RAPID COMMUNICATION Jpn. J. Cancer Res. 83, 929–932, September 1992

Simultaneous Expression of T-Cell and Myeloid Cell Phenotypes in Eight Newly Established HTLV-I-positive T-Cell Lines

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Eight cell lines were established from patients with adult T-cell leukemia, and from normal adults, by cocultivation with human T-cell leukemia virus type I(HTLV-I)-producer cell lines in the presence of interleukin-2. All of these cell lines harbored HTLV-I and showed T-cell markers CD2, CD3 and CD4, but not B-cell markers. Unexpectedly, all eight cell lines expressed a myeloid marker CD13 and three of the eight lines also expressed another myeloid marker CD33. Dual staining showed the simultaneous expression of CD3 and CD13 on the same cells. Thus, evidence was obtained for the expression of myeloid antigens on HTLV-I-harboring T cells.

Key words: HTLV-I — Myeloid antigen — CD13 — CD33

HTLV-I[1] is causally associated with adult T-cell leukemia (ATL),[2–4] which is highly endemic in southwestern areas of Japan. ATL cells have CD3 and CD4 surface markers and presumably originate from mature helper/inducer T cells expressing these antigens.[5,6] HTLV-I can convert normal CD4-positive T lymphocytes into immortal blast cells *in vitro*.[7] This particular virus can also infect human B cells, null cells, or non-hematopoietic cells.[8–10] We recently reported that a novel ATL-derived cell line expressed myeloid cell antigens, despite its T-cell characteristics.[11] In the present study we examined the myeloid antigen expression in HTLV-I-harboring cell lines newly established from ATL patients, and from healthy adults.

Peripheral blood mononuclear cells (PBMCs) from ATL patients and HTLV-I seronegative healthy adults were separated by Ficoll-Conray density gradient centrifugation. Eight HTLV-I-bearing cell lines were established (Table I). Two lines (KOSP and NMSP) were isolated directly from PBMCs of ATL patients (K.O. and N.M., respectively) by cultivation in RPMI 1640 medium containing 20% fetal calf serum (FCS), penicillin (100 IU / ml), streptomycin (50 μg / ml), and recombinant interleukin-2 (rIL-2, 100 U/ml, Shionogi, Osaka). Six cell lines (M2-2, -4, -8 and MUMC-1, -2, -8) were isolated from PBMCs of two healthy adults (M.S. and S.K., respectively) by cocultivation with HTLV-I-producer cell lines, MT-2[7] and MU,[11] respectively. The PBMCs were cultured with lethally X-irradiated (12,000 R) virus-producer cells in RPMI 1640 medium with 20% FCS and antibiotics. rIL-2 was added to the cultures four weeks later. The cell lines were established one to three months after cultivation. All of the cell lines were dependent for their growth on rIL-2 and had diploid chromosomes (data not shown), hence, may have originated from normal blood lymphocytes infected by HTLV-I. The cell lines were analyzed within three months after establishment.

Acetone-fixed cells were examined for HTLV-I antigens $p19^{gag}$, $p24^{gag}$, and $gp21^{env}$ by indirect immunofluorescence using monoclonal antibodies GIN14,[12] H15[13] and F10,[14] respectively, and $F(ab')_2$ rabbit anti-mouse immunoglobulins-fluorescein isothiocyanate (Ig-FITC) (DAKO Immunoglobulins, Copenhagen, Denmark) as the secondary reagent. Cell surface markers were examined using monoclonal antibodies, T11, B4, B1, MY7, MY4, MY9 (Coulter Immunology, Hialeah, FL), OKT3, OKT4, OKT8, OKM13 (Ortho Diagnostic Systems, Rantan, NJ), anti-LeuM7 (Becton-Dickinson, Mountain View, CA), T55 (Nichirei Co., Tokyo), anti-K and anti-λ immunoglobulin light chains (DAKO), and analyzed using a FACScan flow cytometer (Becton-Dickinson). Two-color immunofluorescence was performed as follows. Cells were first reacted with OKT3 for 30 min at 4°C, washed in phosphate-buffered saline (PBS), and suspended with $F(ab')_2$ anti-mouse Ig-FITC for 30 min at 4°C. The cells were washed in PBS and suspended in 20% normal mouse serum for 20 min at 4°C to prevent subsequent nonspecific antibody binding.

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Table I. Profile of Newly Established Cell Lines

Cell line	Source[a]	Cultivation[b]	% positive cells		
			$p19^{gag}$	$p24^{gag}$	$gp21^{env}$
KOSP	ATL (K.O.)		>90	>90	23.1
NMSP	ATL (N.M.)		10.0	35.5	8.2
M2-2	normal (M.S.)	coculture (MT-2)	52.5	19.0	>90
M2-4	normal (M.S.)	coculture (MT-2)	19.8	—	—
M2-8	normal (M.S.)	coculture (MT-2)	65.6	6.0	27.2
MUMC-1	normal (S.K.)	coculture (MU)	21.6	—	—
MUMC-2	normal (S.K.)	coculture (MU)	51.1	25.6	17.5
MUMC-8	normal (S.K.)	coculture (MU)	78.4	56.1	65.8

a) Donors are shown in parenthesis.
b) HTLV-I-producer cell lines used are given in parenthesis.
—; less then 5%.

Table II. Surface Marker Analysis of HTLV-I-harboring Cell Lines[a]

Cell line	CD2 (TII)[b]	CD3 (OKT3)	CD4 (OKT4)	CD7 (T55)	CD8 (OKT8)	CD19 (B4)	CD20 (B1)	sIg	CD13 (MY7)	CD14 (MY4)	CD33 (MY9)
KOSP	>90	>90	>90	—	8.6	—	—	—	>90	7.0	—
NMSP	>90	>90	>90	—	—	—	—	—	11.6	—	43.2
M2-2	>90	>90	>90	47.0	—	—	—	—	40.4	—	14.0
M2-4	>90	>90	>90	n.t.	—	n.t.	n.t.	n.t.	47.8	—	n.t.
M2-8	>90	>90	>90	9.1	—	—	—	—	39.1	—	24.6
MUMC-1	>90	>90	>90	28.4	—	—	—	—	30.1	—	—
MUMC-2	>90	>90	>90	50.8	—	—	—	—	84.5	—	—
MUMC-8	>90	54.6	>90	—	—	—	—	—	38.2	—	—

a) The findings are given as a percentage of positive cells.
b) Monoclonal antibodies used are shown in parenthesis.
—; less than 5%, n.t.; not tested.

The cells were then reacted with PE-labeled MY7 for 30 min at 4°C and subjected to flow cytometric analysis.

Radiolabeling of cells and immunoprecipitation were carried out as described by Fujiwara *et al.*[15] Briefly, 5×10^6 cells were labeled for 3 h with 100 μCi of [^{35}S]-methionine per ml, then lysed in RIPA buffer, and the lysate containing approximately 5×10^7 cpm radioactivity were reacted with 5 μg of monoclonal antibodies. The immunoprecipitates were analyzed by SDS-PAGE using a 6% acrylamide gel.

All of the eight cell lines were positive for HTLV-I antigen $p19^{gag}$, and six lines were positive for $p24^{gag}$ and $gp21^{env}$, hence, they harbored HTLV-I (Table I). These lines were positive for T-cell markers CD2, CD3 and CD4, but not for B-cell markers CD19, CD20 and surface immunoglobulins, thereby indicating that they expressed the helper/induced T-cell phenotype. Unexpectedly, those lines were all positive for a myeloid cell antigen CD-13 and three of the eight lines were positive for another myeloid antigen CD33 (Table II and Fig. 1). Since most of the cells were positive for CD3, it seemed that a proportion of CD3-positive cells simultaneously expressed CD13 and/or CD33. Two-color immunofluorescence analysis clearly demonstrated that CD3 and CD13 were coexpressed on the same cells (Fig. 1C and 1D). The specificity for CD13 was confirmed by positive immunofluorescence of the cells with two other anti-CD13 monoclonal antibodies, OKM13 and anti-LeuM7 (data not shown).

Immunoprecipitation analysis demonstrated protein bands corresponding to CD13 (Fig. 2). Compared to HL-60 cells, in which CD13 was immunoprecipitated as 130 kDa and 150 kDa glycoproteins, all of the HTLV-I-positive cell lines, except NMSP, revealed protein bands of apparent molecular weight 130 and 140 kDa. The negative results seen in NMSP cell line may be due to a weak CD13 expression (see Table II).

The present study demonstrated that myeloid cell antigens are expressed on HTLV-I-bearing T cells. The presence of CD13 molecules (130 kDa and 140 kDa poly-

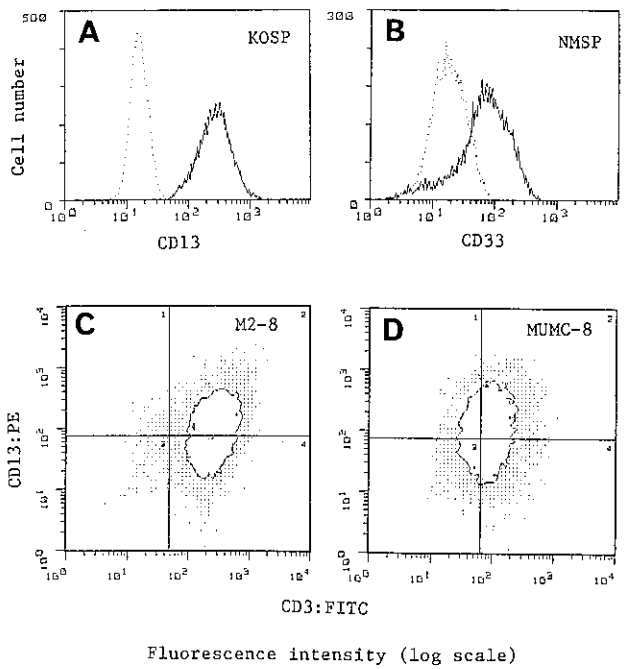

Fig. 1. Flow cytometric analysis. KOSP (A) and NMSP cells (B) were stained for CD13 and CD33, respectively. Two-color fluorescence was performed for M2-8 (C) and MUMC-8 cells (D) with CD3 (FITC) and CD13 (PE) by the method described in the text.

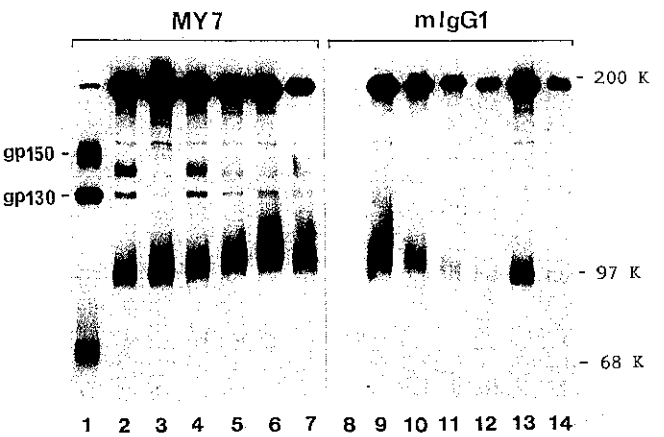

Fig. 2. Immunoprecipitation analysis. HL-60 (lanes 1 and 8), KOSP (lanes 2 and 9), NMSP (lanes 3 and 10), M2-2 (lanes 4 and 11), M2-8 (lanes 5 and 12), MUMC-1 (lanes 6 and 13), and MUMC-8 cells (lanes 7 and 14) were labeled, and the lysates were reacted with MY7 (lanes 1–7) or control mouse IgG1 (mIgG1, lanes 8–14). The positions of molecular weight markers are shown on the right.

peptides), as substantiated by immunoprecipitation, rules out cross-reactivity of monoclonal antibody MY7. In HL-60 cells both 130 and 150 kDa polypeptides are glycosylated forms of CD13 antigen, and the former (gp130) is a precursor of the latter (gp150) that is expressed on the cell surface.[16, 17] In HTLV-I-positive cell lines, 130 kDa and 140 kDa polypeptides may correspond to gp130 and gp150 of HL-60 cells, respectively. The different size (140 kDa vs. 150 kDa) probably reflects differences in the composition of the complex oligosaccharide chains synthesized in the two cell types. Since CD13 is identical to aminopeptidase N[18] and this enzyme is thought to be involved in signal transduction,[19] CD13 expressed on HTLV-I-bearing cell lines might function as a regulator for the signal transduction at the cell membrane.

The expression of myeloid antigens on HTLV-I-positive cell lines seems more frequent than heretofore considered, for the following reasons. 1) All of the eight cell lines reported here, which were isolated from different donors (two ATL patients and two healthy adults) and harbored different virus strains (MT-2 and MU) expressed CD13, and three also expressed CD33. 2) We and others have reported evidence that certain HTLV-I-bearing cell lines are positive for CD13.[11, 20]

Mechanisms involved in the expression of myeloid cell antigens remain elusive. There may be a transactivation by which several cellular genes are induced in HTLV-I-positive cells.[21] In our cell lines, CD13 and CD33 physiologically expressed on cells of myeloid lineage but not those of lymphoid lineage might be induced by trans-acting factors of HTLV-I. Alternatively, HTLV-I might infect biphenotypic cells expressing both T- and myeloid cell antigens. As HTLV-I can infect cells of different lineages[7–9] the latter hypothesis is given support. It is noteworthy that there have been reports of patients with adult acute lymphoblastic leukemia (ALL) whose blasts frequently expressed myeloid markers (e.g. CD13 and CD33) in association with T-cell markers.[22, 23] The expression of myeloid antigens on HTLV-I-harboring T cells may shed new light on undefined targets for HTLV-I infection.

We thank Drs. K. Sugamura, H. Tozawa and Y. Tanaka for providing monoclonal antibodies to HTLV-I, Drs. H. Kobayashi and K. Koda for supplying materials, Dr. R. Masuda for technical advice and J. Matsumoto for typescript services. This work was supported by grants from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, Japan.

(Received March 21, 1992/Accepted June 15, 1992)

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