Jpn. J. Cancer Res. 81, 225–231, March 1990

Heterogeneity of Antigen Molecules Recognized by Anti-tax_1 Monoclonal Antibody Lt-4 in Cell Lines Bearing Human T Cell Leukemia Virus Type I and Related Retroviruses

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Using a monoclonal antibody, Lt-4, directed against human T cell leukemia virus type I (HTLV-I) *trans*-activator (tax_1) antigen, we examined the expression of tax_1 and related antigens in a variety of T cell lines bearing HTLV-I and related retroviruses, simian T cell leukemia virus type I (STLV-I) and HTLV-II, by immunofluorescence and immunoblot assays. Lt-4 reacted with all HTLV-I-bearing cell lines tested and five out of eight simian cell lines bearing STLV-I, but not with an HTLV-II-bearing cell line. Lt-4 detected 40 kd tax_1 antigen molecules in most HTLV-I-bearing cell lines except one cell line that expressed 39 kd tax_1 antigen. In the STLV-I-bearing T cell lines, tax_1-related antigen molecules detected by Lt-4 were heterogeneous, having molecular weights in the range of 36–41 kd.

Key words: HTLV-I — STLV-I — HTLV-II — *trans*-Activator antigen — Monoclonal antibody

Human T cell leukemia virus type I (HTLV-I)[6] is a C-type retrovirus etiologically associated with a human malignant T cell disorder, adult T cell leukemia (ATL).[1-4] The HTLV-I genome contains open reading frames for viral structural components, *gag, pol* and *env* and a unique sequence termed *pX* encoding three non-structural proteins.[5] One antigen called $p40^{tax}$, or tax_1 antigen, acts as a *trans*-activator (tax) of the HTLV-I long terminal repeat (LTR) and has been suggested to be involved in transformation and immortalization of normal T cells infected with HTLV-I, because the tax_1 antigen activates the cellular genes encoding interleukin 2 (IL-2) and IL-2 receptor which are essential for T cell growth.[5-12] Natural antibodies to HTLV-I gag and/or env antigens are found in almost all sera from HTLV-I-infected humans,[13-15] whereas anti-tax_1 antibodies are found in only one-third of the sera.[16]

Although the protein structure of the tax_1 antigen has been deduced from the nucleotide sequence,[17-19] little is known about its antigenic structure. With antisera against synthetic peptides, it was shown that the tax_1 antigen expressed antigenic determinants in the C- and/or N-terminal regions which cross-reacted with other tax antigens encoded by other retroviruses of the HTLV-I family,[7,10,20] simian T cell leukemia virus type I (STLV-I) isolated from several species of Old World monkeys[21-23] and HTLV type II (HTLV-II) isolated from a patient with hairy cell leukemia.[3] The putative tax antigens of some strains of STLV-I and the tax antigen of HTLV-II (tax_2) were shown to be protein molecules of about 41 kd[20] and about 38 kd, respectively.[7,10] However, no report has compared the tax antigen molecules expressed in various cell lines bearing HTLV-I, STLV-I and HTLV-II by using either monoclonal antibodies (mAbs)[24] or monospecific antisera to tax antigen peptides.

To study further the antigenic structure of the tax_1 antigen, preparation of mAbs against various epitopes of the antigen is essential, but a library of mAbs to the tax_1 antigen has not been established. Recently we succeeded in the preparation of a mAb reactive with the tax_1 antigen, Lt-4, from mice immunized with purified native tax_1 antigen.[25] The present study was designed to determine the Lt-4 reactivity with various cell lines expressing *trans*-activator antigens of HTLV-I, STLV-I and HTLV-II, because such studies may be helpful in revealing the antigenicity of not only the tax_1 antigen but also the tax antigens encoded by STLV-I and HTLV-II.

In the present study, we found that Lt-4 reacted with all HTLV-I-bearing cell lines tested and detected 40 kd tax_1 antigen molecules in the cell lines except one that expressed 39 kd tax_1 antigen. Lt-4 also reacted with some STLV-I strain-bearing cell lines, but not with an HTLV-II-bearing cell line. Interestingly, the Lt-4-reactive

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[6] Abbreviations: HTLV-I, human T cell leukemia virus type I; STLV-I, simian T cell leukemia virus type I; HTLV-II, human T cell leukemia virus type II; tax, *trans*-activator.

tax_1-related antigen molecules expressed in the STLV-I-bearing cell lines were heterogeneous.

MATERIALS AND METHODS

Cell lines The cell lines bearing HTLV-I, STLV-I and HTLV-II are listed in Table I. These cell lines were cultured in RPMI1640 medium supplemented with 10–15% fetal calf serum (FCS), 100 units/ml penicillin and 100 μg/ml streptomycin, in the presence or absence of 20 units/ml human recombinant IL-2 (Shionogi, Osaka) at 37°C in 5% CO_2 in humidified air. These cell lines were passaged twice a week.

Monoclonal antibodies HTLV-I-specific mAbs used were GIN-14, anti-gag p19; NOR-1, anti-gag p24; FR-45, anti-gag p15 and TA-21, anti-env gp21.[27,30] A mouse IgG mAb, Lt-4, was prepared recently from mice immunized with affinity-purified tax_1 antigen from an HTLV-I-bearing cell line by using rabbit IgG anti-tax_1 peptide, and was determined to be specific for human HTLV-I-bearing T cell lines and to recognize the tax_1 antigen, $p40^{tax}$.[25]

Immunofluorescence Cells were smeared onto glass slides, fixed with methanol, and stained by an indirect immunofluorescence (IF) method using mAbs and FITC-labeled goat IgG anti-mouse IgG (Cappel, PA) as the secondary reagent.

Immunoblot Immunoblot assays were performed as described previously.[25] Briefly, cell lysates, which were obtained by lysis of cells with a low salt extraction buffer (10 m*M* Tris-HCl, pH 8.0, containing 0.14 *M* NaCl, 3 m*M* $MgCl_2$, 1 m*M* dithiothreitol, 2 m*M* phenylmethylsulfonyl fluoride, and 0.5% NP40) on ice for 20 min, followed by centrifugation at 12,000*g* for 10 min at 4°C, were subjected to SDS-PAGE on 10% separation gels, and blotted electrophoretically onto blot sheets (Clear Blot P membrane, Atto Corp., Tokyo). The sheets were blocked with Block Ace (Dainippon Pharmaceutical Corp., Osaka) at 4°C overnight, incubated with various antibodies diluted with PBS containing 10% Block Ace at room temperature for 45 min, and washed three times with PBS containing 0.05% Tween 20. Bound antibodies were visualized by a modification of Manson's method[31] using a soluble immunocomplex of peroxidase with rabbit anti-peroxidase serum (PAP, Dako, Denmark) (PAP method).

Radio-immunoprecipitation Analysis of antigen molecules was also performed by radio-immunoprecipitation as described previously.[32] Briefly, 1×10^6 cells resuspended in 1 ml of RPMI medium without cysteine were incubated with 100 μCi of ^{35}S-cysteine (specific activity 1250 Ci/mmol, Amersham Japan) at 37°C for 4 h, washed three times with ice-cold PBS, and then lysed in 1 ml of the low salt extraction buffer. Aliquots (200 μl)

Table I. HTLV-I, STLV-I and HTLV-II Retrovirus Bearing T Cell Lines Used in This Study

Cell line	Originated from	IL-2 requirement for cell growth	Reference
HTLV-I-bearing human cell lines			
MT-2	normal human	−	26
HUT102	ATL patient	−	3
F-Taj	ATL patient Taj	−	27
ILT-Taj	ATL patient Taj	+	27
F-Aki	ATL patient Aki	−	27
ILT-Aki	ATL patientAki	+	27
ILT-Som	ATL patient Som	+	27
ILT-500	healthy HTLV-I carrier 500	+	27
ILT-625	healthy HTLV-I carrier 625	+	27
STLV-I-bearing cell lines			
ChM114-1	Chimpanzee (Africa)	+	23
GM0650	Green monkey (Africa)	+	23
FM34	Formosan monkey (Asia)	+	23
Kani 11-6	Cynomolgus monkey (Asia)	−	28
PtM-3	Pig-tailed macaque (Asia)	−	23
JM86	Japanese monkey (Asia)	−	23
BM5	Bonnet monkey (Asia)	+	23
RfM26-1	Red face macaque (Asia)	−	23
HTLV-II-bearing cell line			
Ton-1	normal human	−	29

of cell lysates were reacted with mAbs (100 μl of culture supernatant) for 2 h at 4°C, followed by incubation with 100 μl of 10% (v/v) Protein A-Sepharose 4B (Pharmacia) overnight. Then the Sepharose was washed three times and the eluates were separated by SDS-PAGE on 10% separation gels, and the gels were subjected to fluorography at −70°C.

RESULTS

We previously reported a mAb, Lt-4, specific for HTLV-I *trans*-activator, tax_1, antigen.[25] In this study we have further characterized the reactivity of Lt-4 with various cell lines bearing HTLV-I, various strains of STLV-I and HTLV-II by immunofluorescence and immunoblot assays. For immunoblotting, we separated cell lysate proteins by SDS-PAGE on 10% separation gel, instead of 12.5% gel which was used in the previous experiments, to facilitate comparison of the apparent molecular weights of tax_1 antigen and related antigen molecules migrating to around the 40 kd position.

Fig. 1 shows that T cell lines derived from ATL patients and HTLV-I healthy carriers, irrespective of IL-2 requirement for cell growth, expressed 40 kd antigen molecules detected by Lt-4, except for the HUT102 cell line maintained in our laboratory which expressed 39 kd antigen. In addition, Fig. 1 seems to indicate that there is also microheterogeneity of size of tax_1 antigen molecules expressed in the other HTLV-I-bearing cell lines. That our HUT102 expressed aberrant tax_1 antigen was confirmed by an immunoprecipitation experiment (Fig. 2). From ^{35}S-cysteine-labeled lysates of our HUT102 cells, Lt-4 precipitated 39 kd protein predominantly in addition to 38 kd protein, while from MT-2 and F-Taj cells, Lt-4 specifically precipitated 40 kd proteins. These results

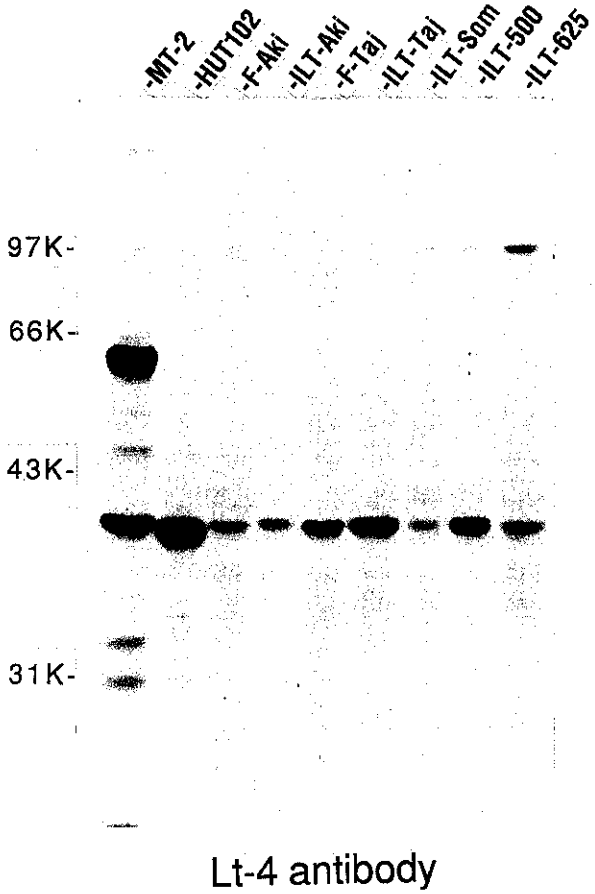

Fig. 1. Immunoblot analysis of tax_1 antigen molecules in various HTLV-I-bearing human T cell lines. Proteins in cell lysates were separated by SDS-PAGE on 10% separation gels and blotted onto blotting sheets. The sheets were then incubated with Lt-4 mAbs bound to the sheets were visualized by using the PAP method.

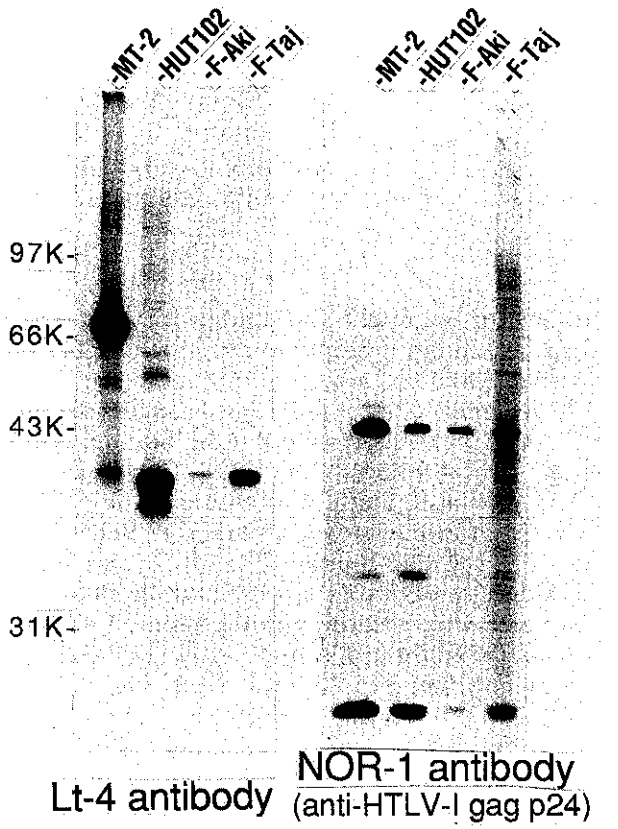

Fig. 2. Immunoprecipitation of tax_1 antigen molecules from HTLV-I-bearing human T cell lines. Lysates of ^{35}S-Cys-labeled cells were reacted with Lt-4 and NOR-1 mAb anti-HTLV-I gag p24. Immune complex was precipitated by using Protein A-Sepharose and analyzed by SDS-PAGE on 10% gel.

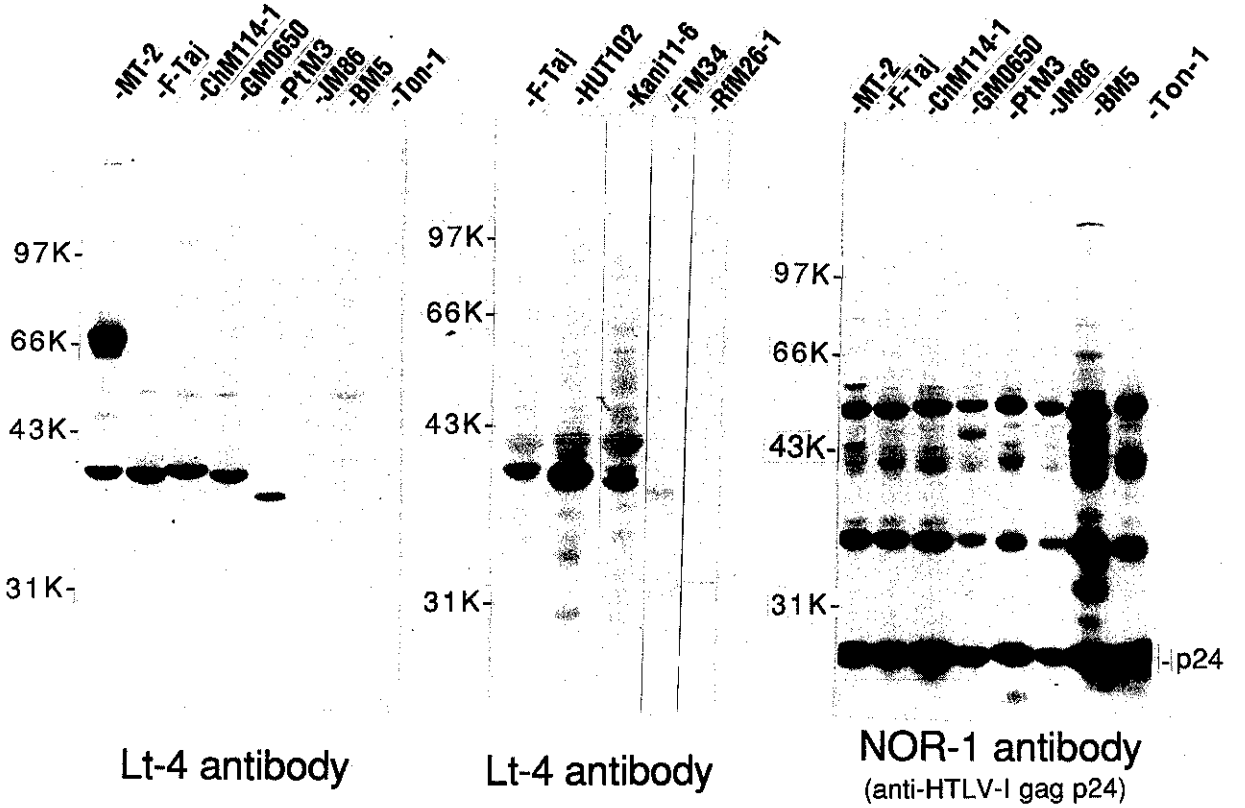

Fig. 3. Immunoblot analysis of reactivity of Lt-4 with T cell lines bearing various strains of STLV-I and HTLV-II. Proteins in the cell lysates were separated by SDS-PAGE, blotted onto blotting sheets, and reacted with Lt-4 or NOR-1.

indicated that most HTLV-I-bearing cell lines expressed 40 kd tax_1 antigen, except our HUT102 cell line which expressed 39 kd tax_1 antigen.

To determine whether tax_1-related antigens recognized by Lt-4 are expressed in cell lines bearing HTLV-I-related viruses, STLV-I and HTLV-II, we screened a variety of simian T cell lines established from simians naturally infected with various strains of STLV-I, and a human T cell line bearing HTLV-II, Ton-1. An immunofluorescence assay showed that while all of the cell lines tested were stained by mAbs against HTLV-I gag antigens (p19, p24 and/or p15) and HTLV-I env gp21 antigen, only four STLV-I-bearing cell lines, ChM114-1, GM0650, Kani11-6 and PtM-3, were stained by Lt-4 (Table II). To examine further the Lt-4 reactivity and to identify Lt-4-reactive antigen molecules, immunoblot assays were performed (Fig. 3). Lt-4 detected antigens with apparent molecular weights of 41, 40, 38 and 36 kd in the cell lysates from ChM114-1, GM0650, Kani 11-6 and PtM3 cell lines, respectively. The FM34 cell line was negative for Lt-4 staining in the immunofluorescence assay, but the immunoblot assay revealed low levels of the Lt-4-reactive 36 kd antigen. However, even in the immunoblot assays, no specific antigen was detected by Lt-4 in the cell lysates from BM5, JM86, RfM26-1 or Ton-1 cell line. There was no apparent difference in the molecular weight of the mature core antigen, p24, expressed in the T cell lines tested. The data of Fig. 3 are summarized in Table III.

DISCUSSION

The present study showed that: (1) mAb Lt-4 directed against the tax_1 antigen detected 40 kd antigen molecules in most HTLV-I-bearing cell lines except our HUT-102 cell line; (2) Lt-4 also reacted with some but not all STLV-I-bearing T cell lines established from monkeys naturally infected with various strains of STLV-I; (3) Lt-4 reactive antigens in the STLV-I bearing cells were hetrogeneous in molecular weight; and (4) Lt-4 did not react with the HTLV-II tax (tax_2) antigen.

The reasons why our HUT102 cell line synthesized 39 kd tax_1 antigen molecules are not known. This is not unique to our HUT102 cell line since an HUT102 cell line

Table II. Immunofluorescence Analysis of the Expression of Lt-4-reactive Antigens in STLV-I- and HTLV-II-bearing T Cell Lines

Cell line	Reactivity with mAb[a]					
	Lt-4 anti-tax_1	GIN-14 anti-p19	NOR-1 anti-p24	FR-45 anti-p15	TA-21 anti-gp21	H-31 anti-IL2R
HTLV-I-bearing cell lines						
MT-2	++[b]	++	++	++	++	++
HUT102	++	++	++	++	++	++
STLV-I-bearing cell lines						
ChM114-1	+	++	++	++	++	++
GM0650	±	+	+	+	+	+
Kani 11-6	+	++	++	−	+	+
PtM-3	+	++	++	+	+	+
FM34	−	±	±	±	+	++
JM86	−	+	+	+	±	+
BM5	−	++	++	±	±	±
RfM26-1	−	+	++	±	++	+
HTLV-II-bearing cell line						
Ton-1	−	++	++	++	++	++

a) Fixed cells were examined by an indirect immunofluorescence method.
b) Reactivities of mAbs were expressed as: −, negative; ±, weakly positive; +, positive; and ++, strongly positive, as determined by fluorescence microscopy.

Table III. Summarized Data on the Reactivity of Lt-4 with Various T Cell Lines Bearing HTLV-I, STLV-I and HTLV-II

Cell line	Antigen detected by Lt-4 (kd)
HTLV-I-bearing cell lines	
MT-2	68, 40
HUT102	39
F-Taj	40
ILT-Taj	40
F-Aki	40
ILT-Aki	40
ILT-Som	40
ILT-500	40
ILT-625	40
STLV-I-bearing cell lines	
ChM114-1	41
GM0650	40
Kani 11-6	38
PtM-3	36
FM34	36
JM86	none
BM5	none
RfM26-1	none
HTLV-II-bearing cell line	
Ton-1	none

maintained in another laboratory also synthesized a similar 39 kd tax_1 antigen (Drs. Nyunoya and Shimotohno, personal communication). It is possible that there is either a deletion of amino acids or an aberrant post-translational modification of the tax_1 antigen molecule in the HUT102 cell lines. Sodroski *et al.*[9] observed a heterogeneity of the tax_1 antigens in some HTLV-I-immortalized human cell lines, and they speculated that there might be a variation in the amino terminus of the tax_1 or post-transcriptional modifications of the tax_1 antigen in the cell lines. Recently, it was reported that the tax_1 antigen was phosphorylated when the antigen was produced with a baculovirus vector in lymphoid cells[33] and insect cells.[34]

The Lt-4-reactive tax_1-related antigens expressed in STLV-I bearing cells may be identical or related to *trans*-activator antigens encoded by STLV-I pX gene. If this is the case, since each of the STLV-I strains was suggested to have diverged separately after divergence from HTLV-I,[35, 36] there may have been alteration in the *trans*-activator gene of each STLV-I strain during evolution, which may explain both the different reactivity of Lt-4 with the panel of STLV-I-bearing cell lines and the heterogeneity in molecular weight of the Lt-4-reactive antigens among STLV-I-bearing cell lines. It has been shown that HTLV-I is a member of the African but not the Asian subtype of STLV-I based on the homology of nucleotide sequences of the long terminal repeat.[36] That Lt-4 reacted with 2 out of 2 cell lines bearing the African subtype STLV-I strains, but only 3 out of 6 cell lines bearing the Asian subtype STLV-I strains, may suggest that the Lt-4-reactive antigenic epitope is conserved better in the African subtype STLV-I strains than in the Asian subtype STLV-I strains.

The present study also showed that Lt-4 had a different specificity from other monospecific antisera prepared against various tax_1 peptides or a fused protein: (1) antisera against tax_1 peptides, OP-1 and OP-4 corresponding to N- and C-terminal regions of both the tax_1 and tax_2 antigens, respectively, reacted both with tax_1 and tax_2 antigens[7, 10]; and (2) antiserum against bGH-p40[XI], that covers 54 amino acids located at the C-terminus of the tax_1 antigen, reacted with the tax_1 but not with tax_2 antigen.[8] However, this antiserum also reacted with the BM5 cell line,[20] with which Lt-4 did not react. Thus, it appears that the tax_1 antigen contains at least four antigenic determinants recognized by antibodies.

Further biochemical analysis of tax_1 antigen and related antigens by two-dimensional gel electrophoresis and partial proteolytic mapping, and a study of the anti-

genicity of these antigens with a library of mAbs specific for various epitopes of the tax_1 antigen are required to elucidate the tax_1 antigenic structure and its relationship to tax antigens encoded by other HTLV-I-related viruses. Such studies are in progress.

ACKNOWLEDGMENTS

We thank Dr. S. R. Jennings for reading the manuscript. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

(Received October 18, 1989/Accepted January 23, 1990)

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