Brief Definitive Report

A TUMOR-ASSOCIATED ANTIGEN SPECIFIC FOR HUMAN *KAPPA* MYELOMA CELLS*

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The great majority of membrane-associated antigens detected on neoplastic lymphoid cells in man lack tumor specificity (1), but, in B cell malignancies, Ig idiotypes represent true clonal-specific markers. While this property has allowed antiidiotype-specific monoclonal antibodies to be used in the treatment (2) of B cell tumors, the potential value of these antibodies is limited to individual patients.

In this paper, K-1-21, a monoclonal antibody with a broader spectrum of reactivity, is described. It binds to free monomers and dimers of human kappa light chains, not to intact Ig molecules. K-1-21 also binds to plasma cells from patients with kappa myeloma but does not react with normal lymphoid cells nor to cells from other malignancies. Thus the K-1-21-reactive determinant, designated KMA (kappa myeloma antigen), can be regarded as a tumor-associated antigen with selectivity for kappa myeloma cells.

Materials and Methods

Human Serum and Urinary Proteins. Serum albumin was obtained from Miles Laboratories Inc., Elkhart, IN. Bence Jones protein (BJP) pools were purchased from Commonwealth Serum Laboratories, Melbourne, Australia. Kappa (NAP and VOR) and lambda (MOS) BJP, IgA paraproteins, and normal human IgG were purified by ion exchange and gel filtration chromatography. Periodic acid-treated VOR kappa BJP were prepared by the method of Shapiro and Erickson (3).

Production and Purification of K-1-21. Spleen cells from BALB/c mice hyperimmunized with purified NAP kappa BJP were fused with P3-NS1-1-Ag4-1 (NS1) myeloma cells (4). One cloned hybrid, K-1-21, secreted a monoclonal IgG1 kappa antibody, as indicated by isoelectric focusing and by immunodiffusion in agarose with anti-mouse Ig-typing reagents (Litton Bionetics Inc., Kensington, MD). Ascites fluid from K-1-21 tumor-bearing BALB/c mice was collected and purified by affinity chromatography on NAP or VOR kappa chains conjugated to Sepharose CL-4B (Pharmacia, Uppsala, Sweden).

Radioimmunoassay. Hybrid culture supernatants or purified K-1-21 antibodies were assayed by indirect solid-phase radioimmunoassay (RIA) using light chains or Ig bound to the wells of polyvinyl chloride microtiter trays at 1 mg/ml. In all assays, an unrelated monoclonal IgG1 antibody was included as a control. Antibody binding was detected by the addition of ^{125}I-rabbit anti-mouse Ig.

Preparation of Cells for Immunofluorescent Staining. Bone marrow aspirates and samples of peripheral blood were obtained from patients with myeloma, or other lymphoid malignancies at the time of routine biopsy. Normal peripheral blood was collected from laboratory workers, while normal bone marrow aspirates were obtained from renal

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transplant donors on routine pretransplant assessment. Tonsillar tissue came from elective tonsillectomies while spleens came from patients undergoing surgery for traumatic rupture or immune thrombocytopenic purpura. Mononuclear cell (MNC) fractions were separated on Ficoll-Hypaque (specific gravity, 1.078). Cells from bone marrow aspirates were then depleted of adherent cells and washed repeatedly to remove any cytophilic Ig.

Human Cell Lines. LICR LON/HMy2 (HMy2) came from the Ludwig Institute of Cancer Research (LICR), London; JURKAT was obtained from Dr. J. Watson, Medical School, Auckland University, Auckland, New Zealand and other cell lines came from LICR, Sydney.

Surface Immunofluorescent Staining. Cells were suspended at 2×10^7 cells/ml in RPMI containing 0.02% (wt/vol) sodium azide (RPMI-NaN_3). Aliquots (100 μl) were incubated (*a*) with 100 μl of a 1:10 dilution of fluorescein isothiocyanate (FITC)-sheep $F(ab')_2$ anti-human Ig heavy or light chain specific antisera (Kallestad Laboratories, Inc., Austin, TX) or (*b*) with 100 μl of K-1-21 or control IgG monoclonal antibodies (at 1 mg/ml) followed by 100 μl of a 1:50 dilution of FITC-sheep $F(ab')_2$ anti-mouse Ig (New England Nuclear, Boston, MA). All incubations were for 30 min at 4°C followed by two washes with cold RPMI-NaN_3. Stained cells were resuspended in 10% phosphate-buffered saline (PBS) in glycerol, pH 8.6, and mounted for examination under a Zeiss photomicroscope II (Carl Zeiss, Oberkochen, Federal Republic of Germany).

Fluorescence-activated Cell Sorter (FACS) III Analysis. Aliquots of washed HMy2 cells (2×10^6 cells in 100 μl) were exposed for 30 min at 4°C to 50 μg of K-1-21 alone or to K-1-21 preincubated with 620 μg VOR or MOS BJ Proteins or to 1,820 μg of normal human IgG. After washing twice in cold RPMI-NaN_3, the cells were incubated for 30 min at 4°C with 100 μl FITC-sheep $F(ab')_2$ anti-mouse Ig (New England Nuclear, Boston, MA) at a 1:50 dilution in PBS, pH 7.2. After two washes in cold PBS-NaN_3, individual samples were analyzed on a FACS III (Becton, Dickinson & Co., Mountain View, CA).

Results

K-1-21 is a hybrid cell line secreting an IgG1 kappa monoclonal antibody that binds all free monomers and disulphide-bonded dimers of human kappa light chains (Table I). No reactivity was seen with free lambda light chains, intact Ig molecules, or human serum albumin. Binding was unaffected by periodic acid treatment of the antigen, indicating that the antibody does not recognize a carbohydrate determinant.

The cell surface distribution of the K-1-21 reactive determinant, KMA, was examined by immunofluorescence of MNC from normal individuals and from patients with a variety of B or T cell malignancies. Two IgG monoclonal antibodies of irrelevant specificity were used as controls and always failed to show any cell surface staining.

K-1-21 antibody did not bind to the surface of normal MNC (Table II). It also failed to react with PBM from patients with kappa myeloma or to PBM or bone marrow plasma cells from patients with lambda myeloma and a variety of non-myelomatous conditions, despite the presence in the latter samples of varying numbers of plasma cells reactive with polyvalent anti-kappa serum (Table II). On the other hand, plasma cells in bone marrow aspirates from eight out of 11 patients with kappa myeloma showed strong fluorescent staining with K-1-21. The proportion of K-1-21-positive plasma cells varied greatly, ranging from 9 to 78% of total plasma cells. Of the three cases where cells did not stain with K-1-21, two were IgG kappa myelomas and the third was an IgA kappa. While cells from one of the IgG kappa secretors also failed to react with polyvalent anti-kappa or anti-Ig sera, anti-kappa antisera did stain 86 and 85%, respectively, of plasma cells from the remaining two patients. Also, BJP from these patients

TABLE I

Reactivity of K-1-21 with Various Human Serum and Urinary Proteins

Protein	Reactivity with K-1-21
Serum albumin	None
IgM kappa paraprotein	None (0/2)*
IgA kappa paraprotein	None (0/1)
Normal IgG (pool)	None
Normal light chain (pool)	Reactive
Lambda BJP (pool)	None
Kappa BJP (pool)	Reactive
Purified lambda BJP	None (0/3)
Purified kappa BJP	Reactive (10/10)
Purified dimers of kappa BJP	Reactive (2/2)
Purified monomers of kappa BJP	Reactive (2/2)
Periodic acid-oxidized VOR kappa BJP	Reactive (1/1)

Binding of K-1-21 was assessed by indirect solid-phase RIA. Antigens tested were adsorbed onto polyvinyl plates. After blocking with bovine serum albumin, purified K-1-21 was added, followed by ^{125}I-rabbit anti-mouse Ig; radioactivity bound on each well was counted on a gamma spectrometer.

* Number reactive per number tested.

TABLE II

Binding of K-1-21 to the Surface of Cells from Normal Donors or Patients with Myeloma and Other Immunological Disorders

Mononuclear cell source	Diagnosis	No. of samples tested	Reactivity with:	
			K-1-21*	Anti-kappa‡
Peripheral blood	Normal	8	−	+
	B kappa CLL	3	−	+
	B lambda CLL	2	−	−
	Kappa myeloma	2	−	+
	Macroglobulinemia	1	−	+
	Sezary	2	−	+
	Multiple sclerosis	2	−	+
	T CLL	2	−	+
Tonsil	Normal	4	−	+
Spleen	Normal	5	−	+
Bone marrow	Normal	5	−	+
Plasma cells only	Nonmyelomatous§	5	−	+
	Myeloma			
	IgG kappa	6	+ (4/6)‖	+ (5/6)
	IgG + M kappa	1	+	+
	IgA kappa	2	+ (1/2)	+ (2/2)
	free kappa	2	+ (2/2)	+ (2/2)
	IgA lambda	1	−	ND¶
	free lambda	2	−	ND

* Cells were stained with K-1-21 or a control monoclonal followed by FITC-sheep F(ab')$_2$ anti-mouse Ig.

‡ Cells were stained with FITC-goat anti-human kappa antiserum. The numbers of kappa-reactive cells fall within the expected normal range for each cell population examined.

§ The five nonmyelomatous bone marrows were: amyloid of Ig origin (52% kappa-reactive plasma cells), IgG kappa benign paraproteinaemia (50% kappa), systemic lupus erythrematosus (40% kappa), B CLL in remission (18% kappa), and acute myeloid leukemia (12% kappa).

‖ Number reactive per number examined.

¶ Not done.

TABLE III

Binding of K-1-21 Monoclonal Antibody to Determinants on the Surface of Various Human Cell Lines as Determined by Immunofluorescence

Cell line	Cell line origin	Reactivity with K-1-21*
LAZ-007	B cell ALL‡	–
RDG	B cell ALL	–
WIL	B cell ALL	–
JP	B cell, EBV transformed§	–
GK	B cell, EBV transformed	–
SC4	B cell, EBV transformed	–
Daudi	B cell, EBV transformed	–
LICR LON/HMy2	IgG kappa myeloma	++‖
PMC-22B	Melanoma	–
CCRF-HSB	T cell ALL	–
F2/F7	T cell ALL	–
MPB ALL	T cell ALL	–
MOLT-4	T cell ALL	–
CCRF-CEM	T cell pseudodiploid ALL	–
JURKAT	T cell leukemia	–

* Cell lines were stained with K-1-21 or a control monoclonal followed by FITC-sheep F(ab')$_2$ anti-mouse Ig.

‡ ALL, acute lymphocytic leukemia.

§ EBV, Epstein Barr virus.

‖ Percentage of cells staining (10 experiments) ranged from 68 to 79%.

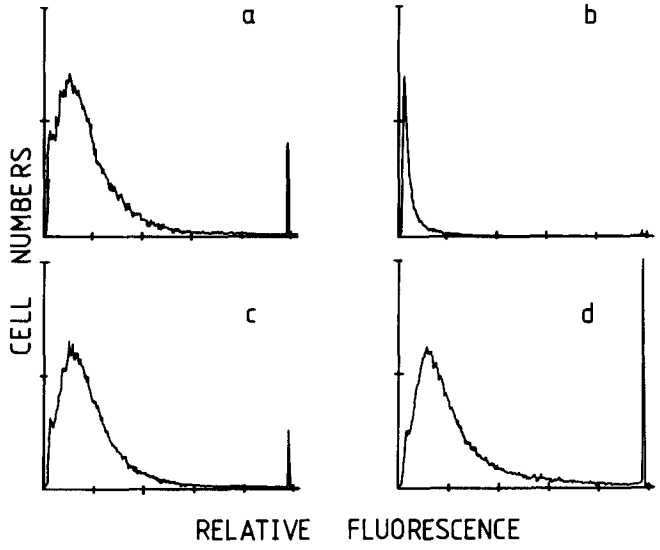

FIGURE 1. Single-parameter histograms of FACS III analysis demonstrating the effect of preincubation with various immunoglobulin fractions on the binding of K-1-21 antibody to LICR LON/HMy2 cells. The cells (2×10^6) were incubated with: (*a*) K-1-21 antibody alone, (*b*) K-1-21 preincubated with VOR kappa BJP, (*c*) K-1-21 preincubated with MOS lambda BJP, (*d*) K-1-21 preincubated with normal human IgG. Labeled cells were then washed and incubated with FITC-sheep F(ab')$_2$ anti-mouse Ig before analysis on a FACS III. The high voltage and gain settings for all samples were identical.

displayed strong reactivity with K-1-21 in an indirect RIA, indicating that a K-1-21-reactive determinant was present on the secreted cell products.

Further analysis of the KMA expression was carried out on human cell lines (Table III). Of these, only HMy2, derived from an IgG kappa myeloma (5), reacted with K-1-21.

The specificity of K-1-21 binding to HMy2 cells was examined by flow cytometry. HMy2 cells were incubated with K-1-21 alone or with K-1-21 preincubated with normal human IgG or with lambda (MOS) or kappa (VOR) BJP. The cells were then incubated with FITC-sheep $F(ab')_2$ anti-mouse Ig. Upon FACS analysis, HMy2 cells incubated with K-1-21 exhibited strong cell surface reactivity (Fig. 1). However, on exposure to K-1-21 preincubated with VOR kappa light chains, the fluorescence curve was shifted sharply to the left. No apparent diminution in fluorescence intensity occurred when K-1-21 was preincubated with equivalent amounts of lambda light chain or with human IgG.

Discussion

The IgG_1 monoclonal antibody K-1-21 described here recognizes an epitope on dimers and monomers of free human kappa light chains but fails to react with intact Ig. Cell surface binding studies of K-1-21 antibody revealed an epitope, KMA, with tumor-specific properties. Thus, K-1-21 bound strongly to the surface of plasma cells from 8 of 11 patients with kappa myeloma but failed to react with plasma cells from normal individuals or from patients with lambda myeloma or nonmyelomatous conditions. Furthermore, no binding was observed to PBM from normal donors or from patients with chronic lymphocytic leukemia (CLL), myeloma, or macroglobulinaemia, or to MNC from normal secondary lymphoid tissue. Present evidence thus suggests that KMA may be regarded in an operational sense as a tumor marker with specificity for kappa myeloma cells.

The proportion of plasma cells in the bone marrow that could be labeled with K-1-21 varied greatly. On the basis of flow cytometry analysis, this appears to be related to the cycling characteristics of the tumor at the time of sampling rather than to a true lack of expression of KMA (unpublished observations). Predictably, therefore, BJP from the two patients whose bone marrow cells failed to bind K-1-21, but did react with a polyvalent anti-kappa antiserum, displayed strong reactivity with the monoclonal antibody in an indirect RIA.

Further analysis of KMA expression was carried out with a panel of human cell lines. Convincing cell surface reactivity was only observed in the IgG kappa myeloma line HMy2, the one myeloma line included in the panel, whereas cells from all other lines were negative. The specificity of binding of K-1-21 was confirmed in inhibition studies with FACS III analysis. When HMy2 cells were exposed to K-1-21 antibody that had been preincubated with purified VOR kappa light chains, complete inhibition of binding occurred, whereas no comparable diminution in fluorescence intensity occurred after preincubation with equivalent amounts of lambda light chain or with human IgG.

These results raise the interesting question of the nature of the K-1-21-reactive determinant on the cell membrane. Since K-1-21 reacts with kappa light chains in free form but not with kappa chains associated with intact Ig molecules, KMA may be an epitope on kappa chains inserted as free entities in the cell membrane of myeloma cells. Consistent with this interpretation is the demonstration of free

light chains on the surface of certain murine plasmacytoma cells (6) and their absence on normal mouse spleen cells (7).

However, it is also possible that K-1-21 may recognize a cross-reactive determinant on a molecule other than free kappa light chains that is unique to the membrane of kappa myeloma cells. Cross-reactivity of this type has been demonstrated with a number of monoclonal antibodies (8).

Summary

A monoclonal antibody (K-1-21) raised against a kappa Bence Jones protein exhibits unique binding properties to malignant plasma cells. K-1-21 is an IgG1 kappa antibody that reacts with human kappa light chains in free form, but shows no reactivity with heavy chain-associated kappa light chains. By immunofluorescence, K-1-21 binds to the surface of LICR LON/HMy2 (HMy2) kappa myeloma cells and to plasma cells from a majority (8/11) of patients with various types of kappa myeloma; it did not bind to the surface of normal cells, nor to malignant cells of non-kappa myeloma origin. Flow cytometry analysis of K-1-21 binding to HMy2 cells indicated that the surface reactivity of K-1-21 could be completely inhibited by preincubation of the antibody with purified kappa light chains, whereas no inhibition occurred after preincubation with lambda chains or intact human IgG. Thus, the epitope recognized by K-1-21 on the cell surface may be similar, if not identical, to the determinant recognized on soluble free kappa light chains, and constitutes a tumor-associated antigen with selectivity for kappa myeloma cells. K-1-21 may therefore have clinical potential in patients with kappa myeloma.

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