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Immunological Studies on the Surface Antigens of Tumor Cell (II) Introduction to Immunological Studies on the Development and Cell Differentiation of the Leukemia Cell

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The CALLA on the surface of leukemic cell lines, recognized by our monoclonal antibody, KP-22(IgG_1, *K*) was one of cell surface glycoproteins having mol. wt. of approximately 100,000 dalton, and could be shed in spent medium or endocytosed when binding the cognate antidoby, KP-22. In the presence of cognate antibodies, 60% of CALLAs recognized by KP-22 MAs were modulated and cleared from the cell surface during 24 hrs, and approximaetely 35% of them was endocytosed and 25% was shed in spent medium. The reappearance of the membrane CALLA after modulation by the KP-22 required at least 6 hours and supposed to be newly synthesized molecules.

KEY WORDS: Monoclonal antibody, Antigen modulation, CALLA, Leukemia

Advances in hybridoma technology, which led to the production of monoclonal antibodies (MAs) recognizing tumor-associated products, have provided new potentials for detection and therapy of primary and metastatic tumour deposits. A major component of this development was the demonstration of *in vivo* localization of parenterally administered anti-tumor MA within tumor deposits. These antibodies which can localize in human tumor xenograft have included those to carcinoembryonic atigen (CEA)(Hedin *et al.*, 1982), colorectal carcinoma (Herlyn *et al.*, 1980, Herlyn *et al.*, 1982), melanoma (Ghose *et al.*, 1982), breast carcinoma (Colcher *et al.*, 1984) and teranoma (Moshakis *et al.*, 1981); the experimental confirmation of the potential of these antibodies for tumor localization has formed the basis for a number of clinical trials of the diagnostic and therapeutic application of monoclonal antibodies (Bernhard *et al.*, 1983, Dillman *et al.*, 1984, Goldberg *et al.*, 1983, Goodman *et al.*, 1985, Houghton *et al.*, 1985, Ramakrishnan 1984, Sears *et al.*, 1982, Vitetta 1986).

Encouraged by recent trends, we have porduced a monoclonal antibody, KP-22, which can recognize common acute lymphoblastic leukemia antigen (CALLA) on the various tumor cells. Although a CALLA has been shown to be a cell membrane glycoprotein with M. W. of 95 to 100 Kds (Kim *et al.*, 1989, Ritz *et al.*, 1980a), Structural and functional characteristics have not been established. Using KP-22 monoclonal antibody, we have demonstrated that CALLA is expressed on various cells, including common acute lymphoblastic leukemia (ALL), Burkitt's lymphoma, human fibroblasts, and cultured normal human fibroblasts, even though their quantitative intensities are highly different from one another. Regardless of a microheterogeneity in molecular weight of CALLAs from different cell types, peptide mapping patterns of them are identical, suggesting that such microheterogeneity seems to be partly due to heterogeneous terminal sialic acid compositions added in the posttranslational modification (Kim *et al.*, 1989).

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The recent development of techniques for production of MA has led to reevaluate of the passive antibody therapy for neoplastic disease. This renewed interest is the result of the ability to prepare virtually unlimited amounts of homogeneous antibody of defined specificity and isotype against variety of tumor associated antigens. In general, the anti-tumor antigens detected by these MAs are also expressed on some normal cells. Nevertheless, they were theoretically useful in a clinical setting provided that their reactivity with normal cells does not produce significant toxicity in the recipient (Dexter *et al.*, 1983). Since we have been interested in the use of KP-22 for prognostic and diagnostic indicator of tumors, we carried out experiments to examine the possibility of passive immunotherapeutic and antibody-induced modulation effects of KP-22 on a leukemia cell line, Reh cells *in vitro*. Antibody-induced modulation has been also described for virus-related antigens on both murine (Ioachim *et al.*, 1979) and human cells (Joseph and Oldstone, 1975) as well as for normal cell membrane antigens, such as surface immunoglobulin and histocompatibility antigens (Pernis, 1985). However, precise mechanisms by which antigenic modulation occur may vary with different antigens and cell types, and the *in vitro* culture conditions used to study this phenomenon (Chatenoud and Bach, 1984). Although modultion of various cell surface antigens has been studied, the significance of antigenic modulation as a defense mechanism of human tumor cells has not been established. Using fibroblasts and leukemic cell lines, we have studied the cellular response to KP-22 monoclonal antibody *in vitro*. In this report, we suggest that the modulation of target antigen may be one of important mechanisms whereby ALL cells can escape antibody-mediated therapy.

Materials and Methods

Preparation of Hybridoma and Production of MA detecting CALLA:

Hybridomas were prepared using the method developed by Kôhler and Milstein (1975) with several modifications as described by Kim *et al.* (1988 b). Anti-CALLA MA, KP-22, was produced as reported previously (Kim *et al.*, 1989).

The immunoglobulin produced by KP-22 hybrid clone was identified as IgG_1, *K* by using mouse-immunoglobulin identification Kit (Boehringer Manheim Biochemicals). Anti-outer membrane proteins of *Vibrio vulnificus* MA (OMP 180; IgG_1, *K*), using as a control MA, were obtained as reported previously (Kim *et al.*, 1988a).

Enzyme Linked Immunosorbent Assay (ELISA):

ELISA was carried out according to the method described elsewhere (Kim *et al.*, 1988b).

Antibody-binding Radioimmunoassay (RIA):

The reactivity of MA for various cultured cell lines was determined in an antibody-binding RIA (Waibel *et al.*, 1988). Briefly 3-5 $\times$ 10^4 target cells in 50 μl of serum-free medium were mixed with 100 μl of culture fluid and incubated for 1 hr at 20°C in U-bottom microtest plates (Falcon, Oxnard, CA). After washing the cells with 150 μl medium three times, 100 μl of ^{125}I-labeled rabbit anti-mouse $F(ab')_2$ or goat anti-mouse IgG antibody (100,000 cpm) was 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 from mouse ascitic fluid was purified using protein A-Sepharose (Pharmacia Fine Chemicals Piscataway, NJ) affinity chromatography according to the method described by Ey *et al.*, (1978).

Immunofluorescence for Localization of CALLA:

To evaluate the clearing of the antigen-antibody complex from the leukemic cell surface, the basic standard procedure (Braun *et al.*, 1980, Schreiner *et al.*, 1976, Choi *et al.*, 1991) was employed. Reh or Nalm-6 cells (5 $\times$ 10^4 cells) were placed in test tubes and incubated with MA (0.1 ml ascites at 1 : 100) for 30 min at 4°C.

Cells were washed 3 times by decantation and addition of media repeatedly and the washed cells were suspended to take to a 37°C water bath and placed in tubes that contain 4% paraformaldehyde at room temperture to fix for 15 min. After

fixing, cells were spinned, and decanted to resuspend in meida with 0.02% sodium azide. To examine the destination of CALLA-KP-22 complex in permeabilized fibroblast cells, the cells grown on the coverslip were incubated with diluted ascite fluid (1/200) containing KP-22 at 37°C for various time intervals. The cells were washed and fixed with 4% paraformaldehyde in PBS (pH 7.4) for 20 min at 37°C, opened with 0.1% Triton X-100 and stained by indirect immunofluorescence. For indirect immunofluorescence, FITC-conjugated goat anti-mouse IgG (Sigma) was used and immunofluorescent microscopy was carried out according to Wang *et al.* (1982) under a Zeiss stndard 16 fluorescent microscope.

Results

Previous studies by Ritz and his colleagues have demonstrated that J-5, an anti-CALLA monoclonal antibody, mediates antigenic modulation of CALLA *in vitro* (Ritz *et al.*, 1980b), and probably *in vivo* (Ritz *et al.*, 1981). As the first step in determining whether KP-22 modulates CALLA, we incubated various concentrations of KP-22 with CALLA postivie target cell line, Reh, for different periods. Free excess monoclonal antibody was washed from the system, and the amount of murine immunoglobulin bound to cell surface was measured by the addition of ^{125}I-labeled rabbit anti-mouse F(ab')$_2$. After incubation with higher concentrations of KP-22 (0.2 to 1 μg/ml), no significant decrease in immunoglobulin binding was observed with lapse of time (Fig.1).

When lower concentrations (0.008 to 0.04 μg/ml) of KP-22 were incubated with the target cells, after initial binding of monoclonal antibody to cell membrane antigen (CALLA), both KP-22 antibody and CALLA were gradually lost from the cell surface during incubation at 37°C, demonstrating antibody-dependent loss of CALLA from the cell surface.

Two explanations might be offered for the progressive reduction in the binding of KP-22 during incubation with the Reh cell line. Antigen might disappear altogether from the tumor cell surface, undergoing modulation comparable to that observed with the common acute lymphoblastic

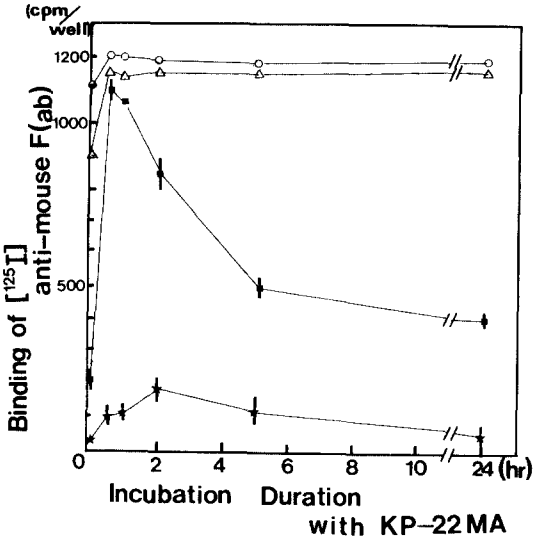

Fig. 1. Quantitative change in the bound KP-22 MA to the target cells (Reh) surface after prolonged incubation of cells with different concentrations of KP-22. Initially 3 × 10^4 target cells were cultured at 37°C for different periods with various concentration of KP-22 in 50 μl medium, free antibodies were removed from the system by cell-washing and antibodies bound to the cell surface were measured in a γ-counter by addition of 50 μl (7 mCi/g) ^{125}I-labeled anti-mouse F(ab')$_2$ to incubate for additional 60 min. Antibody concentrtions: 1 μg/ml, ○: 0.2 μg/ml, △: 0.04 μg/ml, ■: 0.008 μg/ml, ★: Bars, S. D.

leukemia antigen (Ritz *et al.*, 1980a), which is different from capping of antigen. Alternatively, with limiting dilutions of the monoclonal reagent, antigen might be disappeared by shedding or endocytosis and reexpressed at the cell surface but sufficient antibody might not be available in the medium to produce a detectable interaction with the newly synthesized antigen. To test these possibilities, Reh cells were incubated with different concentrations of KP-22 for 24 hrs and the amount of immunoglobulin bound to the surface of tumor cells was measured by incubation with ^{125}I-labeled rabbit anti-mouse F(ab')$_2$, either with or without the addition of saturating concentrations of fresh KP-22 antibody.

After cells were pretreated in the medium containing hyper-saturating amounts of KP-22 (more than 0.2 μg/ml) for porlonged time (24 hrs) and followed by another incubation (1 hr) in the medium with or without additional KP-22 (1 μg/

ml), and the cells were used for indirect radioimmunoassay, the total amounts of radioactive 2nd antibody bound to cells remained relatively constant irrelevant presence or absence of additional KP-22 in the 2nd incubation medium. When pretreatment with limiting dilutions of KP-22 (less than 0.2 μg/ml) for 24 hrs, however, the binding values of 2nd antibody were significantly increased if any additional KP-22 supplemented in the 2nd incubation medium (Fig. 2). These results indicated that antigen-antibody complex may disappear from the cell surface, being substituted by newly expressed CALLAs which are available for free antibody, however in the antibody hyper-saturated medium these newly appeared CALLAs are occupied by pre-existing KP-22 in excess in hyper-saturated medium all the way during incubation time up to 24 hrs.

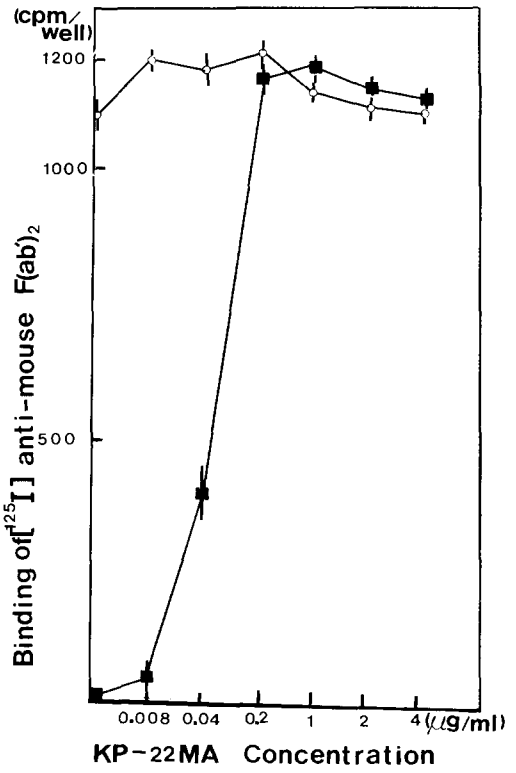

Fig. 2. Estimation of free cell surface determinants after prolonged incubation of cells with different concentrations of KP-22 MA initially. 5×10^4 target cells were cultured at 37°C for 24 hours with various concentrations of KP-22 MA as the conditions described in Fig.1, and free cell surface determinants were estimated by a further 1 hour incubation at 37°C with (○) or without (■) an additional 1 μg/ml amount of KP-22 MA. Total KP-22 MA bound to cell surface was determined by incubation with 50 μl (7 Ci/g) of ^{125}I-conjugated F(ab')$_2$ specific to murine antibody. Bars, S. D.

Consequently, antigenic modulation distinct from capping of cell surface molecules did not occur under conditions where bound antibody was eliminated from the surface of the tumor cells. This raised the possiblity that the bound antiody might be endocytosed or shed and that new antigenic determinants might be reexpressed during 24 hr incubation in the antibody free media. These data demonstrated that target cells for KP-22 could become resistant to antibody after losing the expression of antibody-defined antigenic determinants, CALLAs, similar to leukemia antigens by Ritz (1981), Pesando (1981) and OKT3-defined antigen (Chatenoud, 1984). To determine how long antibody could be retained at the cell surface in interaction with cognate antigen, Reh cells were incubated at 37°C for 30 min with 1 μg/ml of unlabeled KP-22. After washing, cells were incubated in medium without antibodies. At different time points, the amount of immunoglobulin still bound to the tumor cell surface was determined by incubation with ^{125}I-labeled rabbit anti-mouse F(ab')$_2$. The result was that the amount of KP-22 bound to cell surface decreased by 35% over 5 hr, more sharply than thereafter. However, the amount of KP-22 bound by Reh cells continued to decrease over the entire period of incubation, falling to 25% of the initial value after 24 hr (Fig. 3A).

Disappearance of KP-22 from the tumor cell surface could occur either by release into the culture medium with shed antigen or by endocytosis into the tumor cell. To determine whether the bound antibody remain associated with the tumor cells, Reh cells were incubated with ^{125}I-labeled KP-22 for 30 min and washed throughly. Cells were incubated for different durations up to 24 hr, and the amount of residual radioactivity still associated with tumor cells was measured at different intervals. As shown in Fig. 3B, the amount of KP-22 associated with Reh cell decreased by 21% over 5 hr, but no significantly additional decrease was observed over the next 24 hr. Since the fraction of radioactivity by endocytosis still re-

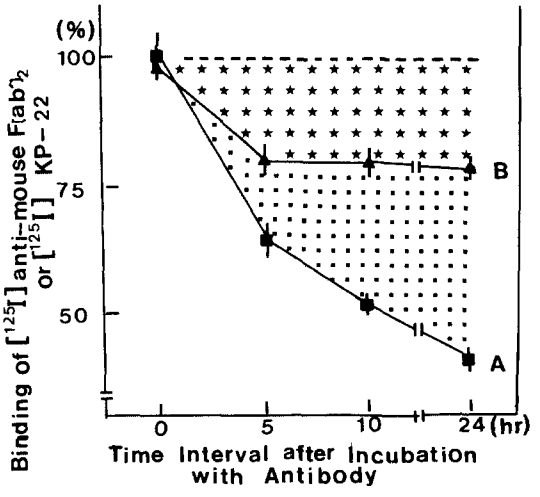

Fig. 3. Persistence of antibody at the cell surface (■) or in the cytoplasm plus cell surface (▲). 5×10^4 target cell (Reh) were incubated at 37°C for 30 min with either of 1 μg/ml of unlabeled or ^{125}I-labeled KP-22 MA. After washing, cells were cultured at 37°C for various periods without antibody. The radioactivity associated with cells was measured by detecting ^{125}I-labeled KP-22 MA and the amount of unlabeled KP-22 MA remaining at the cell surface was detected with ^{125}I-labeled anti-mouse rabbit F(ab')$_2$. Bar, S. D.

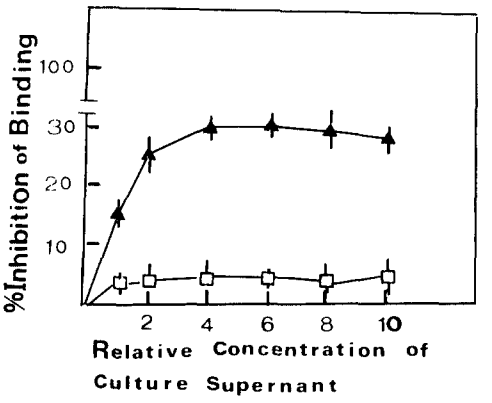

Fig. 4. Competitive inhibition of shedding antigens in culture supernatant to the binding of KP-22 MA to the target cell (Reh) surface antigens. 5×10^8 target cells were cultured in 30 ml medium without changing the medium for 5 days and the supernatants were collected to be concentrated up to 10-fold. Percentage of inhibition has been plotted as a function of relative supernatant concentration. Fresh concentrated culture supernatant ▲; that of heated in a boiling water bath for 45 min, □. Bar, S. D.

sides in the cytoplasm in the case of incubation with ^{125}I-labeled KP-22, the initial decrease over 5 hr must result from shedding only. No additional decrease thereafter up to 24 hrs was observed and it was interpreted as the result of a stagnation of shedding.

From the results in Fig. 3 at incubation time of 24 hr, out of 60% radioactivity disappeared at cell surface, 35% remained in the cells indicating the fraction was endocytosed by the cells, whereas 25% of it (b) disappeared from the cells probably by shedding. Fig. 3 would show each of the two fractions of shedding(asterisk) and endocytosis (dotted) out of total disappearance of antigenic determinant on cell surface. In the early stage of incubation with KP-22, shedding was prominent much more than endocytosis whereas in late stage endocytosis dominated and continually increased in its rate with lapse of time remaining shedding in its stagnation.

To determine whether shedding process actually occur and whether the shed antigens are detected in the culture supernatants, we carried out RIA experiment designed to detect competitive inhibition of the supernatants to the binding of the KP-22 to the cell surface antigens. Reh cell culture supernatant was prepared by the method in which 5×10^8 Reh cells were cultured in 30 ml medium for 5 days without changing medium and subsequent medium was concentrated up to 10 fold using Amicon filter. Aliquots of 4×10^4 Reh cells in 50 μl of serum free medium were mixed with 100 μl of various grades 2-10 fold of concentrated cell culture supernatants or normal culture medium (control) and incubated for indirect antibody-binding radiommunoassay using KP-22 and ^{125}I-labelled goat anti-mouse IgG antibody. The results were that the concentrated culture medium in contrast to normal medium (control; 0% inhibition) showed the significantly inhibitory effect up to 30% on the interaction of the cell-bound antigen to KP-22, indicating the presence of shed antigen in spent culture medium (Fig. 4).

Since the inhibitory activity was associated with supernatant of 100,000 × g culture medium and since the heating of supernatant destroyed the inhibitory activity, antigens were considered to be shedded at molecular level rather than at supramolecular level. When supernatants were analyzed

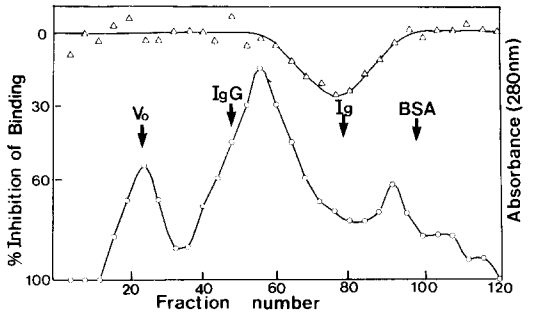

Fig. 5. Gel filtration profile of concentrated culture supernatant and detection of shed antigen. The supernatant was chromatographed on Sephadex G-150 (2 × 80 cm) in PBS. The absorbance at 280 nm (○) and binding inhibition of KP-22 (△) was measured. Arrows indicate the eluted positions of blue dextran 4000 (Vo), mouse IgG (IgG) and bovine serum albumin (BSA) respectively. The inhibitory activity for KP-22 was eluted betweem IgG and BSA, corresponding to a molecular weight of approximately 100,000 dalton.

by gel filteration Sephadex G-150, the inhibitory activity eluted between immunoglobulin and serum albumin suggesting the molecular weight of the shed antigen to be approximately 100 Kd (Fig. 5).

Immunofluorescence techniques with monoclonal antibody can be applied to study the dynamics of antigenic determinant and interrelationships among plasma membrane proteins. The techniques can be used to study the distribution of membrane antigenic determinant prior to any interaction with antibodies, the redistribution and fate of antigenic determinant-antibody complexes, and the interrelationships between two membrane proteins, i.e., their association prior to or after interaction with antibodies and the relationship between surface proteins and cytoplasmic structures (Braun *et al.*, 1980, Schreiner *et al.*, 1976).

To examine the effects of KP-22 on the redistribution of CALLA, permeabilized fibroblasts and unpermeabilized Reh cells were respectively incubated with KP-22 and chased the destination of antigen-antibody complex at 37°C for the various time by indirect immunofluorescence. Fibroblast cells were incubated with KP-22 for the various duration, fixed, opened with Triton X-100, and labeled CALLA with KP-22 was visualized using FITC conjugated anti-mouse IgG. The microscopy of CALLA in shorter incubation time (10 min) with KP-22 shows fine, punctate labelling scattered in the cytoplasm, suggesting that the antibody might be binding to discrete structures in cytoplasm (Fig. 6A).

With lapse of time, the punctate dots were getting fewer and bigger suggesting the aggregation of CALLA-KP-22 complex in clusters (Fig. 6B, C, D). Although CALLA in permeabiliized fibroblasts were localized in cytoplasm in the form of spots stained with monoclonal antibody KP-22, it might be merely the consequence of the fact that antigen-antibody complex on cell surface at 37°C induced the rapid internalization of the cross-linked membrane component. When unpermeabilized Reh cells were incubated with KP-22 for 30 min at 4°C, CALLA immunofluorescence was consistent with retention on the cell surface in small punctate dots scattered throughout cell membrane, demonstrating the existence of CALLA on the cell surface in punctae pattern (Fig. 7A). In contrast, after incubation with KP-22 for 30 min at 4°C and further incubation for 20 min at 37°C, CALLA staining pattern lost their punctate appearance and became concentrated in cross-link structure demonstrating an accumulation of CALLAs in patching and capping (Fig. 7B). When unpermeabilizd leukemic cells were incubated with KP-22 for 30 min at 4°C and cultured for a longer period (150 min), CALLA was almost not visible on the cell surface (data were not shown).

In contrast to that of permeabilized fibroblasts where KP-22 staining CALLAs were confined to compact perinuclear structure and clustered, showing typical Golgi distribution (Fig. 6E). Therefore, the disappearance of antigen from the leukemic cell surface seems to result from the internalization or shedding.

In brief indirect immunofluorescence analysis using KP-22 monoclonal antibody showed the rearrangement of CALLA on the cell surface in concurrence with formation of antigen-antibody complex.

Discussion

CALLA$^+$ leukemic cell lines, Reh and Nalm-6, were used for most of our experiments because these lines provide large numbers of cells that can

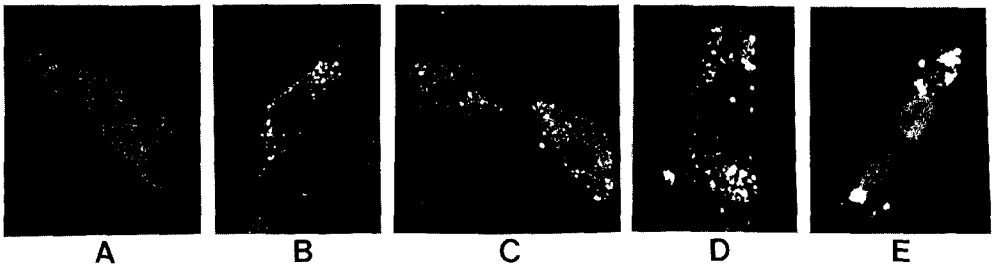

Fig. 6. Immunological localization of CALLA-KP-22 complex in permeabilized fibroblast cells with lapse of time at 37 C. Fibroblast cells grown on coverslip were incubated with KP-22 (0.1 ml mouse ascites at 1 : 200) at 37°C for 10 min (A), 30 min (B, C), 50 min (D) and 150 min (E), fixed with 4% paraformaldehyde in PBS for 20 min at 37 C, opened with 0.1% Triton X-100, and stained with FITC-conjugated goat anti-mouse IgG.

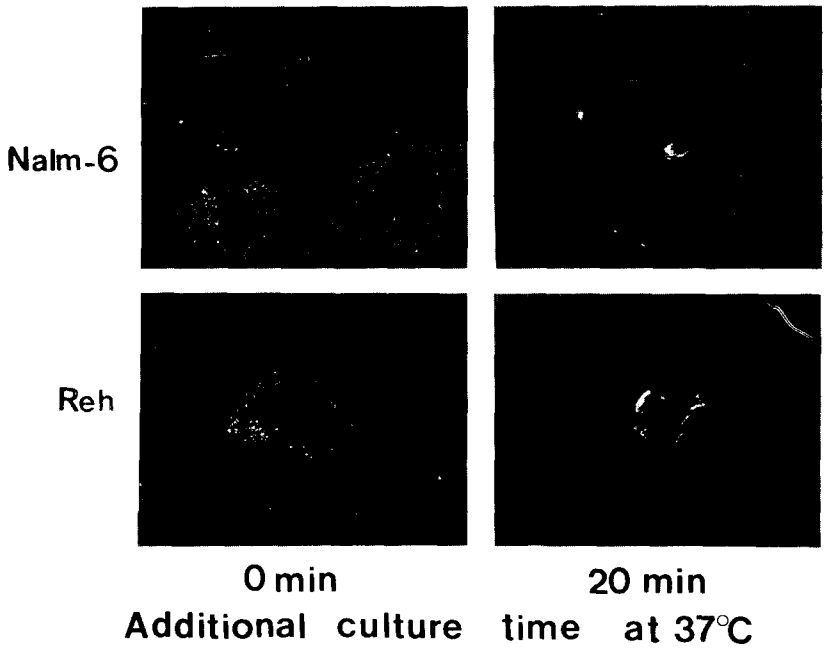

Fig. 7. Time dependent redistribution-pattern of CALLA-KP-22 complexes on the surface of leukemic cells, Nalm-6 (upper) and Reh (bottoms). Cells preincubated with KP-22 (0.1 ml mouse ascites at 1 : 100, equivalent 0.2 μg antibody/ml) for 30 min at 4°C for additional 0 min (left), 20 min (right), 150 min and cells were fixed with 4% paraformaldehyde and stained by indirect immunofluorescence with FITC-conjugated goat anti-mouse IgG. On incubation time of 150 min at 37 C (data were not shown), no detectable staining patterns are visible.

proliferate indefinitely and that have stable phenotypic properties *in vitro*. The potential use of MAs that recognize tumor-associated antigenic determinants is still far reaching. MAs directed against tumor cell surface components can inhibit tumor cell porliferation in culture and in whole animals (Vollmers *et al.*, 1985, Masui *et al.*, 1984). However, administration of MAs directly to patients has been tried with limited success so far. MAs by themselves will most likely be of therapeutic benefit in clearing tumor cells from the blood, in diminishing the amount of circulating tumor antigen, which could have blocking effect on subsequent immunotherapy, and in clearing

the bone marrow of tumor cells so that it can be used for autologous transplantation in patients after they have been treated with bone marrow suppressive therapy (Bast *et al.*, 1985). The mechanism by which antibody forms complex with antigenic determinant and subsequent modulation has been remained to be elucidated. When KP-22 were incubated with the Reh cells, the amount of murine immunoglobulin bound to cell surface CALLA diminished significantly during incubation over 24 hr after initial equilibration as seen in Fig. 1. Tse and Pernis (1984) have shown that a proportion of T lymphocytes in mouse spleen actively internalize their own membrance MHC class-I molecules and that this process is 'spotaneous', i.e., it is not dependent on cross-linking of MHC molecules by added ligand. The disappearance of CALLA from cell surface by KP-22 seems to be different from that of the MHC molecules in T lymphocytes in several respects: (1) the membrane CALLAs of the leukemic cell require cross-linking for internalization; without cross-linking, the internalization of CALLAs do not occur; (2) the internalized CALLA-KP-22 complexs are not likely recycled to the cell membrane but are subject to various degrees of proteolysis in an intracellular compartment that involves lysozymes; (3) the reappearance of the membrane CALLA after modulation by the corresponding antibodies required at least more than 6 hours and synthesis of new molecules (Fig. 2). These characterisitcs suggest that monolconal antibody-induced modulation of cell surface CALLA may be lost from the cell surface by capping, endocytosis and shedding in resemblance with the characteristics that the original immunoglobulin antigenic molecules on the surfaces of B cells and B-cell leukemia are removed from the cell surface by capping in the presence of IgG followed by either endocytosis or shedding, or both (Waldmann, 1985). Because antigenic determinants in the Reh cell membrane are often mobile, many of them can be cross-linked by specific divalent reagents, such as KP-22 antibody molecules, to form areas of two-dimensional precipitation that are termed patches. These patches coalesce into a polar cap, most of which is shed from the cell or endocytosed. The patterns of modulation of CALLs induced by KP-22 seem to be highly different depending on the cell type, leukemic cells or fibroblasts. It has been actually reported that anti-CALLA BA-3 antibody, which recognizes similar or identical epitopes to those reacting with another monoclonal, J5, does not modulate *in vitro* (Lebien, 1982) whereas J5 does (Ritz, 1980b). At this point, we should keep in mind the heterogeneous behaviors of different antigens and antibodies. This heterogeneity is, at least in part, based on qualitative differences in terms of transmembrane anchoring to the cytoskeleton of cell membrane structures. This is suggested, for example, by the phenomenon of spontaneous capping which is cytoskeleton contraction inducing the redistribution of membrane molecules (Chatenoud and Bach, 1984).

For the purpose of indirect immunofluorescent localization of CALLA in the cells, cells were fixed with 4% paraformaldehyde. However, some fixatives may alter the antigenicity of proteins and like glutaraldehyde, may produce autofluorescence. The investigators have consistently used fixation in 4% paraformaldehyde without problems with background fluorescence and loss in antigenicity.

As seen in Fig. 7, plasma membrane antigenic determinants recognized by KP-22 and examined by immunofluorescence were originally distributed punctately throughout the Reh and Nalm-6 cell surface with an apparent organization. In both cell lines, the surface antigenic determinants were found in a delicate, punctate pattern when KP-22 initially bound to the antigenic determinant. However, with lapse of time and apparent involvement of antibody binding, the antigenic determinants seemed to be redistributed gradually. At 50 min after antigen-antibody complexes formed both cells distributed surface antigenic determinants to some poles of the cell and finally showed large plaques of aggregates of fluorescence. With lapse of longer time, all CALLAs disappear from the leukemic cell surface. The significance of this distribution is not clear, but we are tentatively considering it as two basic patterns of redistribution. One is by capping followed by endocytosis; the other is by patching followed immediately by endocytosis of the anigenic determinant.

In fact, antigenic modulation, defined by the action of antibodies reacting with membrane antigens, should probably be placed in the context of

membrane changes induced by ligand-receptor interactions. The redistribution of various hormones or neurotransmitter receptors, after binding of their respective specific hormonal ligand, is porbably closely related to some of the mechanisms of antigenic modulation. The present studies clearly demonstrate that modulation of CALLA on acute lymphoblastic leukemia cell line occurs in response to KP-22 antibody. This property may be related to structural characteristics of this glycoprotein such as its manner of insertion within the cell membrane or to a functional role that has not yet been elucidated. Alternatively, CALLA modulation that results in the loss of antibody bound to cell surface may represent a tumor cell defense mechanism and response to specific antibody. Further studies will be necessary to determine the significance of this phenomenon *in vitro*.

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종양세포 표면항원에 대한 분자면역학적 연구(II)
백혈병세포의 발생과 세포분화에 관한 연구

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본 연구자들이 생성한 단클론항체 KP-22(IgG_1, K)에 의해서 인지되는 leukemic세포주 표면항원 CALLA는 분자량 약 10만달톤의 당단백질로서 KP-22 단클론항체와 결합하면 배양액으로 shedding되거나 세포내로 endocytosis된다. KP-22의 존재하에 KP-22에 의해 인지되는 CALLA의 약 60%가 24시간후에 modulation되어 세포표면에서 사라지는데 실험적으로 약 35%가 endocytosis, 25%가 shedding에 의하는 것으로 나타났다. KP-22에 의해 세포표면 CALLA가 modultion된 후 재합성, 재출현 하는데 최소한 6시간 이상이 소요되는 것으로 추정된다.