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The surface expression of a tumor-associated antigen on human kappa myeloma cells*

The monoclonal antibody K-1-21 defines an antigen, KMA (kappa myeloma antigen) on the surface of human κ myeloma cells. K-1-21 also recognizes human κ light chains in free form but not when covalently bonded to heavy chains. To examine the relationship between KMA and this determinant on free κ chains, the surface expression of KMA was examined on the IgG,κ myeloma line LICR LON/HMy2 (HMy2). No patching or capping was observed in the presence of K-1-21 alone, but KMA could be capped if the cells were incubated with K-1-21 followed by fluorescein isothiocyanate-conjugated sheep $F(ab')_2$ anti-mouse immunoglobulin. Capping was not affected by the inhibitors calcium ionophore or dibucaine. When IgG molecules were removed from the cell surface by capping with anti-IgG antiserum both KMA and free κ light chains could still be detected with K-1-21 and a polyvalent anti-κ antiserum, respectively. By contrast, after removal of all surface κ chains with the polyvalent anti-κ serum, no staining was observed with K-1-21 indicating that KMA may be an epitope on free κ chains inserted in the membrane of κ myeloma cells but absent from normal cells. KMA cell surface expression varied with the stage of the cell cycle. Flow cytometric analysis of K-1-21-stained HMy2 cells from either continuous cultures or from elutriated fractions enriched for various cell cycle phases showed that, within the cycling population, cells in $G_2 + M$ expressed KMA at a higher frequency and density than did cells in G_1.

1 Introduction

Multiple myeloma is a malignant disease characterized by the uncontrolled proliferation of a clone of plasma cells and the accumulation of their secreted immunoglobulin (Ig) in the serum or urine [1]. Recently, we have described a monoclonal antibody (mAb) with clinical potential which recognizes a determinant selectively expressed on the surface of kappa (κ) myeloma cells [2]. This antibody, designated K-1-21, was derived from a fusion of P3-NSI-1/Ag4.1 (NS-1) plasmacytoma cells with spleen cells from mice immunized with human κ Bence Jones proteins. K-1-21 is an IgG_1,κ mAb which reacts with human κ light chains in free form (both as monomers and dimers), but does not bind to κ chains covalently bound to heavy chain. Immunofluorescence analysis revealed that K-1-21 recognized a determinant, KMA (κ myeloma antigen), expressed on the surface of plasma cells from patients with κ myeloma but not detected on normal cells nor on malignant cells of non-κ myeloma origin [2]. However, the relationship between KMA and the K-1-21-reactive determinant on free κ chains was not established. To do so capping studies were undertaken with the human IgG,κ cell line LICR LON HMy2 (HMy2) [3].

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Abbreviations: **B CLL:** B cell chronic lymphocytic leukemia **FACS:** Fluorescence-activated cell sorter **FITC:** Fluorescein isothiocyanate **FITC-SaMIg:** Fluorescein isothiocyanate-conjugated sheep $F(ab')_2$ anti-mouse immunoglobulin **KMA:** Kappa myeloma antigen **PBS:** Phosphate-buffered saline, pH 7.4 **SDME:** Supplemented Dulbecco's modified Eagle's medium **TRITC:** Tetramethyl rhodamine isothiocyanate **Ig:** Immunoglobulin **mAb:** Monoclonal antibody(ies) **FCS:** Fetal calf serum

The proportion of plasma cells that could be labeled with K-1-21 in a bone marrow aspirate varied greatly from patient to patient. Since only a minority of myeloma cells are actively proliferating at any particular time [4, 5] it was possible that the variation in KMA expression could be related to the cycling characteristics of the tumor when the aspirate was taken, rather than to a true absence of this determinant from a proportion of the malignant cells. To clarify the issue, which has importance if the clinical potential of K-1-21 is to be realized, the cell surface expression of KMA and its relation to the cell cycle was examined by flow cytometry using HMy2 myeloma cells. The results of the two groups of experiments indicated that (a) KMA is possibly an epitope on free κ chains inserted in the membrane of myeloma cells and (b) its expression varies with the stage of the cell cycle, being greatest in $G_2 + M$ and S.

2 Materials and methods

2.1 mAb

K-1-21, an IgG_1,κ mAb reactive against free human κ chains was derived from a fusion between NS-1 and spleen cells from mice immunized with human κ Bence Jones proteins. Its production and purification have already been described [2]. F-1-1, an affinity purified IgG_1 mAb of irrelevant antigen specificity, was used as a negative control.

2.2 Cells

The human myeloma line LICR LON/HMy2 (HMy2) was obtained from the Ludwig Institute of Cancer Research, London, GB. When the cells were first obtained, the majority were diploid in nature, as indicated by DNA staining and fluorescence-activated cell sorter (FACS) analysis (see Sect. 2.7). However, after one year in culture the line had become tetraploid. The experiments described in this report were all carried out with the tetraploid population. The cells were grown

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in supplemented Dulbecco's modified Eagle's medium (SDME) [6] containing 10% fetal calf serum (FCS, Flow Laboratories, Sydney, Australia). They were harvested in exponential growth, washed twice in RPMI 1640 (RPMI), then incubated for 1 h at 37 °C and again washed twice before use to remove cytophilic Ig. Mononuclear cells were prepared from the peripheral blood of a patient with B cell chronic lymphocytic leukemia (B CLL) by separation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) [2].

2.3 Surface immunofluorescent staining

HMy2 cells or human mononuclear cells were suspended at 2×10^7 cells/ml in cold (4 °C) RPMI containing 0.02% (w/v) sodium azide (RPMI-NaN_3). Aliquots (100 µl) of this cell suspension were incubated at 4 °C (a) directly with fluorescein isothiocyanate (FITC)- or tetramethyl rhodamine isothiocyanate (TRITC)-conjugated F(ab')$_2$ goat anti-human Ig heavy- or light chain-specific antisera (Kallestad Laboratories, Austin, TX) or (b) with 100 µl of K-1-21 or F-1-1 mAb (at 1 mg/ml) followed by 100 µl of a 1/50 dilution of FITC-sheep F(ab')$_2$ anti-mouse Ig (FITC-SaMIg) (New England Nuclear, Boston, MA). Conditions for staining were as described previously [2]. Stained cells were either examined by fluorescence microscopy on a Zeiss (Oberkochen, FRG) photomicroscope II with an IV/F epifluorescence condenser containing selective filters for red (TRITC) or green (FITC) fluorescence or were analyzed by flow cytometry on a FACS III (Becton Dickinson, Mountain View, CA). In the latter case, staining was carried out as described above, but quantities of cells and antisera were doubled. The cells were then washed twice in phosphate-buffered saline (PBS) containing 0.02% (w/v) NaN_3 (PBS-NaN_3) and resuspended at 1×10^6–2×10^6 cells/ml before analysis. Within any one experiment, the laser power, photomultiplier tube voltage, scatter and fluorescent gains were kept constant (4.88 nm laser line at 400 mW and 520 nm longpass and 520 nm bandpass filters). After FACS analysis the population size of stained and unstained cells were quantitated by cutting out and weighing tracings of the histogram peaks [7].

2.4 Cytoplasmic immunofluorescent staining

Aliquots (125 µl) of HMy2 cells suspended at 5×10^5–10×10^5 cells/ml in RPMI were deposited on slides with a cytocentrifuge (Shandon Instruments, London, GB) and then dried and fixed for 30 min in absolute methanol at 4 °C. After 3 washes in PBS containing 5% pooled, filtered human serum (PBS/HS), the cell deposits were covered with 20 µl of K-1-21 (mg/ml) and incubated for 30 min at room temperature. After 3 washes in PBS/HS, the cell deposits were covered with 20 µl of K-1-21 (1 mg/ml) and incubated for 30 min at room temperature. After 3 washes in PBS/HS, the cell deposits were covered with 20 µl of FITC-SaMIg (1/50 dilution) and incubated for 30 min at room temperature. Finally, they were washed, mounted and examined under the fluorescence microscope.

2.5 Capping of the KMA : K-1-21 complex

Capping of KMA from the surface of HMy2 cells was examined using either K-1-21 alone or with the addition of SaMIg: (a) 1 ml of cells (10^7/ml) was incubated with 200 µl of sterile K-1-21 (1 mg/ml) at 37 °C for up to 30 h. Aliquots (100 µl) were removed at intervals and stained at 4 °C with FITC-SaMIg so that capping could be assessed under a fluorescence microscope, (b) K-1-21 (20 µl at 1 mg/ml) was added to 100 µl of HMy2 cells, which were then incubated for 30 min at 4 °C. After 1 wash in RPMI, they were washed again either in RPMI alone, or in RPMI containing 10^{-4} M dibucaine, 5×10^{-6} M calcium ionophore or 10^{-2} M sodium azide [13]. One hundred µl of FITC-SaMIg diluted 1/50 in RPMI with the relevant inhibitor was added and the cells were incubated for 30, 60 or 90 min at either 4 °C or 37 °C. After washing twice in RPMI-NaN_3, the cells were examined for capping under a fluorescence microscope.

2.6 Co-capping

Sixty µl of PBS or TRITC-conjugated goat antiserum to human IgG or κ chains were added to 300 µl aliquots of HMy2 cells suspended at 8×10^6 cells/ml in RPMI with 20% FCS. The cells were then incubated at 37 °C for 4 h and after 2 washes in RPMI-NaN_3, divided into 3 aliquots, to which 20 µl of either K-1-21, FITC-F(ab')$_2$ goat anti-human IgG or FITC-F(ab')$_2$ goat anti-κ chains (all at 1 mg/ml) were added. After a further incubation at 4 °C for 30 min, the cells were washed twice in cold RPMI-NaN_3. Cells labeled directly with fluoresceinated antisera were examined for capping immediately, while cells labeled with K-1-21 as a second antibody were subsequently stained with 100 µl of a 1/50 dilution of FITC-SaMIg in RPMI-NaN_3 at 4 °C before capping was assessed.

2.7 Flow cytometry DNA analysis

One hundred Kumtz units of RNAase (Type IA, Sigma Chemical Co., St. Louis, MO) were added to 1×10^6 HMy2 cells and 1×10^5 chicken red blood cells suspended in 2 ml of RPMI, 1% Triton X-100 (w/v) (RPMI-TX) after the cells had been stained for DNA content by the addition of 300 µl ethidium bromide (Sigma) in RPMI-TX and 300 µl mithramycin (Pfizer Inc., New York, NY) in RPMI-TX, 75 mM $MgCl_2$ to give final concentrations of 40 mg/ml and 12.5 mg/ml, respectively. DNA content was analyzed on an IPC 22 flow cytometer (FM Ortho Instruments, Westwood, MA) as described by Taylor [8].

2.8 Quantitation of human IgG secretion

Supernatants from HMy2 cell cultures were assayed for secretion of human IgG by an enzyme-linked immunosorbent assay. The wells of a polystyrene microtiter tray (Linbro, Flow, Maclean, VA) were coated with affinity-purified human IgG (50 µg/ml in PBS, 75 µl/well) by incubation for 1 h at 37 °C. Nonspecific binding sites were then blocked with 1% bovine serum albumin (BSA) in PBS. Concurrently, a second tray was prepared by blocking with BSA, and then adding to each well 15 µl of a 1/12 000 dilution of a goat antiserum to human Ig conjugated to alkaline phosphatase (New England Nuclear) and 60 µl of either standard IgG solutions or HMy2 supernatants. After 1 h at 37 °C, 60-µl aliquots were removed from each well of the second tray and placed in an IgG-coated well in the first tray. This tray was incubated for a further 1 h at 37 °C and then washed 3 times in PBS, once in double distilled water and once in sodium carbonate buffer, pH 9.6.

Finally, 100 µl enzyme substrate, p-nitrophenyl phosphate (Sigma) (1 mg/ml in sodium carbonate buffer) was then added to each well and color development was allowed to proceed at 37 °C. Absorbance of the solution in each well was determined on a Titertex Multiskan (Flow, Irvine, GB).

2.9 Expression of KMA on the surface of HMy2 cells grown in stationary culture

HMy2 cells were grown in 75 cm^2 plastic flasks (Corning Glass Works, Corning, NY) in SDME supplemented with 10% FCS from 3×10^3 cells/ml initial concentration, to 2×10^6 cells/ml final concentration. Cells were fed daily and during this period, cell numbers, cell DNA content, secretion of IgG, surface and cytoplasmic immunofluorescence due to the binding of K-1-21 followed by FITC-SaMIg were sequentially assessed.

2.10 Elutriation

Centrifugal elutriation, a technique for separating cells based on differences in cell mass [9] was used to fractionate HMy2 cells into enriched G_1, S or $G_2 + M$ growth phases. For this purpose HMy2 cells (7.5×10^7 cells in 20 ml SDME–10% FCS) which had been obtained from cultures in exponential growth (1.5×10^5 cells/ml) were fractionated in an elutriator rotor (Beckman JE-6, Palo Alto, CA) equilibrated in SDME–10% FCS as described by Kaiser and co-workers [9]. The rotor speed was maintained at 1030 rpm and seven fractions were collected over a linear pump speed gradient varying from 6.25 to 22 ml/min. The separated cells were immediately analyzed for DNA content, while cell surface fluorescence, as a consequence of the binding of (a) K-1-21 or F-1-1 followed by FITC-SaMIg or (b) FITC-goat anti-κ chains, was assessed by flow cytometry.

3 Results

3.1 FACS analysis of K-1-21 binding to HMy2

Cells from cultures of the HMy2 line were stained with K-1-21 or a control mAb F-1-1 followed by FITC-SaMIg. FACS analysis indicated that K-1-21 bound strongly to HMy2 cells (Fig. 1), while F-1-1 did not. As noted previously [2], there was a range in the intensity of staining of HMy2 cells by K-1-21 and a small proportion appeared not to express the K-1-21-reactive determinant, KMA.

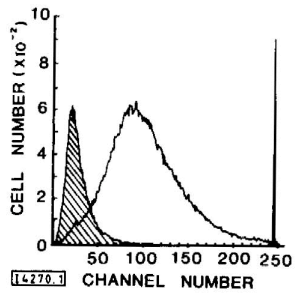

Figure 1. Immunofluorescence profiles of HMy2 cells stained with F-1-1 (hatched area) or K-1-21 (open area) mAb followed by FITC-SaMIg as indicated by FACS III analysis.

In a second experiment the specificity of K-1-21 for myeloma cells was checked by comparing its reactivity with that of a polyvalent FITC-anti-human κ chain antiserum on cells from the peripheral blood of a patient with κ B CLL. As seen in Fig. 2, strong staining was observed on FACS analysis with the anti-κ antiserum, whereas K-1-21 was negative.

3.2 Capping of the KMA determinant

The HMy2 cell line was used as a model for studying the membrane characteristics of KMA. Initially, HMy2 cells were labeled with K-1-21 alone and, after incubation at 37 °C for varying time periods, the cells were stained with FITC-SaMIg

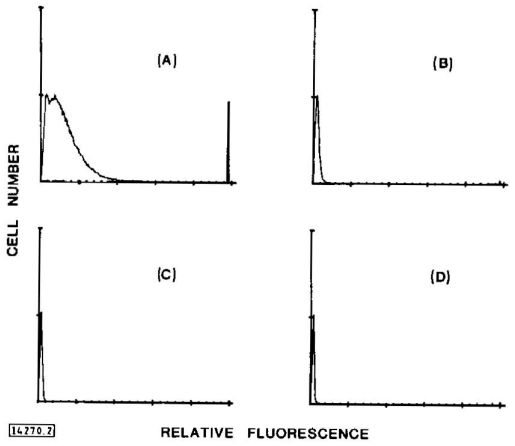

Figure 2. Immunofluorescence profiles of peripheral blood lymphocytes from a patient with B CLL after staining with FITC-goat $F(ab')_2$ anti-human κ (A) or λ light chains (B); or after staining with (C) K-1-21 or (D) F-1-1 followed by FITC-SaMIg.

Table 1. Capping of KMA on the surface of HMy2 with K-1-21 and sheep anti-mouse Ig

Capping antibodies	Inhibitor	Extent of capping at 30 min	60 min	90 min
K-1-21[a]	None	–[b]	–	–
K-1-21 + FITC-SaMIg[c]	None	+	++	+++
K-1-21 + FITC-SaMIg	Calcium ionophore	+	++	+++
K-1-21 + FITC-SaMIg	Dibucaine	+	++	+++
K-1-21 + FITC-SaMIg	NaN_3	–	–	–

a) HMy2 cells were incubated for up to 30 h with K-1-21 at 37 °C. Aliquots were removed at intervals and cells were stained with FITC-SaMIg at 4 °C before examination under a fluorescence microscope. No capping was observed even at 30 h.

b) The symbols indicate as follows: – no capping, + patching, ++ patching with some capping, +++ all cells capped.

c) HMy2 cells were incubated for 30 min at 4 °C with K-1-21, followed by addition of FITC-SaMIg with or without an inhibitor, and the extent of capping was noted after 30, 60 or 90 min incubation at 37 °C.

Table 2. The effect of capping Ig from the surface of HMy2 cells on the expression of KMA

Capping ligand[a]	Test ligand	Red staining	Green staining
TRITC-anti-IgG	FITC-anti-IgG	–[b]	–
TRITC-anti-IgG	FITC-anti-κ	–	+
TRITC-anti-IgG	K-1-21	–	+++
TRITC-anti-κ	FITC-anti-IgG	–	–
TRITC-anti-κ	FITC-anti-κ	–	–
TRITC-anti-κ	K-1-21	–	–
None	FITC-anti-IgG	NA[c]	+++
	K-1-21	NA	+++

a) HMy2 cells were incubated with the capping ligand for 4 h at 37 °C, then washed and incubated with the test ligand for 30 min at 4 °C. Cells labeled directly with a fluoresceinated test ligand were examined for capping, while those labeled with K-1-21 were subsequently stained with FITC-SaMIg before capping was assessed.
b) The symbols indicate as follows: – most cells completely capped, + faint peripheral staining with no capping, +++ good staining with no capping.
c) NA is not applicable.

at 4 °C to enable detection of bound K-1-21. Even after a prolonged incubation period of up to 30 h at 37 °C, no capping was observed (Table 1). However, rapid capping did occur when a second ligand, FITC-SaMIg was introduced before incubation at 37 °C commenced. Thus, after 30 min, nearly all cells were patched and in some cases the patches were beginning to coalesce into caps. By 90 min capping was essentially complete, some cells having shed their antigen-antibody complexes (Table 1). Incubation of the cells with the capping inhibitors, calcium ionophore or dibucaine did not interfere with patching or cap formation, whereas NaN_3 prevented both these events (Table 1).

3.3 Analysis of the relationship between KMA and surface Ig on HMy2 cells by co-capping

Co-capping experiments were carried out to determine the relationship between KMA and membrane-associated IgG on the surface of HMy2 cells. HMy2 cells were stained with TRITC-conjugated goat $F(ab')_2$ antisera to human IgG or κ chains and incubated for 4 h at 37 °C until complete capping had occurred. Aliquots of cells were then removed and the cell surface distribution of IgG, κ chains or KMA was assessed by staining with either FITC-$F(ab')_2$ antisera to human IgG or κ chains, or with K-1-21 followed by FITC-SaMIg.

Capping with TRITC-goat anti-IgG completely removed IgG molecules from the cell surface (Table 2). No red or green staining associated with FITC- or TRITC-conjugated goat anti-IgG reagents could be seen. However, faint staining was detected with FITC-goat anti-κ antiserum which suggested that free, rather than heavy chain-associated, κ chains remained on the cell surface. Strong peripheral green fluorescence was seen after staining these cells with K-1-21 followed by FITC-SaMIg indicating that KMA determinants were not removed when the cells were capped with anti-human IgG.

Capping with TRITC-goat anti-κ antiserum removed the KMA determinant as well as κ chains from the cell surface. Thus no green fluorescence was detected on subsequent staining with either FITC-goat anti-κ or with K-1-21 and FITC-SaMIg (Table 2).

3.4 Expression of KMA on the surface of HMy2 cells grown in stationary culture

The proportion of HMy2 cells reacting strongly with K-1-21 varied substantially in different experiments [2]. Studies were therefore undertaken to determine how the expression of KMA was affected by the stage of the cell cycle. Initially, KMA expression on HMy2 cells was examined on samples withdrawn from a stationary culture which was allowed to pass through logarithmic growth phase and into stationary phase. Cell numbers, DNA content, IgG secretion and surface and cytoplasmic immunofluorescence after staining with K-1-21 and FITC-SaMIg were sequentially assessed. During exponen-

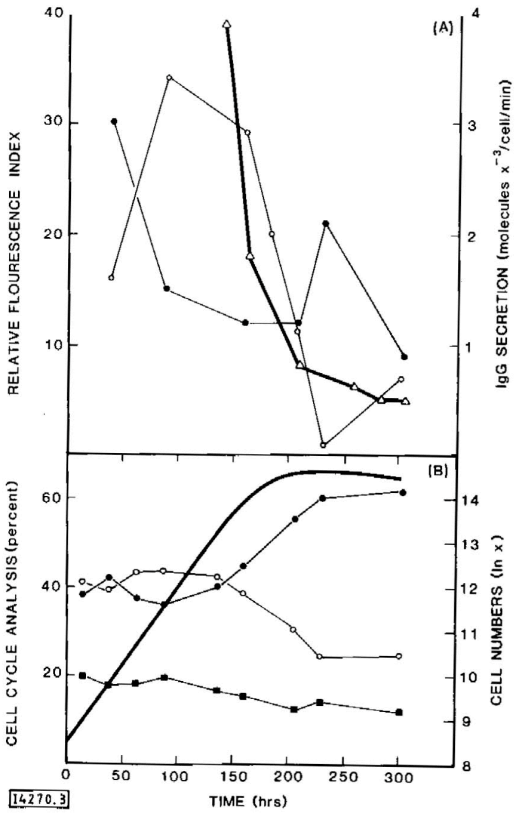

Figure 3. Expression of KMA on the surface of HMy2 cells under optimal or restricted growth conditions. The lower graph shows the increase in cell numbers (—) with time of HMy2 cells in a stationary cell culture. Other lines indicate the percentage of cells in G_1 (●—●), S (○—○) or in G_2 + M (■—■) throughout this period. The upper graph indicates cytoplasmic (●—●) or surface (○—○) immunofluorescence of these cells after staining with K-1-21 followed by FITC-SaMIg and FACS III analysis. All measurements were standardized to the fluorescence level of a negative control, namely, cells treated with K-1-21 preincubated with VOR κ Bence Jones proteins. This eliminated technical variations in the assessment of different samples. IgG secretion by HMy2 cells is also shown (△—△) as determined by an enzyme-linked immunosorbent assay.

tial growth, the number of cells in G_1 approximately equalled the number in S phase (Fig. 3B), but, as the cells became overcrowded and growth reached plateau, the proportion of cells in G_1 markedly increased while the proportion in S phase declined. At the same time, the amount of IgG secreted/cell also decreased. Overcrowding had little effect on the proportion of cells in $G_2 + M$, which remained fairly stable, declining only slightly during the culture period. Both surface and cytoplasmic staining of HMy2 cells by K-1-21 were maximal in early logarithmic growth and declined as the cells became overcrowded (Fig. 3A). Thus, KMA would appear to be preferentially expressed both on the surface and in the cytoplasm of cycling cells (cells in $G_2 + M$ or in S phase) when compared to cells in G_1.

Table 3. Elutriation analysis of KMA on the surface of HMy2 cells at different stages of the cell cycle

Elutriation fraction no.	Cell cycle kinetic parameters[a]			% Unstained cells[b]	
	% G_1	% S	% $G_2 + M$	Anti-κ	K-1-21
Unseparated	40.3	38.1	2.6	ND	10.8
Fraction 1	89.2	14.1	2.6	22.6	35.5
Fraction 4	25.0	52.6	22.3	8.1	14.6
Fraction 5	23.4	36.5	40.0	3.6	10.5

a) As determined by DNA analysis (Fig. 4).
b) Determined by FACS III analysis.

3.5 Expression of KMA on the surface of HMy2 cells fractionated by centrifugal elutriation

Centrifugal elutriation permits the study of cells at different phases of the cell cycle without the gross changes in culture conditions which characterize a stationary culture. This technique was therefore used to check whether KMA determinants are preferentially expressed on cycling cells. HMy2 cells in exponential growth were separated on the basis of mass into seven fractions. When analyzed for DNA content, three fractions were enriched for cells in various phases of the cell cycle (Fig. 4 and Table 3). The original cell population (Fig. 4A) contained approximately equal numbers of cells in G_1 and S phase [40.3 and 38.1% respectively (with few cells in $G_2 + M$) Table 4]. In contrast, the first fraction obtained by elutriation (Fig. 4B) was enriched for G_1 cells, which now represent 83.2% of the total population. The fourth fraction collected (Fig. 4C) was enriched for cells in S phase (52.6%) and for cells in $G_2 + M$ (22.3%), but had a diminished number of cells in G_1. Similarly, fraction five (Fig. 4D) was enriched for cells in $G_2 + M$ (40%) and S phase (36.5%).

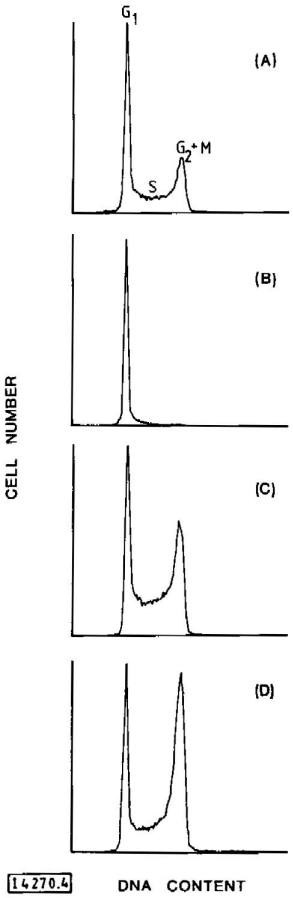

Figure 4. DNA content of HMy2 cells assessed by flow cytometry. (A) Unseparated cells and (B–D) fractions separated by centrifugal elutriation. These histograms represent the first (B), fourth (C) and fifth (D) consecutive 100-ml samples collected. In other fractions cell cycle phases were less clearly separated.

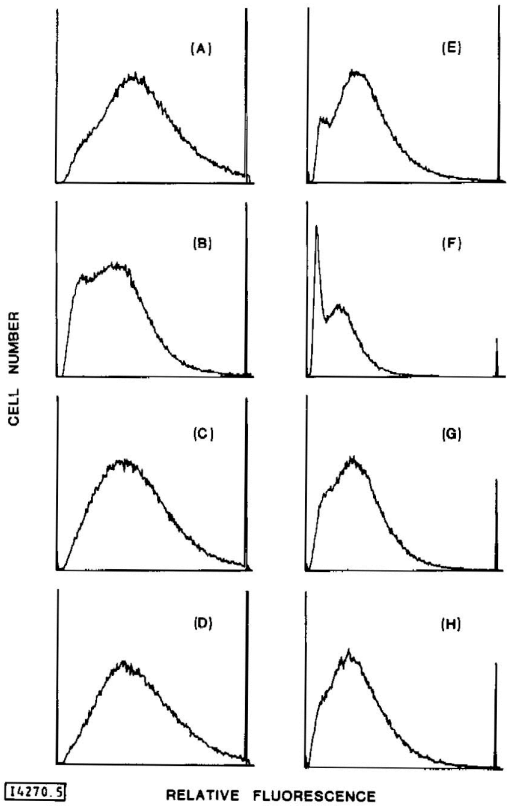

Figure 5. Surface immunofluorescence of HMy2 cells after staining with FITC-conjugated anti-human κ (A–D) or K-1-21 followed by FITC-SaMIg (E–H) as assessed by flow cytometry. The graphs correspond to those in Fig. 4 and represent unfractionated cells (A and E) or cells from the first (B, F), fourth (C, G) or fifth (D, H) fractions collected after centrifugal elutriation.

Unseparated cells and elutriated fractions were incubated with either FITC-goat anti-κ antiserum or with K-1-21 followed by FITC-SaMIg and examined by FACS analysis (Fig. 5). In each fraction, a population of nonstaining cells could be detected that did not appear to express either κ or KMA (Table 3). Fraction 1, which was enriched for cells in G_1, contained 22.6% and 35.5% negatively staining cells after treatment with anti-κ and K-1-21, respectively. Fraction 4, enriched for cells in S phase had fewer nonstaining cells, while fraction 5, which had the highest proportion of cells in $G_2 + M$, had the lowest number of unstained cells (3.6% after anti-κ, 10.5% after K-1-21). Moreover, cells in fraction 5 appeared to express the greatest density of κ or KMA epitope (Fig. 5D and 5H). Taken together these results confirmed the suggestion that both κ chain and KMA determinants are expressed at higher frequency and density on the surface of cycling rather than resting cells.

4 Discussion

K-1-21 is an IgG_1,κ murine mAb which recognizes non-heavy chain-associated human κ chains. In addition, it defines an epitope, KMA, which in an operational sense, appears to be a tumor-specific antigen with selectivity for plasma cells from patients with various types of κ myeloma [2]. In this report, the cell membrane association of KMA was examined using the human IgG,κ myeloma line HMy2, which was shown to consistently express this determinant. Particular attention was focused on its membrane mobility, relationship to surface κ chains and expression during the cell cycle, since these properties clearly require characterization if K-1-21 is to have potential value as a therapeutic agent.

The relative mobility of KMA was examined in capping experiments. Two distinct types of capping have been described. The first, which is exemplified by capping of surface Ig, is a fast, energy demanding process requiring only one type of polyvalent ligand [10] and involves activation of the cells' microfilaments [11]. Type II, which applies to determinants such as H-2 and Thy-1 [11, 12], is a relatively slow process requiring the presence of two successive ligands and takes place independently of the cell microfilaments. Capping of KMA resembled the second process, since (a) it did not occur with K-1-21 alone but depended on the presence of a second antibody with specificity for K-1-21 (Table 1) and (b) it was inhibited by the type I and II capping inhibitor NaN_3, but not by the type I inhibitors, dibucaine and calcium ionophore [13].

The fact that K-1-21 alone could not cause capping of KMA, has implications for the use of this antibody as a therapeutic agent. Thus, binding of K-1-21 to the cell surface would be unlikely to lead to modulation of KMA determinants, thereby permitting positive cells to escape immunodetection or killing as has been described for many other cell surface antigens [14–16]. However, if K-1-21 were coupled to a 'cytotoxin' [17] there is also no guaranteee that the antibody-cytotoxin complex, once localized to the target cell surface, would be internalized. This problem might be overcome by the addition of a second antibody (such as an $F(ab')_2$ anti-mouse IgG_1) to cross-link the KMA/K-1-21 complex. Alternatively, K-1-21 could be conjugated to a cytotoxin active at the cell surface [17]. Also K-1-21 mAb are of the IgG_1 isotype and do not mediate complement-mediated cytotoxicity (C. C. Goodnow, unpublished observations). Thus it might be advantageous to select an IgG_{2a} variant clone from the parent cell line by flow cytometry. Another potential problem with *in vivo* administration of murine mAb is the development of an anti-murine response by the recipient [18], which could result in immune complex formation and removal of antibody from the circulation. However, this difficulty will be minimal in the case of patients with multiple myeloma due to the well documented immunoparesis associated with this particular disease [1].

The relationship between KMA and the determinant recognized by K-1-21 on free κ chains was studied by co-capping with a range of different ligands. These experiments showed that a polyvalent antiserum for κ chains recognized determinants present on the same molecule upon which KMA is found (Table 2) and that the KMA-bearing molecule is independent of intact IgG. Thus, when membrane bound IgG was capped from the surface of HMy2 cells, subsequent surface staining with K-1-21 was unaffected and κ chains, although reduced in amount could still be detected (Table 2). By contrast, capping with a polyvalent anti-κ antiserum removed all reactivity on subsequent staining with both K-1-21 and anti-κ antiserum. One interpretation of this observation is that KMA may be an epitope on κ chains inserted as free entities in the cell membrane of myeloma cells, but absent from the membrane of normal cells. This would agree with the finding that preincubation of K-1-21 with purified κ chains blocks binding to the KMA determinant [2].

K-1-21 was shown previously to bind to a variable number of plasma cells from patients with myeloma and to stain the majority, but not all, HMy2 cells in a conventional culture [2]. This suggested that the expression of KMA might vary with the phases of the cell cycle. Tumor cells can be arrested in G_1 as they run out of nutrients [19]. HMy2 cells were thus followed in a stationary culture system through logarithmic growth and into plateau phase while cell numbers, DNA content, IgG secretion and surface and cytoplasmic expression of KMA were sequentially assessed (Fig. 3). As cell growth reached a plateau, the number of cells in G_1 increased, while the number in S phase declined. At the same time, secretion of IgG decreased, as did the surface and cytoplasmic expression of KMA. In other words, KMA appeared to be preferentially expressed on the surface of cycling cells.

The preferential expression of KMA on cycling cells was confirmed by centrifugal elutriation, which permits the examination of cells at different stages of the cell cycle without the gross changes in culture conditions associated with a stationary culture [19]. FACS analysis of fractions of HMy2 of cells enriched for G_1, $G_2 + M$ or S phase (Fig. 4) with either FITC-goat anti-κ antiserum or with K-1-21 followed by FITC-SaMIg (Fig. 5) indicated that the fraction with the greatest enrichment of G_1 cells also had the highest proportion of negative cells after staining with each antiserum. Conversely, fractions enriched for cells in S or $G_2 + M$ had much lower percentages of negatively staining cells (Table 3). Thus the expression of both κ chains and of KMA determinants was greatest on the surface of HMy2 cells during $G_2 + M$ and S. In this respect, their expression resembles that of intact Ig molecules. Kruth and co-workers [20] for example, found that surface Ig on B lymphoma cells was greater in S phase than in G_1. Thus the wide variation in K-1-21 staining observed in plasma cells from κ myeloma bone marrow aspirates [2] is probably due to the cycling characteristics of the tumor at the time of sampling rather than to a true lack of expression of the KMA determi-

nant on certain cells from the clone. This explanation is in agreement with the previous finding [3] that only 21–45% of bone marrow myeloma progenitor cells and 5.9–7.1% of myeloma cells from patients with active disease are in fact proliferating [4]. For K-1-21 to be a useful therapeutic agent either alone or coupled to a 'cytotoxin' [17, 18], it would therefore be essential to administer the antibody at a time when the cycling population is maximal, perhaps after treatment with non-cycle-specific alkylating agents, which can expand the tumor's growth fraction [21].

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