Immunological Studies on the Surface Antigens of Tumor Cells

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We have produced a new monoclonal antibody detecting common acute lymphoblastic leukemia antigen (CALLA) and designated as KP-22. CALLA detected by KP-22 is expressed on the all of the various cell lines examined including common ALL, Burkitt's lymphoma, human fibroblasts and cultured normal human fibroblasts. However out of cell lines tested, a fraction of T-ALL and all of myelocytic leukemia and all other nonleukemia cell lines except for fibroblast are CALLA negative. Immunoprecipitation of solubilized ^{125}I-labeled membrane proteins from cultured human fibroblasts and leukemia cell lines with KP-22 revealed a major polypeptide chain with an apparent molecular weight of approximately 100 Kd and 95 Kd, respectively.

Even though a microheterogeneity in terms of molecular weight between two CALLAs, the peptide mapping patterns of them are identical indicating that such a microheterogeneity seems to be partly due to heterogeneous terminal sialic acid compositions added by a post-translational modification process.

KEY WORDS: CALLA, Monoclonal antibody, Fibroblast, Leukemia

Common acute lymphoblastic leukemia associated antigen (CALLA) was first defined serologically by Greaves *et al.* (1975) using polyclonal xenoantisera and later by Ritz *et al.* (1980) using a murine monoclonal antibody (MA). The antigen has been charcterized biochemically as a glycoprotein of approximately 100 Kds and is a surface marker expressed primarily on leukemic cells from the majority of patients with non-T, non-B ALL and chronic myelocytic leukemia in lymphoid blast crisis. Subsequently, Greaves *et al.* (1980) were able to show that this antigen is expressed on a small population of normal bone marrow lymphoid cells. These led to the proposal that CALLA was not leukemia specific but was a normal lymphoid differentiation antigen.

Expression of CALLA, however, is not limited to the immature lymphoid cells in the bone marrow. Many investigations have shown that CALLA is present in a variety of unrelated cells such as mature granulocytes (Braun *et al.*, 1983; Pesando *et al.*, 1986; Fujimoto *et al.*, 1988), glomeruli and proximal tubules on the kidney (Metzger *et al.*, 1981; Platt *et al.*, 1983) and fibroblast (Braun *et al.*, 1983). At present limited data are available whether CALLA molecules expressed on quite different cell lineages are related or not, although controversial results were reported (Pesando *et al.*, 1986; Fujimoto et al., 1988; Platt *et al.*, 1983; McCormack *et al.*, 1986; Carrel *et al.*, 1983), and thus present study is designed to produce a new anti-CALLA MA and to examine the correlation between CALLAs on leukemia cell lines and other cells including fibroblasts.

Materials and Methods

Cell lines: All cell lines were grown in RPMI or DMEM medium supplemented with 1 mM glutamine and 10% fetal calf serum. A panel of leuke-

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mic cell lines and human norml fibroblasts were kindly donated by Dr. Roger H. Kennett, School of Medicine, Univ. of Pennsylvania, USA and Dr. Kyung Ja Hong, Catholic Medical College, Korea. All of nonleukemic cell lines were kindly provided by Dr. Soo Do Jung, Nagoya Univ., Japan or purchased from American Type Culture Collection (Rockville, MD.).

Preparation of Hybridoma and Production of MA detecting CALLA: Six-week-old Balb/c mice were immunized by intraperitoneal inoculation of 10^7 cells of Reh cell line, known as a CALLA presenting cell line, emulsified in complete Freund's adjuvant. After 7 and 14 days the mice received intraperitoneally the same amounts of cells in PBS and 7 days later the mice were injected 3×10^6 cells in PBS intravenously. Spleen was harvested 3 days later for cell fusion. Hybrdomas were prepared using the method developed by Kohler and Milstein (1975) with several modifications as described by Kim *et al.* (1988). Briefly the spleen cells and SP 2/0 mouse myeloma cells were washed in RPMI 1640 medium, containing 2.0 mM glutamine, 1.0 mM sodium pyruvate, mixed at a 5:1 ratio, and fused with 40% (v/v) polyehtylene glycol ‹Mr 1,000) plus 5% DMSO (v/v) in DMEM medium. After fusion, individual wells of 96-well microtiter plates (Costar, Cambridge, MA) were seeded with 2.5×10^4 total cells (50 μl) of the cell suspension. Fused cells were then selected for growth with hypoxanthine-aminopterin-thymidine medium. Targets used in the primary screening by ELISA included the cell lines Reh, Nalm 6, Raji (established as CALLA positive cell lines), K562, HL 60 and IMR 5 (known as CALLA negative cell lines). Cells from one mcroculture well were identified as producing an antibody that reacted strongly with Reh, Nalm-6 and weakly with Raji but not with K562, HL-60, nd IMR 5 (Table 1). Cells from this well were cloned by limiting dilution, and the culture supernatants of the clones were retested for antibody production by ELISA. Cells from one stbilized clone (designated as KP-22) were injected intraperitoneally into pristane-primed Balb/c mice in order to generate antibody-containing ascites fluid. the immunogloulin produced by KP-22 hybrid clone was identified as IgG_1 κ by using mouse-immunoglobulin identfication kit (Boehringer Manhein Biochemicals). Anti-outer membrane proteins of Vibrio vulnificus MA (OMP 180; IgG_1, κ), using as a control MA was obtained as reported previously (Kim *et al.* 1988a).

Table 1. Reactivity of a clone, KP-22 culture supernatants on the various established cell lines by ELISA for the primary screening.

Cell Lines	Reactivity
Reh	+++
Nalm-6	+++
Raji	++
K-562	−
HL-60	−
IMR-5	−

Enzyme Linked Immunosorbent Assay (ELISA): Fifty μl of hybridoma supernatants to be tested was added into each target-cell-attatched well of 96-well microplates and incubated for 2 hr at room temperature. Non-specfic sites were blocked with 0.1% bovine serum albumin. After washing with 0.5% Tween 80 in PBS, 50 μl of 1/1,000 peroxidase-conjugated goat anti-mouse IgG in the 4% FCS-containing Tween 80 in PBS was added and incubated for 2 hr at room temperature. After washing extensively, 200 μl of substarate (10 ml of citrate buffer, pH 4.5 + 10 mg of OPD + 4 μl H_2O_2) was added and after 20 min, 175 μl of the reaction mixture was transfered from each well to a clean plate to read OD in wave length 450 nm.

Antiody-binding Radioimmunoassay (RIA): The reactivity of MA for various cultured cell lines tested was performed in an antibody-binding RIA (Waibel *et al.*, 1988). Briefly 5×10^4 target cells in 50 μl of serum-free medium were mixed with 100 μl of cultured fluid and were incubated for 1 hr at 20°C in U-bottom microtest plates (Falcon, Oxnard, CA). After three washings with 150 μl medium, 100 μl of ^{125}I-labeled rabbit anti-mouse $F(ab')_2$ antibody (100,000 cpm) were added and incubated for 1 hr at 4°C. The cells were then washed three times with medium and transferred to tubes for γ-counting.

Purification of Antibodies: When necessary, KP-22 antibody was purified using protein A-Sepharose (Pharmacia Fine Chemicals Piscataway, NJ) affinity chromatography according to the method described by Ey *et al.* (1978) from mouse ascitic fluid.

Determination of the Equilibrium Constant (K) for KP-22: Determination of K value for KP-22 and claculation of the approximate number of CALLA molecules per cell, were done by Scatchard analysis (1949) essentially according to the method described by Lebien *et al.* (1982). All experiments were done at 4°C in Linbro round-bottom microtiter plates, with antibody and cells incubated a multiple concentrations in a final vol of 100 μl. For a given experiment, ^{125}I-KP-22 was added in triplicate to wells containing 10^6 or 10^7 cells/ml. Input cpm ranged from 5×10^4 to 10^6. Plates were incubated for 1 hr at 4°C with mild agitation every 15 min. Upon completion of the experiment, the cells were immediately washed (250 $\times$ g for 2 min) three times in cold PBS containing 2% newborn calf serum and 0.02% sodium azide, and bindable counts were quantitated in a gamma counter. All studies were conducted with Nalm-6 or WI-38.

Data were expressed by plotting the ratio of bound antibody to free antibody divided by the concentration of cells in mol/liter on the ordinate vs the number of antibody molecules bound to a single cell at a given dilution on the abscissa. Regression lines were drawn through the points on the scatter diagram in order to minimize the sum of the squares of the observed deviations about the line. K values were derived from the slope of the line and are expressed as liters/mol (M^{-1}). The number of CALLA molecules present on a single cell was derived from the abscissa intercept.

Cell Cytotoxcity: Cell Cytotoxicity test was carried out according to the method of Okabe *et al.* (1985). Briefly neuraminidase treated target cells were washed twice and brought to a final concentration of 6×10^5 cells/ml in RPMI 1640 containing 5% fetal bovine serum; 3×10^4 target cells (50 μl) were incubated with various dilutions of the monoclonal ascites (50 μl) on ice for 30 min followed by addition of rabbit complement (50 μl, 1 : 3 dilution; Rockland) and further incubation at 37°C in a humidified atmosphere of 5% CO_2 in air for 2 hr. To elude nonspecific lysis, rabbit complement absorbed 3 times against many cell lines was used. To assess the exact percentage of cell lysis, trypan blue dye exclusion tests were used. Thus the surviving cells were counted following the addition of 150 μl of 0.5% trypan blue dye solution.

Immunoprecipitation Experiments: Neuraminidase treated Nalm-6, fibroblasts and Daudi cells were surface labeled with ^{125}I by the lactoperoxidase method (Hubbard *et al.*, 1975) and were extracted wih 0.5% Nonidet P40 in cell lysis buffer containing 0.5% Nonidet P40, 0.15M NaCl, 0.05M Tris-HC1, and 0.005M EDTA, pH 8.0). The lysate was cleared of insolubilized material by ultracentrifugation. The amount of trichloroacetic acid precipitable protein bound ^{125}I was determined for each lysate, and the concenrations of protein radiolabeled were then equalized. Cell lysates were cleared of nonspecific binding proteins by preincubation for 30 min with Sepharose protein A which was then removed by centrifugation. Immune precipitation reactions were initiated by addition of monoclonal antibody (ascites fluid diluted 1:10) (5 μl) to aliquots of cell lysate (approximately 10^6 cpm)in a final volume of 100-200 μl. After overnight incubation at 4°C, immune complexes were collected by incubation with Sepharose protein A for 20 min. The Sepharose protein A pellet was washed 4 times and radiolabeled proteins released by addtion of 50 μl of sample electrophoresis buffer (62 mM Tris-HC1, 2% SDS, 10% glycerol, 0.02% bromphenol blue, with or without 5% 2-mercaptoethanol) and heated at 100°C for 5 min.

Immune-precipitated proteins were applied to 10% polyacrylamide slab gel electrophoresis (SDS-PAGE) under reducing conditions at 150 V for 16 hr. Autoradiographs of dried gels were obtained by exposure for 2 to 7 days at −70°C.

Peptide Analysis: Proteolytic digests of radiolabeled antigens identified by anti-CALLA, KP-22 antibody on fibroblasts and Nalm-6 cells were compared using a modiffication of the

method of Cleveland *et al.* (1977). Nonreducing sample buffer was added to pellets of iodinated antigen purified by immune precipitation, followed by a three-minute incubation at 110°C. Immediately before electrophoresis, samples were treated with fresh solutions of 5 μg *Staphylococcus aureus* V8 in the same buffer for 30 minutes at 22°C and analyzed on 15% SDS-PAGE.

Results

Reactivity of KP-22 MA with Various Cell Lines.

Several factors may play important roles in determining the rate and the efficiency of successful lymphocyte fusion. These factors include the viabilities of myeloma cells and lymphocytes, and their growth phase, an appropriate ratio of about 5 to 1 of lymphocytes to myeloma cells, the addition of medium containing HAT to the fusion wells on the day after the fusion, and the screening for sera which support the optimal growth of hybrid cells. In the experimental condition employed in the present study hybrid cell clones began to appear on day 10 after fusion (Fig.1), and culture supernatants were harvested to be assayed.

The reactivity of KP-22 supernatants was tested on a panel of various cell lines by RIA. The results indicate that KP-22 MA identify CALLAs synthesized by both of hematopoietic cell lines, and established human fibroblast cell lines and cultured normal cells of human fibroblasts that have unique behavioral and chemical properties. Table 2 showed that the MA was highly reactive with four common ALL (Nalm 1, Nalm 6, Nalm 16, Reh) and two Burkitt's lymphoma cell lines (Daudi and Raji); moderately reactive with two T-ALL cell lines (RPMI 8402, JM); nonreactive wih normal (EBV^+) B cell lines (DDI, MAJA, B85) and a fraction of T-ALL cell lines (CCRF-CEM, CCRF-HSB, Molt 4). KP-22 MA did not also react detectably with a chronic erytholeukemia cell line (K562) and with an acute promyelocytic leukemia cell line (HL-60). Although a large fraction of both of lymphoblastic leukemia cell lines tested was positive to KP-22, we found a higher frequency and intensity in malignant cell lines originated from B lymphocytes than T lymphocytes. Our studies with cell lines, along with those reported earlier (Minowada *et al.*; 1978), also indicate that the CALLA antigen is selectively expressed on common ALL, fraction of T-ALL and Burkitt's lymphoma out of hematopoietic malignant cell lines. CALLA antgens recognized by KP-22 are not only selectively expressed on cell lines of malignacy derived from hematopoietic stem cells but also on human fibroblast cell lines and cultured human normal fibroblasts. As shown in Table 3, lung diploid fibroblast cell lines (WI-38, IMR-90) and normal human skin and liver fibroblast cells also bound KP-22 remaining that of a breast carcinoma (BT-20), a pancreas adenocarcinoma (ASPC-1), a fibrosarcoma (HT-1080), two neuroblastoma (SKNSH, IMR 5), and a malignant melanoma (HT-144) in stolidity. Out of non-hematopoietic malignant tumor cell lines tested, cell lines originated from fibroblasts expressed CALLA exclusively. Since normal human cultured fibroblasts also show a significant positive reaction to KP-22 on a single cell level a $CALLA^+$ phenotype does not reliably distinguish a leukemic cell from normal.

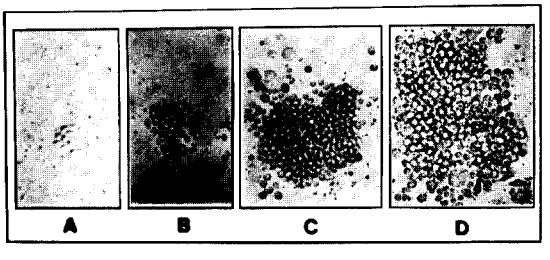

Fig.1 Aspects of a growing clone in a well KP-22 MA producing clone on day 10(A), day 15 (B), day 18 (C) and 21 (D) after fusion.

Characterization of MA KP-22

Scatchard plot was carried out using the data obtained by equilibrium solution radiobinding assay of KP-22 ascitic antibody along wih that of immune serum from the mouse, whose spleen cells were used for fusion (Fig.6 and 7). Scatchard plots derived by testing the ability of ^{125}I-KP-22 to bind Nalm-6 and WI-38. Antibodies were iodinated on a sigle occasion, and the results of four separate experiments for each antibody are

Table 2. Binding of hybridoma anti-CALLA antibody KP-22 and control MA OMP-180 to human malignant hematopoietic cell lines. Results are expressed as cpm bound of ^{125}I-labeled rabbit anti-mouse $F(ab')_2$ antibodies to 5×10^4 target cells with various ascitic fludids.

Cell Lines Used as Target		Hybridoma Products	
		KP-22	OMP-180
B-cell lines			
Normal (EBV$^+$)	DDI	125	120
	MAJA	147	95
	B85	135	130
Burkitt's lymphoma	Daudi	967	133
	Raji	768	120
Common ALL	Nalm-1	1005	101
	Nalm-6	1458	98
	Nalm-16	1205	110
	Reh	1351	105
T-cell Lines			
T ALL/NHL	RPMI 8402	365	123
	JM	442	99
	CCRF-CEM	152	120
	CCRF-HSB2	144	101
	Molt-4	108	132
Myeloid cell lines			
Acute promyelocytic leukemia	HL-60	144	139
Chronic erythroleukemia	K-562	108	126

Table 3. Binding of hybridoma anti-CALLA antibody KP-22 and control MA OMP-180 to human non-leukemia cell lines and human cultured normal fibroblast cells.

Cell Lines Used as Target		Hybridoma Products	
		KP-22	OMP-180
Breast carcinoma	BT-20	123	100
Pancreas adenocarinoma	ASPS-1	111	145
Fibrosarcoma	HT-1080	150	156
Neuroblastoma	SKNSH	174	132
	IMR5	160	128
Malignant melanoma	HT-144	182	181
Lung fibroblast cell lines	WI-38	502	184
	IMR-90	415	145
Normal human fibroblast	Skin	431	162
	Liver	491	131

shown. KP-22 ascitic fluid produced linear plot, while the immune serum antibody produced concave-up plot. (Fig.7) The K values derived for KP-22 ascites antibody and calculation of the approximate number of CALLA molecules per Nalm-6 and WI-38 cell are shown as the mean of the four separate experiments. Fig.6 shows that KP-22 has an approximate K value of 3.1×10^7

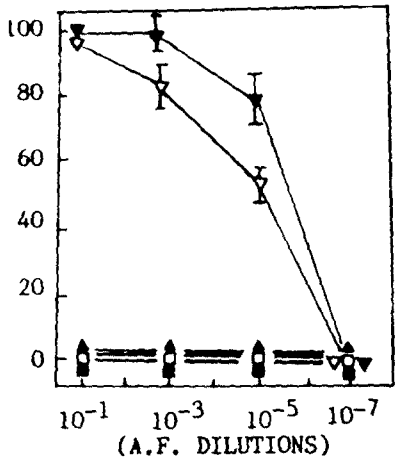

Fig.2 Complement-dependent cytotoxicity of monoclonl KP-22. *Neuramidase treated* 3×10^4 *target cells* (50 μl) were incubated with MA in serial dilutions and 50 μl rabbit complement (1:3) and the surviving cells were counted following the addition of 50 μl of 0.5% trypan *blue dye solution*: ▼; Reh, ▽; *fibrolast*, □; CCRF-CEM, ■; HL-60, ▲ IMR5.

M^{-1} to WI-38 and 8.6×10^7 M^{-1} to Naml-6. Caculation of the approximate number of CALLA molecules per WI-38 and Nalm-6 cell yielded values of 1.0×10^5 and 3.2×10^5 respectively. No effort was made to standardize two cell lines before assay, i.e., by always taking them from the same stage of growth in culture.

Cytotoxicity Tests for Cells.

Various cell lines were treated with KP-22 ascites and complement. As shown in Fig.2, the CALLA presenting cell lines, Reh and fibroblast, showed a high sensitivity to immune cytolysis whereas no cytotoxicity was shown to the other cells that did not epress the antigen. Since greater than 50% lysis was observed with ascites dilution of 10^{-5} on the positive cell lines such as fibroblast and Reh, and no lysis was noted at any dilution on negative cell lines such as MOLT-4, HL-60, and IMR 5, specific reactivity of KP-22 ascites to CALLA was also demonstrated by the assay.

Immunoprecipitation

For the localization of antigenic determinant recognized by KP-22 on the SDS-PAGE gel, immunoprecipitation experiments were carried out. It was proved in the preliminary experiments (data

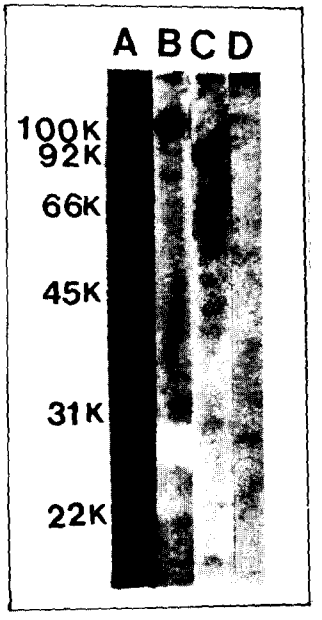

Fig.3 Comparison of two species of CALLA in molecular weight. SDS-PAGE of immunoprecipitates from neuraminidase treated and ^{125}I-labeled fibroblasts (B) and NALM-6 (C,D) membrane proteins were precipitated with KP-22 (B,C) and negative control OMP-180(D) *ascites*. Lane A: marker protein.

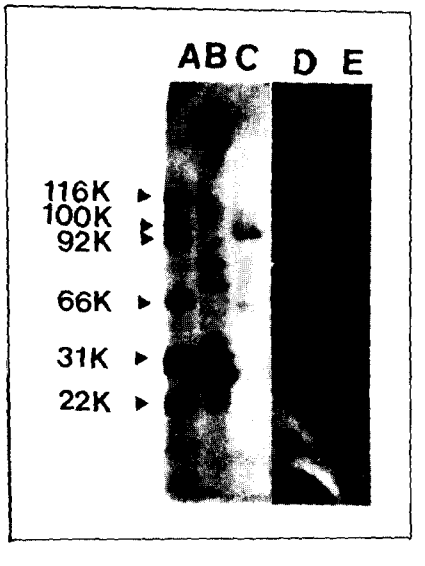

Fig.4 Autoradiography after SDS-PAGE of ^{125}I-labeled surface proteins from fibroblasts (C, neuraminidase treated) and Dauid (D,E). For precipitation, monoclonal KP-22 (C,E) and negative control OMP-180 (D) ascites were used. Lanes A and B are marker proteins and concentrated surface proteins from fibroblasts devoid CALLA, respectively.

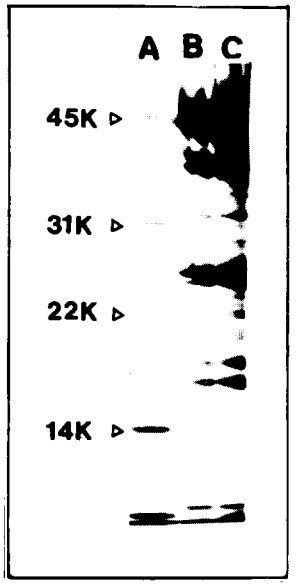

Fig.5 Proteolytic digests of iodinated antigens isolaed from fibroblast ells (B) and Nalm-6 Leukemia (C) by KP-22. 5 ug of *staphylococcus aureus* V8 protease was used for the proteolysis and analyzed on 15% SDS-PAGE. Lane A: marker proteins.

were not shown) that in the case of humn fibroblasts the antigenic determinant on the cells were more effectively recognized when the cells were treated with neuraminidase to digest sialic acid moieties of the determinant partially thus neuraminidase was introduced in the immunoprecipitation study of fibroblasts. Immunoprecipitation of ^{125}I-labeled membrane proteins solubilized from Daudi, Nalm 6 and neuraminidase treated fibroblasts provided further evidence for that the molecules identified by KP-22 MA were CALLAs. As seen in Fig.3 and 4 the KP-22 MA precipitates a single polypeptide chain with an apparent mol. wt of approximately 100 Kd or 95 Kd from labeled proteins of fibroblasts (Fig.3 lane B and Fig.4 lane C), Nalm 6 (Fig. 4 lane C) and Daudi (Fig.4 lane E). In contrast control MA OMP-180 did not show any positive band in Daudi (Fig.3 lane D) or in Nalm 6 (Fig.3 lane D). Furthermore an autoradiograph of SDS-PAGE obtained from immunosupernatants (the remaining cell lysates omitted the KP-22 immunoprecipitates) of solubilized ^{125}I-labeled membrane proteins from neuramini-

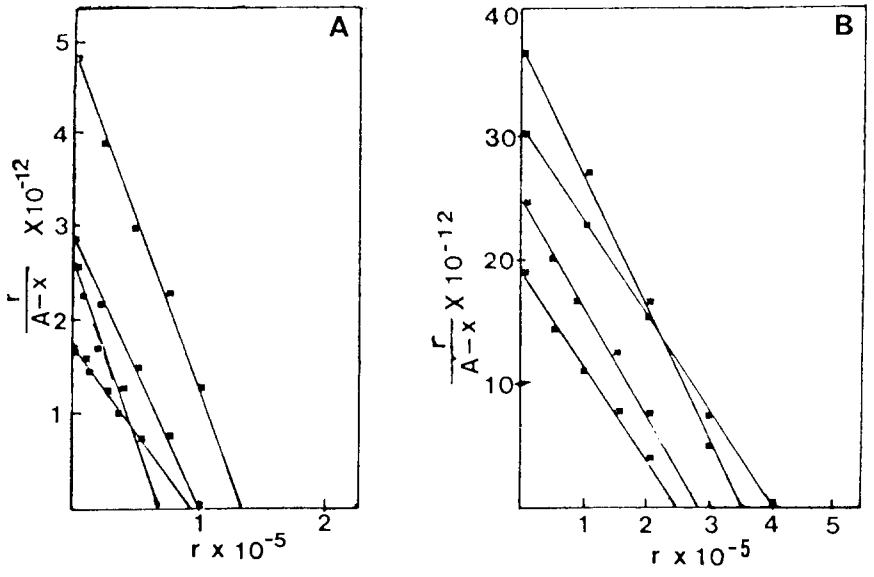

Fig.6 Determination of the euilibrium constants (K) for KP-22 to WI-38(A) and Nalm-6 (B) and calculation of the approximate number (n) of CALLA molecules per WI-38 and Nalm-6 cells. Abscissa indicates the approximate number of CALLA molecules per cell and ordinate indicates the ratio of bound antibody to free antibody divided by the concentration of cell in mol/liter. Experiments were conducted as described in Materials and Methods. Standard deviations of triplicate values usually less than 7%.

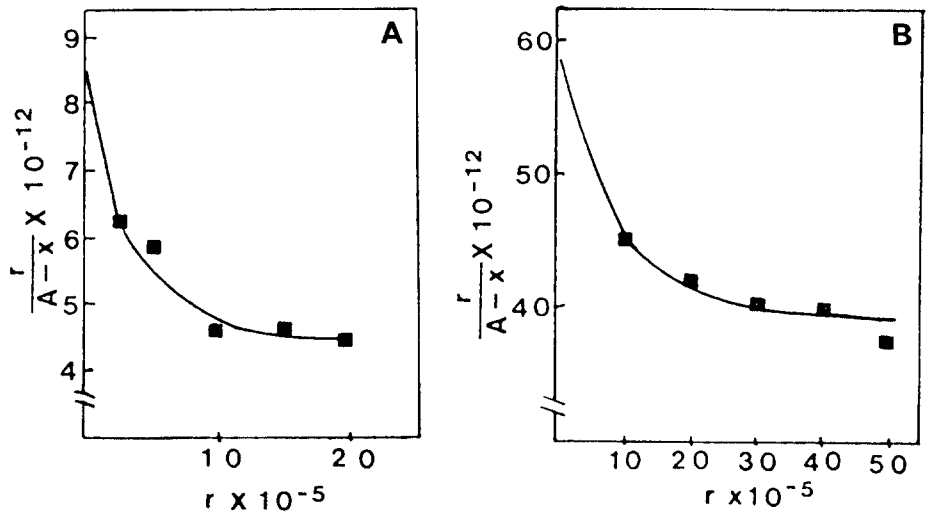

Fig.7 Scatchard anaysis of immune serum for CALLA to WI-38 and Nalm-6 (B). Experimentals were conducted as described in Materials and Methods. Standard deviations of triplicate values less than 10%.

dase treated fibroblast cells showed a ladder-like bands pattern deleted a band of mol.wt. 100 Kd charactristic for CALLA (Fig.3 lane B). This virtually excludes the possibility that the antigenic determinant recongnized by KP-22 is other component than CALLA.

Our immunoprecipitation data also showed a heterogeneity in mol.wt. of CALLA precipitated from different cell lines. CALLA precipitated from cultured human fibroblasts had apparently a higher mol.wt. while CALLA from Daudi and Nalm 6 had slightly lower mol.wt. (Figs.3 and 4)

Peptide Mapping

Despite the difference in mol.wt. between CALLAs from fibroblasts and B cell lines, it is still not known whether these antigens differ in their protein strucures. Since it is known that CALLA molecules contain approximately 25% carbohydrate (Newman *et al.* 1981), the unique mol.wt. of these two antigens might well result from post-translational modifications of a common peptide such as the addition of carbohydrate residues (Fukuda *et al.* 1984) thus the difference seen in the CALLA molecule in the present study could reflect cell-dependent variation in posttranslational glycosylation. To examine this posibility proteolytic digests of CALLAs identified by anti-CALLA KP-22 antibody on neuraminidase treated and iodinated human fibroblasts and NALM-6 leukemia cell line were analyzed by SDS-PAGE, followed by visualized by autoradiography and it was found that these two species produced identical peptide fragments as shown in Fig.5. Proteolysis of CALLA isolated from NALM-6 cells produced multiple lower molecular weight species. Despite the relative absence of discrete peptides especially in low mol.wt. species from digests of fibroblast antigen, overall proteolysis of the fibroblast antigen revealed the same pattern to that of NALM-6. Thus at least some of the differences in the bands between the two samples might be quantitative. From these results we tentatively concluded that CALLA on malignant lymphoid cell lines and fibroblasts was the same protein.

Discussion

Specificity of antibody KP-22 for CALLA was further verified in the fact that the pattern of reactivity of KP-22 antibody with panels of various cell lines by RIA were shown to be overall identical to those of known monoclonal anti-CALLA antibodies A-12 (Carrel *et al.*, 1983), J5 (Greaves

et al., 1983) and 24.1 (Braun *et al.*, 1983). The results indicate that the binding of KP-22 to these cells is consistent with the known cellular distribution of CALLA.

Antibody affinity measures the tendency of antigen and antibody to form a stable complex. In our experiments antibody affinity would reflect the amount of KP-22 bound to cell surface CALLA conpared to the amount of that was unbound. Theoretically, MA should have a single affinity to single antigenic determinant of CALLA and should thus yields a linear Scatchard plot (Scatchard, 1949). Purified anti-CALLA antibodies from immune serum, even if they are all specific for same determinant, will be heterogeneous in affinity and yield a curved, concave-up Scatchard plot. As shown in Fig.6 and 7 our MA produced a straight line in he plot, whereas the polyclonal anti-CALLA antibody did not. These results indicate that our MA is the product of monoclone having a single affinity for a single determinant of CALLA molecule. The typing (IgG1, K) for a unique heavy and light chain subclass identified by using the mouse immunoglobulin identification kit (Boeringer Mannhein Biochemicals) provided further evidence for homogeneity.

Our data clearly indicated that CALLA-highly positive malignant hematopoietic cells consisted mostly of common ALL, Burkitt's lymphoma and some T-ALL cell lines. The most plausible interpretation from the currently available data therefore is that within the hematopoietic system, the CALLA is normally expressed on precursor cells and rlatively immature cells in the T and B lineages and can be considered operationally as a differentiation antigen of these cells (Greaves *et al.* 1983: Greaves *et al.* 1978).

The fact that some hematopoietic cell lines are CALLA positive and others are CALLA negative suggests that the different hematopoietic cell lines may e derived from different precursors. Alternatively, if one accepts the hypothesis that CALLA represents a differentiation antigen that is expressed at early stages of maturation, then the variable expression of CALLA by different hematopoieic cell llines may reflect their various stages of differentiation, Leukemic lymphoblasts cell lines are partial or complete maturation arrest and maintain on overall fidelity of antign expression including CALLA and these cell lines are appropriate for relatively early compartments of T-or B-celldifferentiation.

We have also identified CALLA on normal established fibroblast cell lines sparsely though. Furthermore CALLAs on the surface of cultured human fibroblast cells were documented in immunoprecipitation study. Recent studies indicate that some nonhematopoietic cells, including the proximal tubule, mammary epithelium and fibrolasts also express CALLA (Greaves *et al.*, 1983; Greaves *et al.*, 1980; Braun *et al.*, 1983). These results and our data implicated that CALLA is not specific for either leukemic or normal hematopoietic cells and suggest that this antigen may be expressd by cells widely distributed in tissues of mesenchymal and ectodermal origin. Thus the therapeutic use *in vivo* of MAs such as KP-22 is a more complicated issue, since some pathologic conseuences could potentially arise fromcross-reactivity. An important implication of these observations is that CALLA prbably does not represent a suitable target for *in vivo* therapy of ALL with monoclonl antibodies or with immunotoxin conjugates.

It remains a possibility that CALLA is expressed by culturec fibroblasts, but not by fibroblasts *in vivo*. Metzgar et al. (1981) studied the reactivity of antibody J-5 on frozen tissue sections by the immunoperoxidase method and were unable to demonstrate any expression of CALLA in fetal or adult connective tissue. However, it is also possible that the techniques used were not sufficiently sensitive to detect low levels of antien expression by fibroblast that are sparsely distributed in connective tissue. Our results demonstrate the requirement for extensive studies using sensitive techniques to detect antigen expression in multiple tissues before considering clinical trials that utilize monoclonal antihodies for *in vivo* therapy.

The incubation time required for immune cytolysis of CALLA positive cells was examined (data were not shown). The ascites concentration of more than 10^{-3} showed maximal killing of target cells within 40 min. however, with lower doses of ascites, longer treatment was required to achieve maximal killing. Exposure to lower than 10^{-7} of ascites could not show any killing at all with 2 hr treatment. The difference of CALLA in

mol.wt. between fibroblasts and malignant lymphoid cell lines were evident however the molecular basis for this variation, has not been established. In the present study proteolytic digests of the two antigens produce identical and overlapping banding patterns on SDS-PAGE. The experimental results clearly indicated that CALLA on fibroblasts and mlignant lymphoid cells are related at peptide level although they are different in sugar moieties produced by posttranslationl modifications. Consistently, microheterogeneity of mol.wt. was also reported to exist on CALLA isolated from other cell lineages. CALLA from fibroblasts, malignant melanomas, and polymorphonuclear neutropils has mol.wt. of 95 Kd (Braun *et al.*, 1983), 100 Kd (Carrel *et al.*, 1983), and 90 Kd (Cossman *et al.*, 1983), respectively. Thus, it is very likely that CALLA expressed on different cell lineages are the same although it is evidently necessary to compare each species of CALLA at peptide level as is shown in this study to further confirm their homology.

References

Braun, M. P., P. J. Martin J. A. Ledbetter, and J.A. Hansen 1983. Granulocytes and cultured human fibroblasts express common acute lymphoblastic leukemia-associated antigens. *Blood* **61:**718-725.

Carrel, S., A. Schmidt-Kesson, and J. P. Mach, 1983. Expression of CALLA on human malignant melanom cell lines. *J. Immunol.* **130:**2456-2460.

Cleaveland, D. W., S. G. Fischer, M. W. Kirschner and U.K. Laemmli, 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis *J. Biol. Chem.* **252:**1102-1106.

Cossman, J., L. M. Neckers, W. J. Leonard, and W.G. Greene, 1983. Polymorphonuclear neutrophils express the common acute lymphoblastic leukemia antigen. *J. Exp. Med.* **157:**1064-1069.

Ey, P. L., S. J. Prowse, and C. R. Jenkin, 1978. Isolation of pure IgG_1, IgG_2a and IgG_2b immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry* **15:**429-437.

Fujimoto, J., K. Ishimoto, K. Nobutaka, T. Shigeki, I. Eizaburo, and H. Jun-Ichi, 1988. Immunocytochemical and immunochemicl analysis on the CALLA. *Hybridoma* **7:**227-236.

Fukuda, M., E. Spooncer, J. E. Oates, A. Dell, and J.C. Klock, 1984. Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. *J. Biol. Chem.* **259:**10925-10935.

Greaves, F., G. Hariri, R. A. Newman, D.R. Sutherland, M.A. Ritter, and J. Ritz, 1983. Selective expression of CALLA on immature lymphod cells and their malignant counterparts. *Blood* **61:**628-639.

Greaves, M., D. Delia, G. Janossy, N. Rapson, J. Chesels, M. Woods, and G. Prentice, 1980. Acute lymphoblastic leukemia associated antigen IV. Expression on non-leukemic lymphoid cells. *Leuk. Res.* **4:**15-22.

Greaves, M.F., G. Brown, N. Rapson, and T.A. Lister, 1975. Lymphoblastic Leukemia cells. *Clin. Immunol. Immunopathol.* **4:**67-72.

Greaves, M. F. and G. Janossy, 1978. Patterns of gene expression and the cellular origins of human leukemias. *Biochim. Biophys. Acta* **516:**193-199.

Hubbard, A. C. and Z. A. Cohn, 1975. Externally disposed plasma membrane proteins. I. Enzymatic iodination of mouse L cells. *J. Cell Biol.* **64:**438-460.

Kim, H. D., S. S. Kang, and J. W. Ju, 1988a. Immune response of Balb/c and production of monoclonal antibodies against outer membrane proteins from *Vibrio vulnificus J. Kor. Soc. Microbiol.* **23:**365-376.

Kim, H. D., H. S. Kang, H. M. Kim, O. S. Bang, and S. S. Kang 1988b. Studies on the generation and application of monoclonl antibodies against tumor marker antigen. *Korean J. Zool.* **31:**300-308.

Köhler, G. and C. Milstein, 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256:**495-497.

Lebien, T. W., D. R. Boue, J. G. Bradley, and J.H. Kersey, 1982. Antibody affinity may influence antigenic modulation of the common acute lymphoblastic leukemia antigen *in vitro*. *J. Immunol.* **129:**2287-2292.

McCormack, R.T., R.C. Nelson, and T.W. LeBien, 1986. Sturcture/function of the CALLA (CD-10) expressed on human neutrophils. *J. Immunol.* **137:**1075-1082.

Metzgar, R.S., M. Borowitz, N. Jones, and B. Dowell, 1981. Distribution of common acute lymphoblastic leukemia antigen in nonhematopoietic tissues. *J. Exp. Med.* **154:**1249-1254.

Minowada, J., G. Janossy, M. F. Greaves, T. Tsubota, B.I.S. Srivastava, S. Morikawa, and E. Tatsumi, 1978. Expression of an antigen associated with acute lymphoblastic leukemia in humn leukemia lymphoma cell lines. *J. Natl. Cancer Inst.* **60:**1269-1275.

Newman, R. A, R. Sutherland, and M. F. Greaves, 1981. The biochemical characterization of a cell surface antigen associated with acute lymphoblastic leukemia and lymphocyte precursors. *J. Immunol.* **125:**2024-2030.

Okabe, T., K. Tsutomu, K. Ozawa, A. Urabe, and F.

Takaku, 1985. Elimination of small cell lung cancer cells *in vitro* from human bone marrow by monoclonal antibody. *Cancer Res.* **45:**1930-1933.

Pesanddo, J. M., P. Hoffman, N. Martin, and T. Conrad, 1986. Anti-calla antibodies identify unique antigens on lymphoid cells and granulocytes. *Blood* **67:**588-591.

Platt, J. L., T. W. LeBien, and A.F. Michael, 1983. Stages of renal ontogenesis identified by monoclonal antibodies reactive with lymphohemopoietic differentiation antigens. *J. Exp. Med.* **157:**155-172.

Ritz, J., J. M. Pesando J. Notis-McConarty, H. Lazarus, and S. F. Schlossman, 1980. A monoclonal antibody to human acute lymphoblastic leukemia antigen. *Nature* **283:**583-585.

Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51:**660-672.

Waibel, R., J. Carl, O'Hara, A. Smith. and R. A. Stahel, 1988. Tumor-associated membrane siloglycoprotein on human small cell lung crcinoma identified by the IgG 2a monoclonal antibody SW 20. *Cancer Res.* **48:**4318-4323.

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종양세포 표면항원에 대한 분자면역학적 연구

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CALLA 항원에 대한 단일클론 항체는 백혈병의 진단이나 치료에 대한 활용가능성을 잠재하고 있기 때문에 구미, 일본 등지에서는 이에 대한 연구가 활발하다. 이 점에 유의하여 본 연구자들은 CALLA에 대한 새로운 단일클론항체 KP-22를 개발하고, 이 단일클론항체를 이용 CALLA의 분포를 여러 세포주에 대하여 조사한 결과 common ALL, Burkitt's lymphoma, T-ALL 세포주는 현저한 CALLA양성 반응을 보였으나 사람의 섬유아세포 계열을 제외한 모든 비백혈병성 암세포주 및 myelocytic leukemia 세포주들은 음성이었다.

막 단백질을 ^{125}I 방사능 표지한 후 KP-22를 이용, 면역 침전법으로 백혈병세포주와 사람의 정상 섬유아세포에서 CALLA를 정제하여 전기영동한 결과 각각 분자량이 약 95Kd 및 100 Kd 인 단일 band로 확인되었으나 이들의 peptide mapping 양상은 같았으므로 분자량에서의 미세한 차이는 posttranslational modification 과정에서 첨가되는 sialic acid에 기인 하는 것으로 사료된다.