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A MONOCLONAL ANTIBODY WITH SELECTIVITY FOR HUMAN KAPPA MYELOMA AND LYMPHOMA CELLS WHICH HAS POTENTIAL AS A THERAPEUTIC AGENT

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ABSTRACT

K-1-21 is a monoclonal antibody which binds to human free kappa light chains and recognises a determinant, KMA selectively expressed on kappa myeloma and lymphoma cells. KMA is absent on plasma cells and resting B cells from normal adults but can be detected on some foetal B cells and a small proportion of activated B cells. Expression of KMA is greatest on cycling cells. K-1-21 is an IgG_1 antibody that elicits ADCC but will not cap the KMA determinant unless a second ligand is present. K-1-21 has potential for practical application.

INTRODUCTION

K-1-21 is a murine IgG_1 monoclonal antibody raised against a human kappa Bence Jones protein. It binds to free monomers and dimers of human kappa light chains but does not bind to intact kappa positive immunoglobulin molecules (Boux and Raison et al., 1983). K-1-21 has also been found to react with an epitope designated KMA (kappa myeloma antigen) selectively expressed on the surface of kappa myeloma and lymphoma cells. Since K-1-21, in contrast to anti-idiotypic antibodies is not individual patient specific, the antibody has clinical potential. Studies have been made of factors which might affect this practical application.

MATERIALS AND METHODS

Lymphoid Cell Populations and Human Cell Lines

Mononuclear cell (MNC) suspensions from peripheral blood or bone marrow obtained from patients with myeloma and other malignancies were prepared by separation on Ficoll-Hypaque gradients. MNC suspensions were also prepared from normal peripheral blood or bone marrow, from electively removed tonsil, from spleens removed after traumatic rupture or in cases of idiopathic thrombocytopenic purpura, from cord blood, and from foetal liver and spleen. MW 28, an EBV transformed line was obtained from Dr J. Gibson, (Clinical Immunology Research Centre, Sydney) while other cell lines came from the Ludwig Institute of Cancer Research, Sydney and London.

Antibody-dependent Cell Mediated Cytotoxicity (ADCC)

LICR LON/HMy2 (HMy2) cells were stained with Rose Bengal (O'Neill and Parish, 1983) and allowed to bind K-1-21 antibody which had been preincubated with an excess of either kappa or lambda Bence Jones protein (BJP). Effector cells were adherent cell depleted normal peripheral blood MNC. Effector cells were incubated with antibody-coated target cells for 1 h at 37°C. After centrifugation, the cell supernatant was removed and optical density at 560nm was measured to give an estimate of the dye release from lysed target cells.

Capping of the KMA:K-1-21 complex

To determine whether KMA would be capped from the cell surface after binding by K-1-21, HMy2 cells were incubated with K-1-21 at 37°C for up to 30 h. The cells were then stained at 4°C with FITC-conjugated sheep anti-mouse immunoglobulin (FITC-SaMIg) to detect the KMA:K-1-21 complex. To determine the effect of adding a second ligand, HMy2 cells were incubated at 4°C with K-1-21 followed by incubation with FITC-SaMIg at 37°C. This incubation was also carried out in the presence of capping inhibitors 10^{-4}M dibucaine, 5×10^{-6}M calcium ionophore or 10^{-2}M sodium azide. Stained cells were analysed by flow cytometry.

Elutriation

HMy2 cells were fractionated into enriched growth phases by centrifugal elutriation as described elsewhere (Boux et al., 1984). Separated cells were then analysed by flow cytometry for DNA content and for surface immunofluorescence after staining

with either K-1-21 and FITC-SaMIg or with a polyvalent FITC conjugated goat anti-human kappa reagent (FITC-GaHK).

TABLE I

Binding of K-1-21 to the surface of cells from patients with myeloma and other immunological disorders and to a panel of human continuous cell line.

Cell Type	Source	Reactivity with K-1-21	
		No.Positive/ No.Samples	% Cells Positive (Range)
Bone Marrow Plasma Cells	Non-Myelomatous*	- (0/8)	-
	Kappa Myeloma	+ (8/11)	5 - 89
	Kappa Macroglobulinemia	+ (2/2)	0.5 - 45
	Lambda Myeloma	- (0/5)	-
Peripheral Blood MNC	Kappa B CLL	- (0/6)	-
	Lambda B CLL	- (0/3)	-
	Kappa Myeloma	+ (4/8)	0.5 - 3
	Lambda Myeloma	- (0/7)	-
	Kappa Lymphoma	+ (6/7)	1.0 - 8.5
	Lambda Lymphoma	- (0/3)	-
	Kappa Macroglobulinemia	- (0/4)	-
	Lambda Macroglobulinemia	- (0/2)	-
	Sezary Syndrome	- (0/4)	-
	Multiple Sclerosis	- (0/2)	-
	T CLL	- (0/3)	-
	Hairy Cell Leukemia	- (0/1)	-
	T ALL	- (0/1)	-
	Benign Monoclonal Gammopathy	- (0/1)	-
Human Continuous Cell Lines	B ALL(LAZ-007,RDG,WIL)	- (0/3)	-
	Burkitt Lymphoma (Daudi)	- (0/1)	-
	B Lymphoblastoid (HMy2,MW28)	+ (2/2)	62 - 80
	Melanoma (PMC-22B)	- (0/1)	-
	T ALL (CCRF-HSB,F2/F7 MOLT-4,CCRF-CEM,MPB ALL)	- (0/5)	-
	T Leukemia (Jurkat)	- (0/1)	-

Cells were stained with K-1-21 or a control monoclonal antibody followed by FITC-SaMIg.
* These included amyloid, benign paraproteinemia, SLE, B CLL, AML and lymphoma. All contained Kappa positive cells as detected with an FITC-goat anti-human Kappa reagent.

In Vitro Stimulation of Human B cells

MNC from human tonsil or spleen were subjected to double rosetting with AET-treated SRBC to yield a B cell enriched (E^-_1) population which was cultured in RPMI-10%FCS at 10^6 cells ml^{-1} in the presence or absence of formalin fixed Staphylococcus aureus Cowan I strain (SAC) (Muraguchi and Fauci, 1982). On day three, half the culture medium was removed and replaced with fresh medium with 20% MLA 144 supernatant as a source of TRF. On day six, cells were stained for KMA with K-1-21 preincubated with kappa or lambda BJP, followed by FITC-SaMIg. Cell staining was analysed by flow cytometry.

RESULTS

The data given in Table 1 indicate that KMA can be detected by surface immunoflorescence on the bone marrow plasma cells from patients with kappa myeloma and kappa macroglobulinemia and on peripheral blood MNC from patients with kappa myeloma and kappa

TABLE II

Binding of K-1-21 to the surface of normal adult tissues, foetal tissues, or to cells stimulated in vitro.

"Normal" MNC Source	Surface Immunofluorescence	
	K-1-21 (No.positive/No.tested)	GaHK (No.positive/No.tested)
Peripheral Blood	- (0/9)	+ (9/9)
Tonsil	- (0/6)	+ (6/6)
SAC activated Tonsil	+ (3/4)*	ND
Spleen	- (0/5)	+ (5/5)
Bone Marrow	- (0/5)	+ (5/5)
Foetal Liver (14-20wk)	- (0/6)	+ (6/6)
Foetal Spleen (16wk)	+ (3/3)**	+ (3/3)
Cord Blood	- (0/15)	+ (15/15)

* 3.0 - 17.0% cells positive
** 1.0 - 17.8% cells positive
Cells were stained with K-1-21 preincubated with Kappa or Lambda PBL followed by FITC-SaMIg; or with a polyvalent FITC-GaHK reagent.

lymphoma. KMA was not detected on equivalent lambda expressing malignancies nor on cells from patients with B CLL or with a

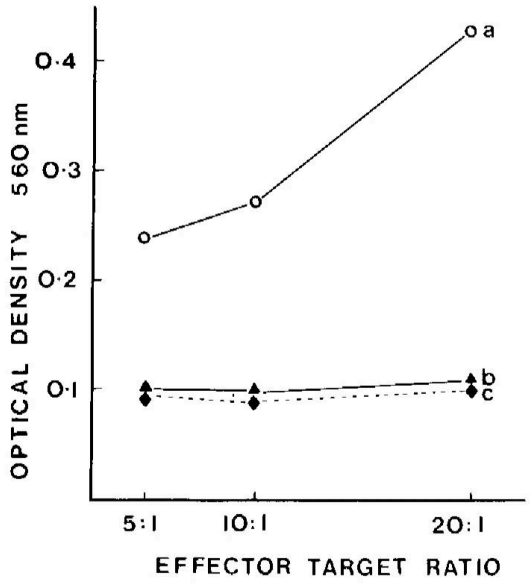

FIGURE 1

K-1-21 (a and c) or K-1-21 preincubated with excess free kappa light chain (b) was bound to HMy2 target cells stained with Rose Bengal. Cell lysis, estimated by dye release, was measured after 1 h incubation of the target cells with adherent cell depleted MNC (a and b) or with unstained HMy2 cells (c).

variety of other immunological disorders including one case of benign kappa paraproteinemia. KMA is also expressed on the surface of cells from two lymphoblastoid human cell lines (LICR/LON HMy2 and MW28) but was absent on T cell derived lines and on lines derived from B ALL or Burkitt's lymphoma. Table 2 indicates that KMA could not be detected on normal cells from adult peripheral blood, tonsil, spleen or bone marrow, although the determinant was expressed on some foetal B cell and some activated adult B cells. It was found on cells from 16 week foetal spleen where 1.0 - 17.8% of cells were positive but was absent from foetal liver and from cord blood. KMA was also found on a small proportion (4 - 17%) of normal adult tonsillar B cells after stimulation with Staph. aureus for six days in vitro culture.

The KMA positive human lymphoblastoid cell line LICR LON/HMy2 (HMy2) has been used in studies on the cytotoxic efficiacy of K-1-21 and on the membrane mobility of KMA and its expression during the cell cycle, properties which are relevant to the potential value of K-1-21 as a therapeutic agent. It was found that: (a) K-1-21, which is a monoclonal antibody of the IgG_1 subclass, did not mediate complement dependent cytotoxicity of HMy2 cells but will elicit ADCC (Figure 1); (b) K-1-21 alone does not cap KMA. Even after a prolonged incubation of up to 30 h at 37°C, no capping occurred (Table 3). However, the addition of a second ligand FITC-SaMIg, induced rapid capping which was inhibited by NaN_3 but not by calcium ionophore or dibucaine. The capping is thus of Type II rather than Type I origin (Loor et al., 1981); and (c) the expression of KMA on HMy2 cells is dependent on the stage of the cell cycle (Table 4). Flow cytometric analysis of K-1-21 stained HMy2 cells from elutriated fractions showed that cells enriched for the G_1 growth phase contain the lowest number of cells staining with K-1-21 or GaHK or conversly that KMA is expressed at greatest frequency on cycling cells.

TABLE III

Capping of KMA from the surface of HMy2 with K-1-21 and sheep anti-mouse immunoglobulin.

Capping Antibodies	Inhibitor	Type of capping inhibited	Capping at 90 min
K-1-21	None		-
K-1-21 + FITC-SaMIg	None		+++
K-1-21 + FITC-SaMIg	Calcium ionophore	Type I	+++
K-1-21 + FITC-SaMIg	Dibucaine	Type I	+++
K-1-21 + FITC-SaMIg	NaN_3	Type I and II	-

- indicates that no capping was observed even after 30 h incubation.

+++ indicates that after 90 min incubation all cells were capped.

TABLE IV

Elutriation analysis of KMA on the surface of HMy2 cells at different stages of the cell cycle.

Elutriation fraction	Cell cycle Kinetic parameters			% stained cells*	
	% G_1	%S	% G_2 + M	GaHK	K-1-21
unseparated	40	38	3	ND	89
G_1 enriched	89	14	3	77	64
S and G_2 + M enriched	25	52	22	92	85
G_2 + M enriched	23	36	40	96	89

* Cells were stained with FITC-GaHk or with K-1-21 and FITC-SaMIg.

DISCUSSION

KMA may be regarded in an operational sense as a tumour-associated marker with selectivity for human kappa myeloma, macroglobulinemia and lymphoma cells. However, KMA is not confined to neoplastic tissues. The determinant has been detected on some foetal B cells and a small proportion of in vitro activated B cells although it is absent from resting B cells and plasma cells from freshly isolated lymphoid tissues and from bone marrow. These findings suggest that KMA may be a differentiation antigen expressed at a limited stage or B cell development but represented in an expanded form by the tumour cell population (Figure 2). Thus, KMA, while absent from the surface of resting mature B cells, may be transiently expressed in activated B cells, but lost in normal lymphoblasts or plasmacytes. Malignancies arising at early stages of B cell development (ALL, B CLL) do not exhibit KMA while malignancies arising at or after the stage of B cell activation express this determinant. Relation of KMA to the expression of other B cell differentiation markers such as IgD and the antigens revealed by monoclonals PCA-1 and 4F2 remains to be determined.

The K-1-21 antibody appeared to have potential for practical application as a therapeutic agent. Factors which might affect the

KMA AS A B CELL DIFFERENTIATION ANTIGEN

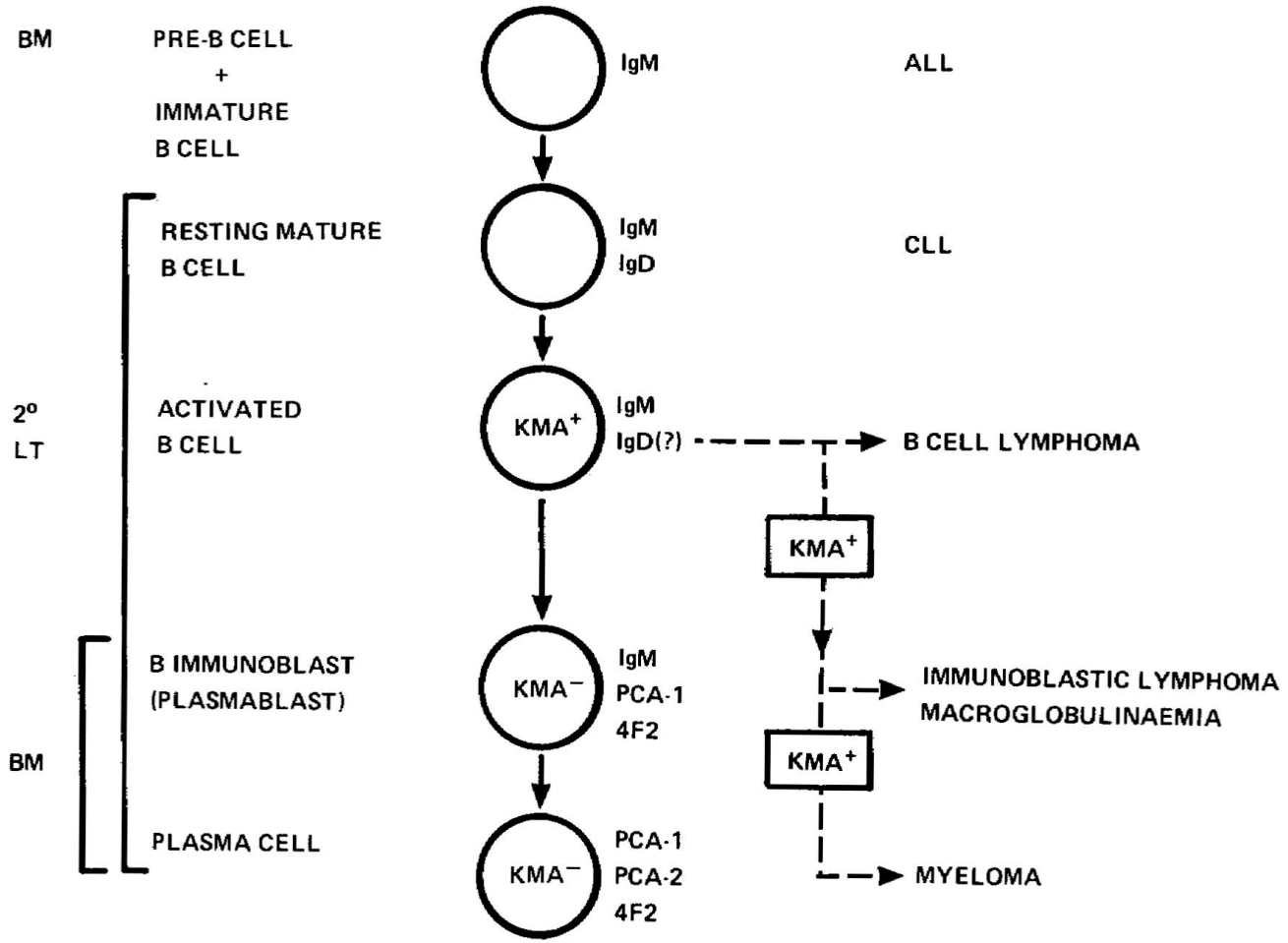

FIGURE 2

KMA as a differentiation antigen expressed transiently on normal B cells and on malignant B cells after the stage of B cell activation.

use of the antibody in a clinical situation have been examined using the KMA positive cell line HMy2. While K-1-21 did not mediate complement dependent cytotoxicity, it did elicit ADCC which might thus be the mechanism of lysis if K-1-21 were used in serotherapy. The fact that K-1-21 alone does not cause capping of KMA suggests that binding of the antibody is unlikely to lead to modulation of antigenic determinants with consequent escape from immunodetection. But if K-1-21 is coupled to a 'cytotoxin', the agent should not require internalisation for its effect. Experiments are therefore underway to couple K-1-21 to the surface active drug adriamycin (Tritton and Yee, 1982). The expression of K-1-21 was shown to vary with phases of the cell cycle, being greatest in S and G_2 + M and least in G_1. This highlights the importance of giving K-1-21 at a time of maximal expansion of the tumour growth fraction such as occurs after administration of conventional chemotherapy. Experiments are in progress to test the efficacy of K-1-21 in a xenograft model using nude mice and its homing potential to the marrow in patients with kappa myeloma. K-1-21 has additional practical applications unlike conventional anti-kappa reagents. First it can be used for precise quantitation of free kappa light chains in serum and urine. Secondly, it has diagnostic potential in discriminating between benign monoclonal gammopathy and true myeloma.

REFERENCES

Boux, H. A., Raison, R. L., Walker, K. Z., Hayden, G. E., and Basten, A., 1983, A tumor-associated antigen specific for human kappa myeloma cells, J. Exp. Med., 158:1769.

Boux, H. A., Raison, R. L., Walker, K. Z., Musgrove, E., and Basten, A., 1984, The surface expression of a tumour-associated antigen on human kappa myeloma cells, Eur. J. Immunol., 14:216.

Loor, F., Martin-Pelissard, C., and Angman, L., 1981, Capping revisited II. Low sensitivity to sulfydryl reagents, Cell. Immunol. 57:73.

Muraguchi, A., and Fauci, A. S., 1982, Proliferative responses of normal human B lymphocytes. Development of an assay system for human B cell growth factor (BCGF), J.Immunol. 129:1104.

O'Neill, H. C., and Parish, C. R., 1983, A rapid automated colorimetric assay for measuring antibody binding to cell surface antigens, J. Immunol. Meth. 64:257.

Tritton T. R., and Yee, G., 1982, The anticancer agent adriamycin can be actively cytotoxic without entering cells, Science (N.Y.) 217:248.