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Production and Characterization of a Human Monoclonal Antibody Recognizing a New Antigen Expressed on Some Lymphoid Cells

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A cell line secreting a human monoclonal antibody was established by Epstein-Barr virus transforming B cells derived from an enlarged cervical lymph node excised from a patient bearing a carotid body tumor. The reactivity of the monoclonal antibody, designated as mNISP, was tested on various cells and cell lines. An antigen defined by the mNISP was expressed on some Burkitt's lymphoma cell lines and on a non-T non-B acute lymphoblastic leukemia cell line. Furthermore, this antigen was expressed on leukemic cells from 2 of 8 patients with chronic myelocytic leukemia, 2 of 10 patients with acute myeloblastic leukemia, one of 13 patients with acute lymphoblastic leukemia, and two patients with adult T cell leukemia, but it was not expressed on normal T, B and adherent (macrophage) cells. In addition, mNISP reacted with T cells obtained from human T-cell leukemia virus type I carriers. We found that the antigen defined by mNISP was distinct from any previously reported antigen in terms of its pattern of cellular expression and molecular weight, suggesting that mNISP recognizes a new antigen expressed on some lymphoid cells.

Key words: Human monoclonal antibody — Epstein-Barr virus transformation — Lymphoid cells

The carotid body, located at the bifurcation of the common carotid artery, is a chemoreceptor organ about the size of a grain of wheat.[1)] Its only known pathologic lesion, carotid body tumor (CBT), is the most common of the chemodectomas. It is nonetheless an extremely rare tumor of unknown incidence, but is infrequently malignant and occasionally familial in occurrence.[2)] It is apparently more common in persons living at high altitudes and also appears to be associated with a disease causing chronic hypoxia.[3)] Familial CBT is believed to have an autosomal dominant mode of genetic transmission,[4)] and is characterized by a highly increased frequency of bilateral tumors. Furthermore, hereditary deficiencies of clotting factors VII and X were reported to be associated with CBT.[5)] However, little else is known about the causes of CBT occurrence.

Recently, we experienced in succession two familial cases of CBT,[6)] and attempted to establish cell-lines originating from B-cells separated from an enlarged cervical lymph node of one of these patients. In this report, we describe the properties of a human monoclonal antibody (mAb), designated as mNISP secreted from an Epstein-Barr virus (EBV)-transformed B cell line established in this way. From the reactivity of mNISP, it was concluded that mNISP recognizes a new antigen expressed on some lymphoid cells.

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MATERIALS AND METHODS

Patient A 15-year-old male patient bearing a CBT was admitted to Kitasato University Hospital with a neck mass and low grade fever. An operation was performed in December, 1986 under general anesthesia accompanied with controlled hypothermia. One of his enlarged cervical lymph nodes was used for establishing the cell line.

Preparation of normal lymphoid or leukemic cells Peripheral blood (PB) and bone marrow (BM) aspirates obtained from patients with leukemia or from normal healthy donors were separated by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density sedimentation,[7)] and the interface layers were collected. Leukemic cells obtained from the PB or BM of patients with leukemia were cryopreserved in liquid nitrogen as previously described.[8)] Normal peripheral blood mononuclear cells (PBMCs) isolated from the PB of normal healthy donors were depleted of macrophages by adherence to plastic as previously described.[9)] The adherent cells were recovered and used as a macrophage-enriched population. The macrophage-depleted PBMCs were separated into erythrocyte (E)-rosette-positive (E+) and E-rosette-negative (E−) populations using sheep erythrocytes.[10)] Fresh leukemic cells were obtained from the Clinical Laboratories in Yokosuka Kyosaikai Hospital.

Preparation of T cells from human T-cell leukemia virus type I (HTLV-I) carriers Donors' sera were screened by means of the particle agglutination (PA) test using Serodia-ATLA (Fujirebio) at Shonan Red Cross Blood Center. PBMCs from HTLV-I carriers were isolated by Ficoll-Paque density sedimentation, and T cells were separated by E-rosette formation. The T cells were cultured in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (FCS) for 48 h.

Human cell lines All human cell lines except five EBV-transformed B cell lines, two neuroblastoma cell lines, and seven cancer cell lines, which were used in the present investigation, were supplied by the Japan Cancer Research Resources Bank (Yoga, Tokyo). The EBV-transformed B cell lines were kindly provided by Dr. F. Ohtani of the Department of Transplantation Immunology, Kitasato University School of Medicine. The neuroblastoma cell lines were kindly provided by Dr. M. Sekiguchi of the Department of Clinical Oncology, Institute of Medical Science, University of Tokyo. The cancer cell lines were supplied by the Japan Immunoresearch Laboratories (Takasaki, Gunma).

Antibodies Mouse mAbs, designated as CD3 (T3),[11] CD10 (J-5),[12] Phycoerythrin (PE)-conjugated CD10 (J-5),[12] CD13 (MY7),[13] CD19 (B4),[14] CD20 (B1),[15] and CD25 (IL-2R1),[16] were purchased from Coulter Immunology Laboratories (Hialeah, FL). CD10 (BA-3)[17] was obtained from (Hybritech, La Jolla, CA). L243[18] and CD23 (H107)[19] were purchased from Becton Dickinson Immunotech Laboratories (Mountain View, CA) and Nichirei Laboratories (Higashimurayama, Tokyo) respectively. CD25 (H31)[20] was kindly provided by Dr. Y. Tanaka of the Department of Immunology, Kitasato University School of Hygienic Science. The specificity of the mAbs used in this study is summarized in Table I. Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig(G+M+A)F(ab′)2, FITC-conjugated goat anti-human IgMF(ab′)2, non-conjugated goat anti-human IgG, non-conjugated goat anti-human IgM, non-conjugated goat anti-human IgA and horseradish peroxidase (POD)-conjugated goat anti-human Ig(G+M+A)F(ab′)2 were purchased from Cappel Laboratories (Malvern, PA).

Table I. The Specificity of the Mouse mAbs Used in This Study

CD no.	mAb	Specificity	Ig class
CD3	T3	Mature T	IgG1
CD10	J-5	CALLA	IgG2a
CD10	BA-3	CALLA	IgG2b
CD13	MY7	Normal and leukemic myeloid cells	IgG1
CD19	B4	Early B cells	IgG1
CD20	B1	Pan-B cells	IgG2a
CD23	H107	Fc ε receptor	IgG2a
CD25	IL-2R1	Interleukin-2 receptor	IgG2a
CD25	H31	Interleukin-2 receptor	IgG1
—	L243	HLA-DR	IgG1

Establishment of a human mAb-producing B cell line A cervical lymph node about the size of a broad bean, but apparently free from metastasis with neoplastic cells was excised, and chopped as finely as possible with a pair of scissors. The finely minced tissues were washed twice in RPMI1640 medium, and suspended in the medium. The cell suspension was layered on Ficoll-Paque and centrifuged at 1500 rpm for 30 min at room temperature. The lymphocyte-enriched interface was aspirated and resuspended in RPMI1640 medium. Then the T and B cells were separated by means of E rosette formation. The E− cells (composed mainly of B cells) were suspended in complete RPMI1640 medium supplemented with 20% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 m*M* L-glutamine. The suspension was distributed in 24-well plates (Costar, Cambridge, MA) with approximately 10^5 cells in 900 μl. To transform the B cells, filtered EBV-containing culture supernatant (100 μl) derived from a marmoset cell line, B95-8, was added to each well, and incubated for 60 min at 37°C, after which 1 ml of complete RPMI 1640 medium was added to each well, and the plates were kept under the same conditions. The medium was refreshed 2 to 3 times per week by adding 1 ml of fresh medium. EBV-transformed B cells usually began to appear within three weeks. The amount of human Ig in the culture supernatants was determined by enzyme-linked immunosorbent assay (ELISA).[21] After multiplying, cell populations from the wells showing higher optical density in tissue culture flasks were selected, and the transformed cells were cloned by limiting dilution. The selected cells were then recloned twice, resulting in the establishment of a cell line which has stably produced a large quantity of human Ig, even in the new culture medium GIT (Wako Pure Chemicals, Tokyo) which lacks FCS. The cell line and its mAb were named NISP and mNISP respectively.

Flow cytometric analysis The binding activity of the mAbs was analyzed by direct or indirect immunofluorescence using either a microscope or a fluorescence-activated cell sorter (FACStar, Becton Dickinson) as previously described.[21] In some experiments, cells were tested by two-color flow cytometry.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting Cells from two cell lines, Burkitt's lymphoma Raji and T cell leukemia TALL-1 were solubilized in 50 m*M* Tris-buffer (pH 8.0)

containing 0.5% E911, 2 m*M* phenylmethylsulfonyl fluoride (PMSF), 150 m*M* NaCl, 3 m*M* $MgCl_2$ and 1 m*M* dithiothreitol (DTT). After centrifugation, the solubilized proteins were separated on 10% SDS gel[22] and transferred electrophoretically to a nitrocellulose sheet,[23] which was then incubated with mNISP, CD10 (J-5) or L243. After the incubation, immunoperoxidase staining was performed using an avidin-biotin complex (ABC) kit[24] (Vector Laboratories Inc., Burlingame, CA), followed by visualization by means of the diamino-

Table II. Binding of mNISP for Neoplastic and Hematopoietic Cell Lines

Cell lines	Degree of binding with mAbs[a]									
	mNISP	J-5	BA-3	MY7	B4	B1	IL-2R1	H107	L243	T3
T cell leukemia										
CEM	−	−	−	−	−	−	−	−	−	−
Molt4	−	−	−	−	−	−	−	−	−	±
TALL-1	−	−	−	−	−	−	−	−	−	−
Burkitt's lymphoma										
Raji	++	++	++	−	++	++	−	+	++	−
Daudi	++	++	++	−	++	++	−	−	++	−
Ramos	++	++	++	−	+	+	−	−	++	−
BT-1	−	−	−	−	−	+	NT[b]	+	++	−
EBV-B cell line										
AKIBA	−	−	−	−	+	+	−	++	++	−
KT12	−	−	−	−	+	+	−	++	++	−
MANN	−	−	−	−	±	++	−	++	++	−
B85	−	−	−	−	+	+	−	+	++	−
HID	−	−	−	−	±	++	−	++	++	−
B lymphoblastoid										
IM9	+	−	−	NT	NT	++	−	++	++	−
Myeloid cell										
HL60	−	−	−	++	−	−	−	−	−	−
Erythroid cell										
K562	−	−	−	±	−	−	−	−	−	−
Neuroblastoma										
GOTO	−	+	±	NT	NT	−	NT	NT	NT	−
NB-1	−	+	±	NT	NT	−	NT	NT	NT	−
Non-T non-B										
P30/OHK	+	±	±	NT	NT	−	NT	NT	+	−
CML										
KU812	−	−	−	NT	NT	NT	NT	NT	−	NT
KU812E	−	−	−	NT	NT	NT	NT	NT	−	NT
Cancer cell										
KATO-III[c]	−	−	−	−	NT	−	−	−	++	−
MKN28[c]	−	−	−	NT	NT	−	NT	NT	−	−
MKN45[c]	−	−	−	NT	NT	−	NT	NT	−	−
QG56[d]	−	−	−	NT	NT	−	NT	NT	−	−
PC-3[d]	−	−	−	−	NT	−	−	−	−	−
KB[e]	−	−	−	NT	NT	−	NT	NT	−	−
HeLa[f]	−	−	−	NT	NT	−	NT	NT	−	−

a) Binding of mAbs was scored as follows: (−) indicates <10%, (±) indicates 11 to 20%, (+) indicates 21 to 70%, (++) indicates >71%.
b) NT: not tested.
c) Stomach cancer.
d) Lung adenocarcinoma.
e) Epidermoid carcinoma, oral.
f) Epitheloid carcinoma, cervix.

bentidine-chromogen reaction. Control immunoblotting was also done using nonreactive human IgM or mouse IgG.

RESULTS

Characteristics of NISP and mNISP NISP has been maintained for more than three years without loss of Ig-secreting activity. The doubling time of this cell line in GIT medium is approximately 24 h. The HLA type (HLA-A24, A11; Bw61, Bw62; Cw3.1, Cw4; DR4; DRw53; DQw3; DQw4) was determined by means of a standard microcytotoxicity test.[25] The isotype of mNISP is IgM. The binding titer of mNISP to the Raji cell line was 1:16 in the unconcentrated culture supernatants.

Binding activity of mNISP for a panel of established neoplastic and hematopoietic cell lines The reactivity of mNISP was assessed with a group of 27 established cell lines by immunofluorescence assay. Three Burkitt's lymphoma cell lines, Raji, Daudi and Ramos were found to bind strongly. (Table II). Non-T non-B ALL cell line (P30/OHK) and B lymphoblastoid cell line (IM9) were found to bind moderately. The sero-reactivity of mNISP to cell lines except neuroblastoma cell lines (GOTO and NB-1) and B lymphoblastoid cell line (IM9) was found to be similar to that of CD10 (J-5 or BA-3), but not CD3(T3), CD13(MY7), CD19(B4), CD20(B1), CD23(H107), CD25(IL-2R1) or L243. In addition, Raji, P30/OHK and IM9 cell lines were examined for CD10 (J-5) and mNISP reactivity using two-color analysis. We found that although a large cell population (>99%) was double-positive ($CD10^+$·$mNISP^+$) on Raji cells, only a small population (<3%) was $CD10^+$·$mNISP^+$ on P30/OHK cells (Fig. 1).

Reactivity of mNISP with leukemic cells from patients with various leukemias Leukemic cells were tested by indirect immunofluorescence microscopy using mAb, mNISP or CD10 (J-5). As indicated in Table III, the antigen defined by mNISP was expressed on leukemic cells from 2 of 8 patients with CML, 2 of 10 patients with AML, one of 13 patients with ALL and two patients with ATL. On the other hand, control mAb, CD10(J-5) reacted with the leukemic cells from 2 patients with CML, from one patient with CLL and from 2 patients with ALL.

Binding activity of mNISP with T cells from HTLV-I carriers T cells from 6 HTLV-I carriers were tested by FACS analysis. Fig. 2 shows that the antigen defined by mNISP is highly expressed on T cells from HTLV-I carriers, but it was not expressed on T cells from a normal healthy donor. These T cells also expressed IL-2R weakly but did not express common acute lymphoblastic leukemia antigen (CALLA), when tested using a CD25(H31) mAb.

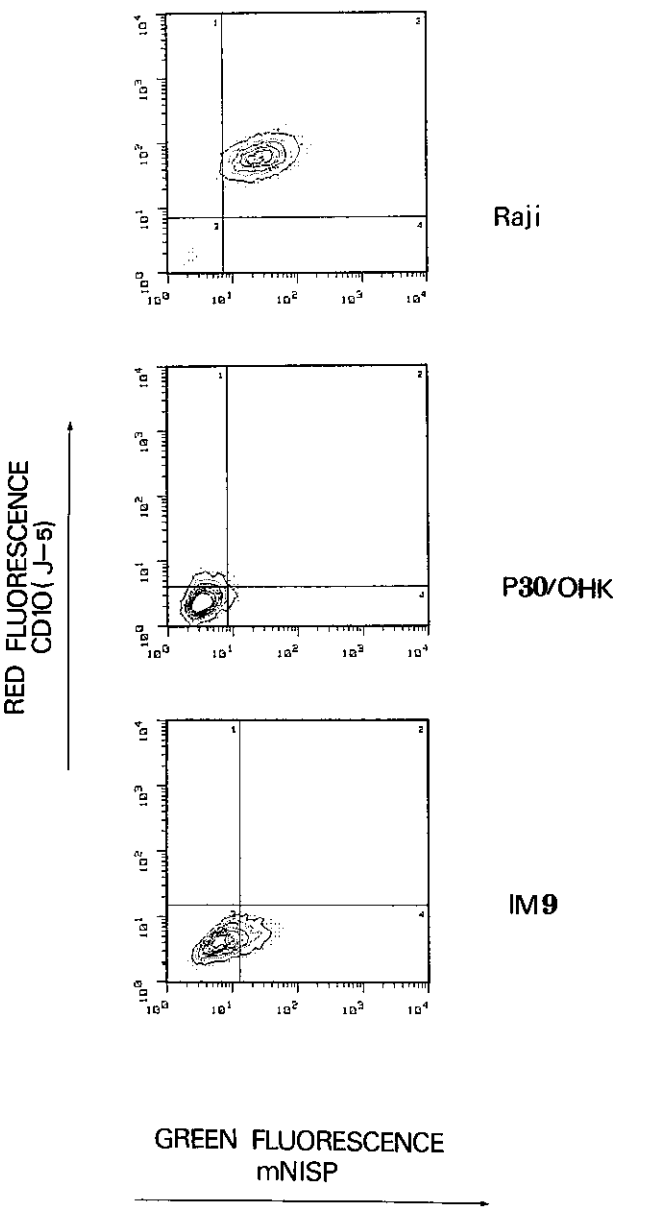

Fig. 1. Two-color analysis of Raji, P30/OHK and IM9 cell lines. The cells were stained with PE-conjugated CD10 (J-5) and mNISP at the first stage. For the second stage, FITC-conjugated goat anti-human IgMF(ab')2 was used. Relative fluorescence intensity is expressed on a logarithmic scale.

Reactivity of mNISP with normal lymphoid cells and erythrocytes The binding activity of mNISP to T, B and adherent (macrophage) cells obtained from the PB of normal healthy donors was tested by FACS analysis, and that to erythrocytes (human and sheep) by means of the agglutination test. All lymphoid cells and erythro-

Table III. Binding of mNISP to Leukemic Cells

Patient[a)]	No. samples positive/No. samples tested[b)]	
	mNISP	CD10 (J-5)
CML	2/8	2/8
AML	2/10	0/10
CLL	0/3	1/3
ALL	1/13	2/13
ATL	2/2	0/2

a) CML, chronic myelocytic leukemia; AML, acute myeloblastic leukemia; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; ATL, adult T cell leukemia.
b) Analyzed by indirect immunofluorescence microscopy.

cytes gave negative results in the presence of mNISP (Table IV).

Biochemical characterization of cell surface antigen recognized by mNISP using SDS-PAGE coupled with Western blotting Solubilized proteins from Raji and from TALL-1 cells were electrophoresed on 10% polyacrylamide gel, transferred to nitrocellulose sheets, and immunoblotted with mNISP, CD10 (J-5) and L243. As illustrated in Fig. 3, mNISP precipitated a polypeptide with the apparent molecular weight of 55,000 to 56,000 daltons from among the proteins of Raji cells, but it showed negative results with an extract of TALL-1 cells. In addition, a polypeptide with the apparent molecular weight of 50,000 daltons was regarded as non-specific, because normal human IgM used as a negative control

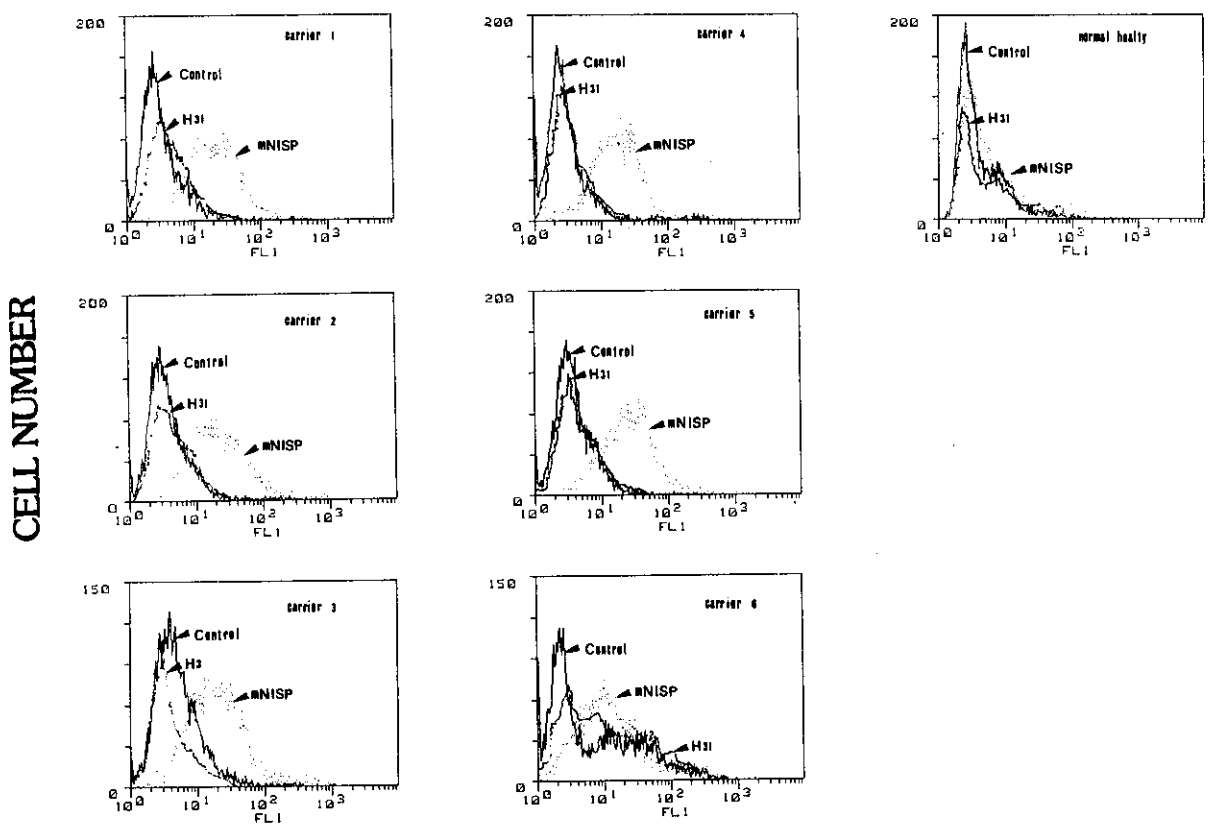

Fig. 2. Expression of an antigen defined by mNISP on T cells from HTLV-I carriers and from a normal healthy donor. A cell suspension was successively treated with mNISP and CD25 (H31) at the first stage. For the second stage, FITC-conjugated goat anti-human IgMF(ab')2 and FITC-conjugated goat anti-mouse IgGF(ab')2 were used. Control samples were treated without mNISP and CD25 (H31). Cell surface immunofluorescence of individual cells was determined by means of a FACStar.

Table IV. Binding of mNISP to Normal Lymphoid Cells and Erythrocytes

mAbs	% of positive cells[a]			Degree of agglutination[b]				
	PBMCs[c]		Adherent	Erythrocytes				
	T cells (n=3)	B cells (n=3)	Macrophages (n=3)	Human				Sheep
				A	B	O	AB	
mNISP	0.8	0.2	1.5	−	−	−	−	−
B1	5.6	82.5	8.5	−	−	−	−	−
T3	92.8	8.5	9.8	−	−	−	−	−

a) Analyzed by FACS. The average of positive cells of three healthy donors is shown.
b) Analyzed by aggultination test: −, negative.
c) PBMCs: Peripheral blood mononuclear cells.

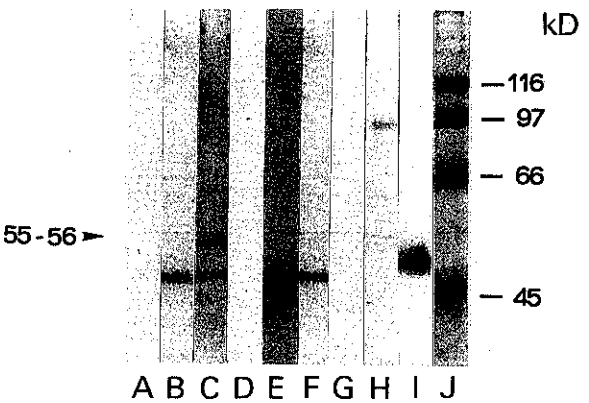

Fig. 3. Western blotting analysis of solubilized Raji and TALL-1 cells with mNISP, CD10 (J-5) or L243. Solubilized proteins from Raji (lanes A, B, C, G, H, I) and TALL-1 cells (lanes D, E, F) were subjected to 10% SDS-PAGE, and electrotransferred to nitrocellulose sheets. Strips were immunoblotted with antibodies as follows: lanes A, D, GIT medium; lanes B, E, GIT medium containing normal human IgM (1 μg/ml); lanes C, F, mNISP; lane G, GIT medium containing normal mouse IgG (1 μg/ml); lane H, CD10 (J-5); lane I, L243. Molecular weight markers stained with Amido black (lane J) are as follows, β-galactosidase, 116,000; phosphorylase b, 97,000; bovine albumin, 66,000; egg albumin, 45,000.

precipitated a component of 50,000 daltons from among the proteins of Raji and TALL-1 cells. On the other hand, CD10 (J-5) and L243 precipitated polypeptides with molecular weights of 97,000 and 54,000 daltons, respectively, from among the proteins of Raji cells.

DISCUSSION

In these studies, we succeeded in establishing a cell line that produces a human mAb, mNISP, from lymph node cells obtained from a patient bearing a CBT, an infrequently malignant tumor. The reactivity of mNISP was tested on various cells and cell lines, suggesting that mNISP recognizes an antigen expressed on some leukemia/lymphoma cells or cell lines. In addition, the reactivity of mNISP was found to be similar to that of CD10 (J-5 or BA-3), which is reactive with CALLA. In the two-color analysis, it was found that Raji cells have a large $CD10^+$·$mNISP^+$ population, while only a small cell population was $CD10^+$·$mNISP^+$ among P30/OHK cells. CD10 (J-5 or BA-3) is specific for a CALLA that has been identified as a cell surface glycoprotein with a molecular weight of 95,000 to 100,000 daltons, and is expressed on lymphoblasts from patients with the common type of ALL and on cells from patients with CML in lymphoid blast crisis. It is also known to be expressed on cells of a number of surface Ig malignancies, including Burkitt's lymphoma[26] as well as of several established human glioma cell lines.[27] In this experiment, although both CD10 (J-5) and mNISP reacted with some Burkitt's lymphomas, CALLA but not an antigen defined by mNISP was also expressed on neuroblastoma cell lines, GOTO and NB-1. In contrast, we obtained evidence that the antigen defined by mNISP was highly expressed on T cells from HTLV-I carriers in which CALLA was not detected by FACS analysis. Furthermore, the antigen defined by mNISP was not identical to CALLA in terms of molecular weight based on Western blotting analysis. Thus, it can be concluded that an antigen defined by mNISP is clearly distinct from CALLA and any previously reported antigens in terms of molecular weight and pattern of cellular expression, and mNISP may recognize a surface molecule which can be induced by HTLV-I infection. Although the cell surface products detected on HTLV-I-infected cells have already been characterized,[28-30] the nature of the HTLV-I-

induced new surface antigen defined by mNISP on T cells from HTLV-I carriers is not clear at the present time. Our studies suggest that mNISP reacts with a common epitope on the cell surface of some leukemia/lymphoma cells or cell lines, and T cells from HTLV-I carriers. However, the immunological significance of these cellular specificities exhibited by this new antigen remains to be established, and is under investigation in our laboratories.

Finally, further studies will be necessary to analyze the biological function of mNISP, and the relationship between CBT and mNISP.

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