured with or without both PHA and PMA, suppressed dose-dependently the incorporation of ^{3}H-thymidine by IL-2 dependent cells than the control. And in the response to IL-2, IL-2 inhibited the proliferation of tumor cells dose-dependently. These findings suggest that IL-2 promoted the inhibitory effect of a factor produced by tumor cells. One possibility is that this factor inhibited the proliferation via IL-2 receptor (IL-2 R). And IL-2 upregulated the IL-2 R expression resulting in the promotion of the inhibitory effect of the factor. Burton et al. (14) reported that in the late phase of ATL, leukemic cells no longer produced IL-2. Tatsumi et al. (15) reported leukemic cells from ATL suppressed the proliferative response of normal T cells to alloantigens. Mori, et al. (16) also reported the culture supernatant from ATL suppressed lymphocyte proliferative responses to stimulation with mitogens. Honda et al. (17), Hardt et al. (18) and Lelchuk et al. (19) reported that IL-2 inhibitor produced in the supernatant of MLR in rodents blocked production of IL-2 from helper T cells and expression of the activities. Furthermore, production of IL-2 inhibitor by cancer cells was reported by Fontana et al. (20). Since these IL-2 inhibitors have not been fully characterized, it is not clear whether IL-2 inhibitor like factor in this patient was same as the factors reported previously. However as cells in ATL are transformed from OKT4^{+} cells, the factors inhibiting IL-2 dependent cell lines may be similar to suppressor factors produced by OKT4^{+} suppressor T cells or abnormal IL-2 by OKT4^{+} helper T cells. The effect of contaminated normal lymphocytes is supposed low, and same conclusion is obtained if the contaminated normal lymphocytes had functioned to some extent. Thus, the leukemic cells induced an immunodeficiency state in the host resulting in the occurrence of multiple primary cancers. The incidence of synchronous double carcinoma of the vulva and uterine cervix is very low. We experienced only one case in a total of 1156 patients with gynecologic carcinomas in the last of 13 years in our clinics. In fact, high occurence of primary malignant neoplasms have been reported in patients with ATL (21), especially smouldering type (5/18 cases (28%)) (4). ATL is a profoundly immunosuppressive malignancy (22). In patients with other leukemia, the incidence of synchronous primary malignancies is not rare, but not frequent. Mansow, et al. (23) reported that the risk of all cancers developing in patients with CLL is threefold that for the age- and sex-matched population. In another report (24), from 2134 patients with leukemia, synchronous primary lesions was diagnosed in 27 cases (1.3%). Human immunodeficiency virus (HIV) was known as another human T-Iymphotropic virus (HTLV). In AIDS patients, Kaposi's sarcoma, opportunistic infection and malignant lymphoma are commnon causes of complications and mortality. The immunodefficient state in AIDS patients is accounted for by the depletion of T 4^{+} cells. Whereas humoral factor in the culture supernatant of HIV-infected cells which inhibits PWM-driven lgG synthesis, and T-cell proliferation responding to Tetanus Toxoid was reported

by Laurence, et al. (25). Thus, the onset of double carcinoma found in this patient seems to be based on the immunodeficient state caused by the leukemic cells. To my knowledge, this is the first report of an ATL patient complicated with double cancer of the uterine cervix and vulva.

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