—RAPID COMMUNICATION—

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A MONOCLONAL ANTIBODY THAT RECOGNIZES A COMMON ANTIGEN IN MYELOID AND HUMAN T-CELL LEUKEMIA VIRUS TYPE I-CARRYING CELLS

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A novel monoclonal antibody, designated D-213, was generated to human myeloid cell line PL-21. D-213 defined a 47-kD antigen which was specifically expressed in normal and leukemic cells of the myeloid lineage and in T-cell lines carrying human T-cell leukemia virus type I. This antibody was also reactive with adult T-cell leukemia/lymphoma (ATLL) cells in formalin-fixed, paraffin-embedded tissues. D-213 would be useful for making an immunohistochemical diagnosis of ATLL in cases where serological or virological information is not available.

Key words: Monoclonal antibody — Myeloid cells — HTLV-I

Adult T cell leukemia/lymphoma (ATLL)[1] is a T-cell neoplasia associated with human T-cell leukemia virus type I (HTLV-I).[2-4] Usually, the diagnosis of ATLL is not difficult because of its characteristic cytological features and the presence of serum antibodies to HTLV-I.[5] However, some difficulties arise in distinguishing ATLL from other types of malignant lymphoma when serological or virological information is not available. Recently, we generated a monoclonal antibody that reacts specifically not only with myeloid cells but also with HTLV-I-carrying cells. The production and characterization of this unique monoclonal antibody is reported in this communication.

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Monoclonal antibody was produced according to the standard method.[6] Briefly, BALB/c mice were injected with 1×10^6 cells from human myeloid leukemia cell line PL-21[7] twice intraperitoneally and once intravenously at one-week intervals. Three days after the last immunization, spleen cells were fused with NS-1 cells. Hybridomas that secreted antibodies reactive with PL-21 cells but not with human peripheral mononuclear cells were cloned, and two clones were selected. One of the two hybridomas secreted IgM antibody and was designated D-213. Indirect immunofluorescence study showed that the D-213 antibody reacted with human myeloid cell lines (PL-21, KCL-22, K-562, HL-60 and ML-3) and HTLV-I-carrying cell lines (MT-1 and MT-2) (Figs. 1 and 2). No other hematopoietic cell lines except for a few populations of HPB-ALL reacted with this monoclonal antibody (Table I). Immunoblotting analysis[8] showed that D-213 detected a 47-kD antigen in both myeloid and HTLV-I-carrying cell lines (Fig. 3). Furthermore, this monoclonal antibody was found to be applicable to the immunohistochemical method.[9] In 8 of 12 ATLL cases, the cytoplasm and surface membrane of leukemic cells, especially of pleomorphic ones, were stained in formalin-fixed, paraffin-embedded tissue sections (Fig. 4). In contrast, neoplastic cells from HTLV-I-negative T-cell lymphoma, B-cell lymphoma, immunoblastic lymphoadenopathy (IBL), IBL-like T cell lymphoma and Hodgkin's disease were all negative for D-213 (Table II). None of the normal lymphoid tissues including thymuses, tonsils, lymph nodes, and spleens was reactive with D-213. In fresh smear preparations, D-213 stained normal myeloid cells in various differentiation stages and leukemic cells from 5 of 7 cases of acute myelocytic leukemia, but did not react with normal or concanavalin A-stimulated lymphocytes, megakaryocytes, erythroblasts or leukemic cells from 6 cases of acute lymphocytic leukemia and 2 cases of acute monocytic leukemia. We also examined the reactivity of

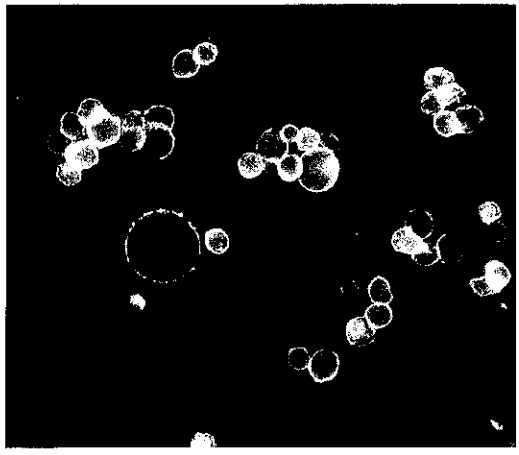

Fig. 1. D-213 is strongly reactive with the PL-21 myeloid cell line used as the immunogen. Indirect immunofluorescence staining. ×400.

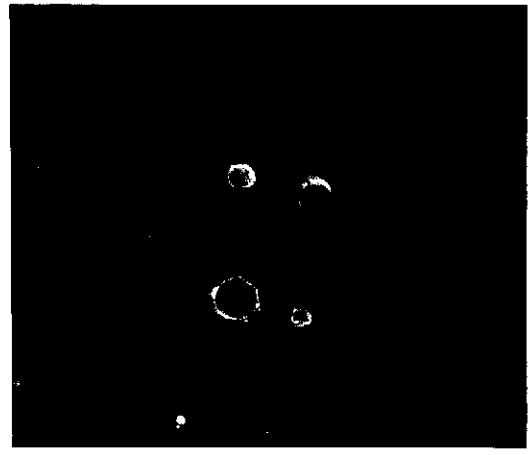

Fig. 2. D-213 is also reactive with the HTLV-I-producing MT-2 cell line. Indirect immunofluorescence staining. ×400.

Table I. Reactivity of D-213 with Human Hematopoietic Cell Lines

Cell line	Cell type	Origin	HTLV-I genome	% positive
PL-21	myeloid	APL	−	100
KCL-22	myeloid	CMLbc	−	71
K-562	myeloid	CMLbc	−	41
HL-60	myeloid	APL	−	65
ML-3	myeloid	AML	−	100
MT-1	T-cell	ATL	+	31
MT-2	T-cell	human cord lymphocyte	+	63
Transformed cell lines[a] (n=6)	T-cell	normal lymphocyte	+	11–70 (mean 31%)
HPB-ALL	T-cell	ALL	−	5
TALL-1	T-cell	ALL	−	0
MOLT-4	T-cell	ALL	−	0
CCRF-HSB-2	T-cell	ALL	−	0
NALL-1	null-cell	ALL	−	0
KEN-L-1	null-cell	ALL	−	0
RC-K8	B-cell	lymphoma	−	0
NALM-1	B-cell	CMLbc	−	0
Raji	B-cell	BL	−	0
BALL-1	B-cell	ALL	−	0
J-111	monocyte	AMoL	−	0
THP-1	monocyte	AMoL	−	0

a) Six T-cell lines were established by cocultivation with MT-2.
APL, acute promyelocytic leukemia; CMLbc, chronic myelogenous leukemia blastic crisis; AML, acute myelogenous leukemia; BL, Burkitt lymphoma; AMoL, acute monocytic leukemia.

D-213 with fresh peripheral lymphocytes from ATLL patients and healthy HTLV-I carriers. Unfortunately, D-213 gave weakly positive or negative reactions in these cases. It may be that the antigen recognized by D-213 is expressed only in ATLL cells in the mitotic cycle.

These results demonstrate that D-213 is a novel monoclonal antibody that recognizes a 47-kD antigen expressed in both myeloid and HTLV-I-positive cells. D-213 is distinct from previously reported myeloid-specific monoclonal antibodies which do not react with HTLV-I-carrying cells. D-213 appears to be particularly useful for the immunohistochemical diagnosis of ATLL as well as myeloid neoplasia. It is noteworthy that D-213 stained ATLL cells in formalin-fixed, paraffin-embedded tissue sections which lacked viral antigen expression. The monoclonal antibody would allow us to reevaluate in retrospect lymphoma cases in which data on HTLV-I

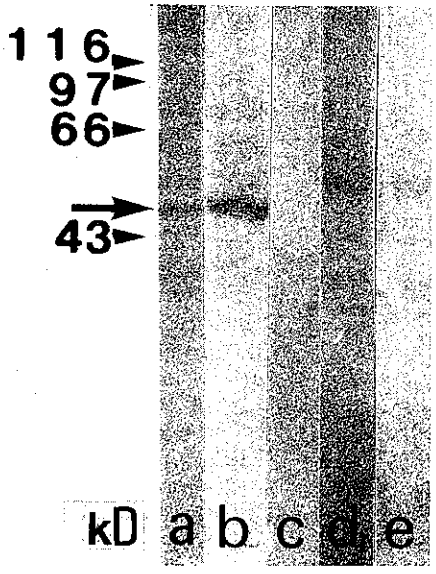

Fig. 3. Western immunoblotting analysis of lysates of MT-2 (lanes a and d), PL-21 (lanes b and e), and HTLV-I-negative TALL-1 (lane c) under reducing conditions. Lanes a, b, and c were reacted with D-213 and lanes d and e with normal mouse IgM. A-47 kD protein band is demonstrated in lanes a and b (arrow) but not in lanes c, d, and e.

Table II. Immunohistoreactivity of D-213 in Formalin-fixed, Paraffin-embedded Tissue Sections of Lymphomas and Related Disorders

Diagnosis	No. positive/ No. evaluated
ATLL	8/12
T-cell lymphoma	0/11
B-cell lymphoma	0/8
IBL	0/3
IBL like T cell lymphoma	0/2
Hodgkin's disease	0/3

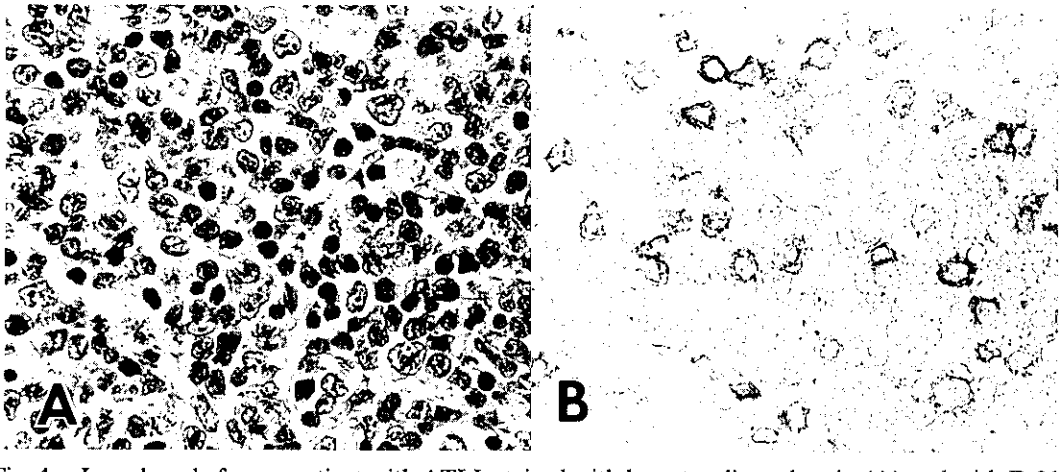

Fig. 4. Lymph node from a patient with ATLL stained with hematoxylin and eosin (A) and with D-213 (B). ×400.

serology or virology are lacking. At present, we do not know the mechanism by which the 47-kD antigen is expressed in both myeloid and HTLV-I-carrying cells. It has been reported that some cellular genes may be activated by transacting factors of HTLV-I.[10, 11] The antigen recognized by D-213 may represent such a cellular gene product, which is physiologically expressed in myeloid lineage cells but not in other hematopoietic cells including lymphocytes. Tsubai *et al.*[12] recently reported a monoclonal antibody, which detected a protein expressed in HTLV-I-related lymphoid cells and tissues irrespective of viral antigen expression. This monoclonal antibody successfully stained ATLL cells in frozen sections, whereas D-213 reacted with both myeloid and ATLL cells even in formalin-fixed, paraffin-embedded sections. Thus, D-213 promises to be a useful diagnostic aid in the study of biopsy or autopsy tissues from patients suspected of having ATLL.

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