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QUANTITATION OF LIGHT CHAIN SYNTHESIS IN MYELOMA × SPLEEN CELL HYBRIDS AND IDENTIFICATION OF MYELOMA CHAIN LOSS VARIANTS USING RADIOIMMUNOASSAY[1]

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A radioimmunoassay specific for the MOPC 21 kappa (K) myeloma chain of NSI and X63 myeloma × spleen cell hybrids was used to study light chain secretion in myeloma-hybrid lines. The M1 series of rat spleen cell × NSI mouse myeloma hybrid lines was chosen to illustrate the application of the radioimmunoassay for K chain quantitation and identification of K chain loss variants. Most of these lines secrete H (specific heavy), L (specific light), and K (myeloma kappa) chains, i.e., are HLK lines. Assays specific for rat L chain and mouse K chain showed that the ratio of L/K chain secreted by 6 different hybrid HLK lines ranged from 1.1 to 12.4. Using the rapid radioimmunoassay screening procedure, HL clonal variants which had lost K chain secretion were isolated at a frequency of $\sim 10^{-2}$ and characterized. K chain loss was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of radiolabelled secreted products. Stability of one HL line and its HLK parent was examined during 9 months of growth *in vitro*. The HL line remained stable, while antibody secreted by the HLK line became inactive, apparently due to overgrowth by clonally dominant HK cells which no longer secreted specific L chains. The radioimmunoassay appears to detect MOPC 21 κ chain variable region determinants. Therefore, although used here with rat-mouse hybrids, it should also be possible to use the assay to obtain mouse-mouse variant hybrid lines secreting antibody of improved homogeneity.

INTRODUCTION

Continuous myeloma-spleen cell hybrid lines secreting monoclonal antibodies offer great advantages over classical methods of antibody production (Köhler and Milstein, 1976; Milstein et al., 1977, 1979). One of the few drawbacks of the myeloma hybrid method is that both myeloma and specific

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[2] *Abbreviations used in this paper:* H: specific heavy chain; L: specific light chain; K: P3 myeloma kappa chain; G: P3 myeloma gamma chain; BSA-TS: 0.25% bovine serum albumin, 0.01 M Tris-HCl, pH 7.8, 0.14 M NaCl; DMEM: Dulbecco's modified Eagle's medium; FBS: fetal bovine serum; GF: gluteraldehyde fixed; IEF: isoelectric focusing; MRBC: mouse red blood cells; P3: P3-X63-Ag8; PAGE: polyacrylamide gel electrophoresis; SDS: sodium dodecyl sulfate; SRBC: sheep red blood cells.

antibody chains are codominantly expressed in the myeloma-spleen cell hybrids (Köhler and Milstein, 1976; Milstein et al., 1977). For example, using the P3-X63-Ag8 (P3)[2] myeloma line as the fusion partner, myeloma γ_1 (G) and kappa (K) chains and the specific antibody heavy (H) and light (L) chains are usually all secreted. Hybrid molecules are assembled in which all permutations of heavy-light chain combinations occur and random heavy-heavy associations also occur unless the heavy chains are of differing classes (Köhler and Shulman, 1978).

Variants which have spontaneously lost synthesis of one of the antibody chains have previously been screened either by PAGE of radioactively labelled Ig chains, or in the case of anti-sheep red blood cell (SRBC) lines, by plaque-forming activity (Köhler and Milstein, 1976; Köhler et al., 1977). Each chain loss occurred with a frequency of about 2% and in a stepwise fashion in which heavy chain loss was a prerequisite before light chain loss could be found. Selection of chain loss variants is highly advantageous, both in terms of the increased purity of the antibodies and the stability of the lines. Also, loss variants are highly valuable for chromosomal assignments of heavy and light chain genes (Hengartner et al., 1978). However, screening for variants by radioactive labelling and PAGE analysis of the secreted chains of a large number of clones (Köhler and Milstein, 1976; Köhler et al., 1977) is time consuming and expensive. Furthermore, the data obtained is of a qualitative nature. The amounts of myeloma and specific light chains secreted by hybrid cells have not been previously quantitated.

This report describes a rapid radioimmunoassay for the P3 or NSI κ chain. It has been used to determine quantitatively the amount and percentage of K chains present in molecules secreted by hybrid cells, demonstrating unequal secretion of K and L chains, and to screen for K chain loss variants. The radioimmunoassay is much more sensitive than PAGE analysis for small amounts of K chain. The method was originally designed for use with rat-mouse hybrid cells, but appears equally applicable to mouse-mouse hybrids.

METHODS

Myeloma proteins, IgG, monoclonal antibodies, and cell lines

MOPC 21 (P3) IgG was the kind gift of Dr. Alan Munro. Other myeloma proteins were homogenous preparations obtained from Litton Bionetics (Kensington, MD). Rat and mouse IgG were from Cappel Laboratories (Cochranville, PA). Fab fragments were prepared by papain digestion and DEAE chromatography as described (Stanworth and Turner, 1978) and separation from Fc verified by immunoelectrophoresis. P3-X63-Ag8 (P3) and P3-NSI-Ag4-1 (NSI) were derived from an *in vitro* culture line (P3) of the MOPC 21 BALB/c myeloma tumor (Milstein et al., 1977). Properties of the M1 monoclonal lines used in this study are summarized in Table 1. The derivation and characterization of these rat anti-mouse, rat spleen cell ×

NSI myeloma hybrid cell lines has been described previously (Springer et al., 1978a,b). The suffixes H, L, or K after the cell line's name denote secretion of specific heavy, specific light, or myeloma kappa chains, respectively. The M1/87.27 HLK line is being made available to other research laboratories through the Salk Cell Distribution Center. Supernatants were obtained from cultures grown to saturation density in 10% fetal bovine serum, Dulbecco's modified Eagle's medium (10% FBS-DMEM). In cases where HLK and HL variants were directly compared, cultures were initiated at identical cell densities between 10^4 and 10^5 cells/ml and grown in parallel for identical lengths of time.

Antisera

Rabbits were immunized to P3 IgG and rat Fab by 3 monthly injections of 1 mg in complete Freund's adjuvant distributed to multiple intramuscular sites. Bleeds were taken biweekly thereafter for several months.

^{125}I-labelled antibodies

Rabbit anti-P3 (MOPC 21) IgG serum was adsorbed by passing 9 ml through a 3.5 ml column of rat serum coupled to Sepharose CL 4B (Pharmacia) (19 mg protein/ml settled beads) ([^{125}I] anti-P3 Fab). A 10 μl bed of 2 mg P3 Fab or rat Fab/ml Sepharose in a column made from a 46 mm × 5.7 mm Sarstedt microfuge tube was saturated with 0.2 ml of absorbed rabbit anti-P3 IgG or 0.4 ml rabbit anti-rat Fab serum, respectively. As described elsewhere (Miles and Hales, 1968; Herzenberg and Herzenberg, 1978), antibodies were iodinated with 1—1.5 mCi of Na^{125}I while bound to the column, and eluted with glycine-HCl pH 2.3 buffer.

Target cells

Glutaraldehyde fixed (GF) sheep or mouse red blood cells (S or MRBC) were prepared as described (Williams, 1973), suspended in 10% BSA at 10^9/ml, and could be stored for at least 1 year (and probably much longer) at −30° C.

P3 K chain inhibition assay

Aliquots (10 μl) of clonal supernatants, myeloma proteins, or IgG to be tested for P3 K chain content, as well as P3 (MOPC 21) IgG standard, were 5-fold serially diluted in 0.25% bovine serum albumin (BSA), 0.01 M Tris-HCl pH 7.8, 0.14 M NaCl (BSA-TS) and 10 μl aliquots were placed in V well polystyrene microtiter plates (Linbro) (if increased sensitivity were desired, aliquots of up to 100 μl could be used). ^{125}I-anti-P3 Fab (5 μl, 5 -10,000 cpm/μl) was added using a repeating dispenser syringe (Hamilton), the plates

sealed with tape (Cooke) and shaken for 45 min at 4°C (Microshaker II, Cooke). During this incubation period, GF SRBC were coated with M1/87.27. M1/87.27 spent culture supernatant (0.2 vol) was added during vortexing to 1 vol of 10^9/ml GF SRBC. This was allowed to stand at room temperature for 20 min or longer, washed twice with 10 vol of BSA-TS immediately before use, and resuspended to 10^9 cells/ml. Aliquots (5 μl) of M1/87.27 coated GF SRBC were added as above, the plates resealed with tape, and shaken for a further 45 min at 4°C. Cells were then washed thrice by addition of 200 μl BSA-TS, centrifugation for 5 min at 200 × *g*, and aspiration of the supernatant. They were suspended in 150 μl of BSA-TS and transferred to tubes for γ-counting.

Subcloning

Soft agar (0.3%) cloning of 1000 cells/100 mm petri dish was carried out as previously described (Springer et al., 1978b), and agar plugs containing single clones transferred to 96 well microculture plates (Costar) containing 0.2 ml medium/well. Clones grew at slightly differing rates. After some clones grew sufficiently to lower the pH of the medium, the medium in each culture was replaced every 2 days, and at least three changes were made before 10 μl aliquots of spent culture medium were assayed for P3 K chain content.

Mancini radial immunodiffusion

Diffusion was carried out as described (Ouchterlony and Nilsson, 1978) in agar containing rabbit anti-rat Fab at a concentration (100 μl/20 ml) yielding rings 8.5 mm in diameter with 0.8 μg of rat IgG. Ring diameter was measured under indirect illumination. Other methods were as described previously (Springer et al., 1978b).

RESULTS

A P3 K chain radioimmunoassay was designed as follows. Inhibitor substances hypothesized to contain the P3 K chain would first be incubated with the second layer reagent, rat IgG-absorbed ^{125}I-rabbit anti-P3 Fab (^{125}I-anti-P3 Fab). As a target for the unbound ^{125}I-anti-P3 Fab, cells coated with first layer HLK monoclonal antibody (containing the P3 K chain) would be added. Thus P3 K chain would block the ^{125}I-anti-P3 Fab from binding to the first layer antibody on the coated cells, which would be measured as a decrease in the cpm bound to washed cells. Absence of the P3 gamma (G) chain from the first layer HLK antibody renders the anti-P3-Fd component of the anti-P3 Fab antibodies unreactive in this assay system.

In preliminary experiments, a number of anti-cell surface monoclonal antibodies secreted by the M1 series of rat spleen cell-NSI myeloma hybrids (Table 1 and Springer et al., 1978b) were compared for their efficiency as

TABLE 1

Rat monoclonal antibodies to mouse differentiation antigens secreted by the M1 rat spleen cell X mouse NSI myeloma hybrid cell lines (Springer et al., 1978b).

Clone	Antibody class (Springer, 1980)	Cellular recognition	Antigen
M1/87	IgM	Sheep RBC but not MRBC	Forssman glycosphingolipid
M1/22.25	IgM	Mouse teratocarcinomas, minor cell subpopulations and early embryos (Stern et al., 1978)	
M1/75	IgG2c	MRBC, not thymocytes	Heat stable, no iodinated component
M1/69	IgG2b	MRBC and most leukocytes	
M1/22.54	IgG2c	Thymocytes but not peripheral T cells	
M1/89.1	IgG2b		
M1/9.47	IgG2b		
M1/9.3	IgG2a	Leukocytes	210,000 mol. wt. [a]
M1/89.18	IgG2b		
M1/70	IgG2b	Phagocytes (Springer et al., 1979)	190,000 mol. wt. [a] 105,000 mol. wt.

[a] Determined by SDS-PAGE after reduction.

first layer antibodies in the indirect binding assay. These lines all secrete rat antibodies to mouse cell surface differentiation antigens, and the two anti-Forssman antigen clones, M1/22.25 and M1/87.27, also cross-react with sheep red blood cells (SRBC). All lines had previously been typed for L and K chain secretion by IEF and SDS-PAGE (Springer et al., 1978b). The M1/22.25 anti-Forssman IgM was inactive in binding the ^{125}I-anti-P3 Fab reagent, but bound ^{125}I-anti-rat Fab, confirming its typing as a spontaneously arising HL variant (Table 2, see legend for details of assay). M1/87.27, the other anti-Forssman IgM, bound both reagents, confirming its typing as an HLK. The input ^{125}I-anti-P3 Fab was 28% active in binding to M1/87.27 coated cells. Five HLK monoclonal antibodies of the IgG class recognizing a heat stable antigen on MRBC were also tested. They were considerably less effective than M1/87.27 as a first layer for ^{125}I-anti-P3 Fab.

Having chosen the M1/87.27 HLK IgM for the first layer, the cellular radioimmunoassay was tested in the inhibition mode (see Methods for details). Serial dilutions of antigen were preincubated with ^{125}I-anti-P3 Fab and tested for their ability to inhibit binding of the ^{125}I-anti-P3 Fab to M1/87.27 HLK coated SRBC (Fig.1). Calibration with P3 IgG showed 50% inhibition was given by 0.3 μg/ml in 10 μl, or 3 ng. Strikingly, mouse

TABLE 2

Efficiency of different monoclonal antibodies as the first layer in an indirect ^{125}I-anti-P3 Fab cell binding assay. Target cells (5 μl of 5 X 10^7/ml) were incubated with 10 μl of spent culture medium containing the monoclonal antibodies, washed, incubated with ^{125}I-anti-P3 Fab (5 μl containing 5 X 10^4 cpm), washed, and γ emissions were counted. Details of incubations and washings were as described in Materials and Methods.

Monoclonal antibody	Target cell	Target antigen	^{125}I-anti-P3 Fab bound (cpm X 10^{-2})	^{125}I-anti-rat Fab bound (cpm X 10^{-2})
M1/87.27.7 HLK	SRBC	Forssman	143	200
M1/22.25.8 HL	SRBC	Forssman	2	198
Control [a]	SRBC	Forssman	1	6
M1/69.16.2 HLK	MRBC	Heat stable antigen	12	ND [b]
M1/69.16.11 HLK	MRBC	Heat stable antigen	21	ND
M1/89.1.5 HLK	MRBC	Heat stable antigen	13	ND
M1/22.54.4 HLK	MRBC	Heat stable antigen	27	ND
M1/9.47.8 HLK	MRBC	Heat stable antigen	17	ND
M1/75.21.4 HLK	MRBC	Heat stable antigen	6	ND
Control[a]	MRBC	Heat stable antigen	2	ND

[a] R5/18.2, an irrelevant HLK rat monoclonal antibody.
[b] ND: not done.

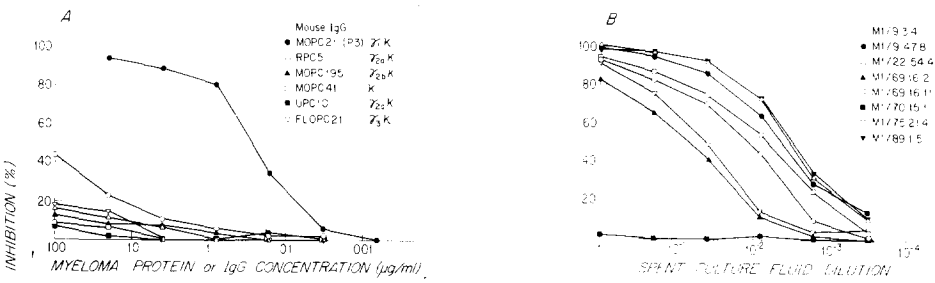

Fig. 1. Characteristics of the P3 light chain assay. A: Inhibition by MOPC 21 (P3), other myeloma proteins containing κ chains, and mouse IgG. B: Inhibition by products secreted in tissue culture by mouse myeloma-rat spleen cell hybrids. Serial dilutions of inhibitors were mixed with ^{125}I-anti-P3 Fab and inhibition of binding to M1/87.27 HLK sensitized SRBC was measured as described in Methods.

IgG was 500-fold less inhibitory than P3 IgG. Furthermore, 5 different κ chain containing mouse myeloma proteins gave no or extremely little inhibition (Fig.1A). This suggests that the assay recognizes K chain V_L determinants expressed on 1/500 of the normal mouse IgG population. Thus, the assay appears useful for detection of P3 K chain loss variants in mouse-mouse as well as rat-mouse hybrids.

Clonal supernatants from the 8 different M1 hybrids which do not cross-react with SRBC were next tested in the inhibition assay (Fig.1B). M1/70

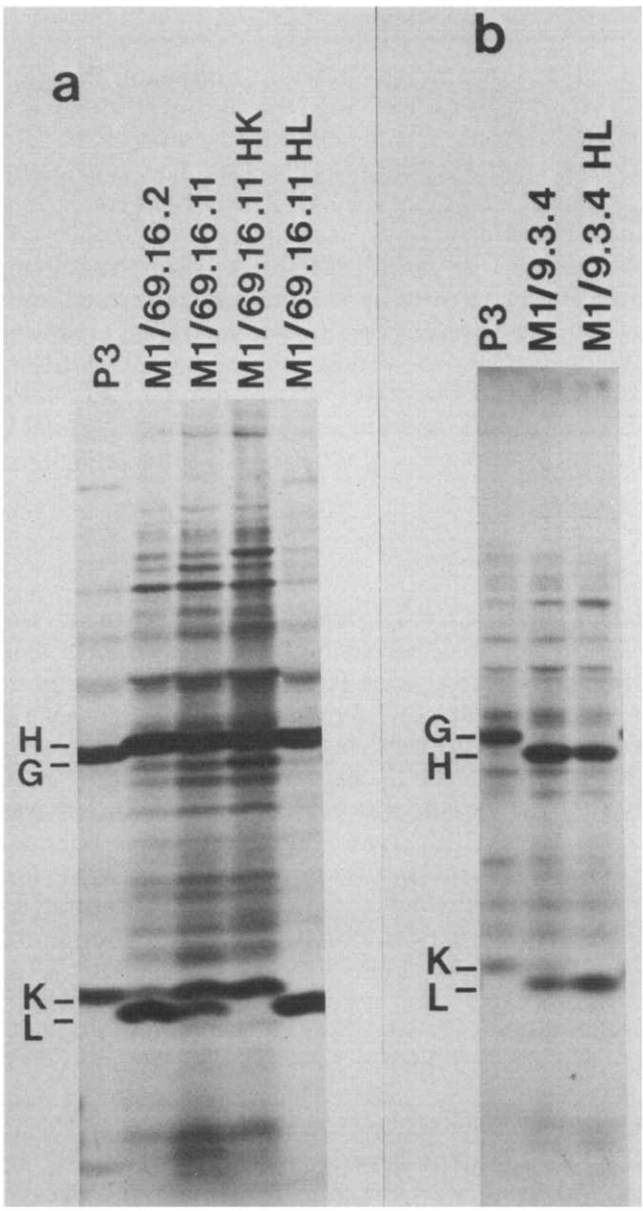

Fig. 2. SDS-PAGE of internally labeled secreted clonal products. A: Clones were labeled with [^{14}C]leucine (0.05 μCi) and supernatants (10 μl) electrophoresed on 5—15% gradient polyacrylamide gels which were dried and exposed to Kodak XR-5 film for 55 days. M1/69.16.11 had grown in culture 7 months, was frozen, and then thawed at 9 months for labelling; M1/69.16.11 HL and M1/69.16.11 HK (a clonally dominant loss variant of M1/69.16.11) had grown continuously in culture 9 months. B: Clones were labeled with [^{3}H]leucine (0.25 μCi) and supernatants (10 μl) electrophoresed on 8—15% polyacrylamide gels, prepared for fluorography as described (Laskey and Mills, 1975) and exposed to pre-flashed film for 65 days. Only the relevant portion of each gel is shown.

was found not to secrete K chain, confirming a previous suggestion that this clone is a spontaneous HL variant (Springer et al., 1978b). Seven other M1 clonal supernatants had concentrations of K chain varying over a 25-fold range and exhibited similarly shaped inhibition curves. All had previously been thought to contain K chain except M1/69.16.2. No K chain in the products of M1/69.16.2 had previously been seen in IEF or SDS-PAGE autoradiograms (Fig. 3 of Springer et al., 1978b). A sister subclone, M1/69.16.11, was previously found to secrete K chain, as confirmed here (Fig. 1B), in addition to L chain. The present results demonstrate a quantitative difference between subclones 2 and 11, rather than a qualitative difference as previously thought. M1/69.16.2 secretes ~ 1.5 μg/ml of K chain, 10-fold less than M1/69.16.11 (Fig. 1B), and trace quantities of M1/69.16.2 K chain are seen after prolonged exposure of SDS-PAGE autoradiograms (Fig. 2A).

Screening for HL variants

Two HLK lines, M1/69.16.11 and M1/9.3.4, were chosen to illustrate the use of the radioimmunoassay in obtaining K chain clonal loss variants. The M1/69.16.11 line was subcloned in soft agar at 1000 cells per 100 mm petri dish. After transfer of clones in agar plugs to 0.2 ml culture wells and several medium changes, which were essential to remove K chain secreted into the agar, culture supernatants were tested in the K chain inhibition assay (Fig. 3). Of 191 clones tested, only one HL variant was found. The HL variant was clearly separated by the assay from HLK clones, which showed a bimodal distribution. Microscopic examination of the HLK clones which gave full inhibition (500—1000 cpm bound) showed they had reached saturation density, while HLK clones which gave partial inhibition (2000—5000 cpm) had not. The HL variant (8000 cpm) had reached saturation density. After further growth, inhibition assays confirmed absence of K chain. Antigen-binding activity was demonstrated in indirect ^{125}I-anti-rat Ig binding assays, in which M1/69.16.11 HL exhibited a higher titer than its M1/69.16.11 parent.

The stability of M1/69.16.11 and its HL variant were compared during growth in tissue culture for 9 months. At various time points, [^{3}H] or [^{14}C] leucine was incorporated into Ig chains, and cells were also frozen in liquid nitrogen. After 6 months, a dramatic decline in the ratio of L to K chain was noted in autoradiograms of M1/69.16.11. After 7 months, this trend had reversed somewhat, since K chain was only slightly more intense than L chain (Fig. 2A). However, after 8 months, and again at 9 months, all traces of L chain secretion disappeared, and the line was designated M1/69.16.11 HK (Fig. 2A). M1/69.16.11 HK had also lost all activity in the ^{125}I-anti-rat IgG indirect binding assay. However, M1/69.16.11 HL which had been grown for the same length of time showed no loss in activity or L chain secretion (Fig. 2A).

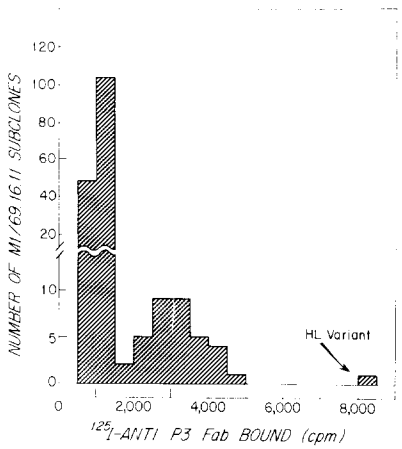

Fig. 3. Identification of an HL variant subclone of M1/69.16.11 using the P3 K chain inhibition assay. The M1/69.16.11 subclone had grown in culture for 2.5 months with intervening storage in liquid nitrogen for 11 months before further subcloning and screening using the P3 K chain inhibition assay was carried out as described in Methods.

The results of M1/9.3.4 screening were similar to those for M1/69.16.11. Initially, 10 microculture well clones and 101 agar clones were assayed and no HL variants found. Further subcloning was carried out, and one HL variant identified among another 116 agar subclones. The HL variant had about 2-fold more activity than the HLK parent in the ^{125}I-anti-Ig binding assay, and loss of the K chain was confirmed by SDS-PAGE analysis (Fig. 2B). The M1/9.3.4 parent clone secretes small amounts of K relative to L chain (Fig. 2B) as reported previously (Springer et al., 1978b), and was designated with the lower case k as an HLk clone. Thus, the obtainment of the M1/9.3.4 HL variant validates the K chain radioimmunoassay for loss variant selection even from clones secreting only low levels of K chain.

K and L chain quantitation in M1 rat-mouse myeloma hybrids and chain loss variants

The amount of L and K chain secreted by the different M1 HLK and HL hybrids, and the total amount of IgG secreted (Table 3) were determined as follows. Mancini radial immunodiffusion of the M1 monoclonal antibodies against rabbit anti-rat Fab was used to measure L chain. The anti-Fab was specific for rat L chain, since (1) it was unreactive in both immunodiffusion and ^{125}I-anti-rat Fab binding assays with M1/89.18, a rat IgG2b which appears to be an unusual example of an HK antibody retaining antigen-binding activity, and (2) it was unreactive with P3 IgG in immunodiffusion. The results quantitatively confirm that light chain secretion in a number of the lines is unbalanced. The most outstanding example is M1/75.21.4 HLk, which makes 12 times more L than K chain. A number of lines secrete

TABLE 3

Chain content of antibodies secreted by M1 monoclonal lines in tissue culture.

Monoclonal antibody		Rat specific light chain [a] (μg/ml)	Mouse MOPC 21 kappa chain [b] (μg/ml)	Total IgG [c] (μg/ml)
M1/9.3.4	HLk	52	8	182
M1/9.3.4	HL	78	<0.1	235
M1/69.16.11	HLK	73	14	260
M1/69.16.11	HL	84	<0.1	252
M1/9.47.8	HLK	33	22	164
M1/22.54.4	HLK	31	28	178
M1/70.15.1	HL	17	<0.1	50
M1/75.21.4	HLk	26	2.1	83
M1/89.1.5	HLK	42	28	210

[a] Measured by Mancini radial immunodiffusion against rabbit anti-rat Fab.
[b] Measured in the standard P3 K chain inhibition assay.
[c] Calculated as (L chain + K chain) × 75,000/25,000.

similar amounts of K and L chain, but none secretes more K than L. The results also confirmed loss of K chain secretion in the M1/9.3.4 HL and M1/69.16.11 HL variants. It is unlikely that K chain secretion was merely altered in a quantitative fashion in the variants, because the assay is sensitive to 100 times less than the original level of K chain secretion. Furthermore, the level of L chain secretion was increased in the HL variants. Using the combined K and L chain values, it was found that the total IgG concentrations of the spent culture supernatants ranged from 50 to 260 μg/ml.

DISCUSSION

Numerous hybrid lines have been produced by laboratories throughout the world using the P3-X63 and NSI myeloma lines. Much effort has been devoted to the characterization and description of the properties of particular lines. A drawback of many of these lines is that myeloma chains are secreted in addition to the specific antibody chains. Thus, methodologies of screening for myeloma chain loss variants are of considerable importance, despite the recent introduction of 'fusoma' lines in which hybridization does not reactivate myeloma chain secretion (Köhler and Shulman, 1978).

In this report, a rapid cellular radioimmune assay for the P3 myeloma K chain was described. It is based on the ability of K chain antigen to inhibit ^{125}I-anti-P3 Fab from binding to M1/87.27 HLK IgM sensitized SRBC. M1/87.27 was superior to a number of other anti-RBC HLK antibodies in acting as a bridge between cells and ^{125}I-anti-P3 Fab, probably because of its IgM structure. Specificity of the ^{125}I-anti-P3 Fab for the P3 K chain was divorced from that for the Fd domain, since only the former is present in M1/87.27 HLK antibody. Use was thereby avoided of purified P3 K chain,

which in the absence of P3 γ_1 chain aggregates in physiological buffers (C. Milstein, personal communication).

The assay was initially developed for use with rat-mouse hybrids. However, mouse IgG was found to be only 0.2% as inhibitory as P3 IgG in the assay, suggesting that the K chain reactivity of the rat IgG-absorbed rabbit anti-P3 serum is directed to mouse V_L determinants. Specificity for mouse V_L determinants which are not dependent on association with a particular heavy chain has also been reported for V_L subgroup-specific antibodies (Weigert and Riblet, 1978). Absorption of anti-mouse C_L activity by rat IgG is not surprising in view of the similarity between the C_L regions of these species (Starace and Querinjean, 1975). The assay system appears appropriate for detecting K chain loss variants in 99.8% of mouse-mouse NSI or P3-X63 myeloma hybrids, and it is possible that absorption with mouse IgG would raise this percentage even higher. Furthermore, the design of the assay could easily be adapted for use with the K chains of other fusoma myelomas such as MPC-11, with which anti-SRBC hybrids have already been produced (Diamond et al., 1978). With the substitution of ^{125}I-anti-rat Fab, the assay can also be used to measure rat L chain at the 1 ng level. However, it is more convenient to measure higher rat L chain concentrations with Mancini radial immunodiffusion.

The radioimmunoassay is much quicker for screening hundreds of clones than the previously used methods of SDS-PAGE or IEF or radioactively labelled secreted Ig, but the latter remain useful as confirmatory techniques for selected clones. Using the cellular radioimmunoassay, the supernatants from several hundred clones can be drawn and assayed in a single day. Another advantage of the radioimmunoassay is its greater sensitivity. Previously using SDS-PAGE and autoradiography, K chain secretion by M1/69.16.2 of about 1.5 μg/ml was missed, while secretion of about 2.1 μg/ml by M1/75.21 was identified. The sensitivity of radioactive chain analysis therefore appears to lie somewhere in this range, although it can be somewhat increased by prolonged autoradiogram exposure. The immunoassay is sensitive to 1 ng of K chain at a concentration of 0.1 μg/ml, and if desired, this could easily be increased to about 0.01 μg/ml by using 100 instead of 10 μl aliquots. A major qualitative advantage of the radioimmunoassay is in the case of L and K chain comigration in SDS-PAGE and/or IEF.

A disadvantage of the immunoassay is that since P3 K chain secretion does not occur in the absence of heavy chain (Milstein et al., 1977), in HLK cells both H and K chain loss have the same effect on the P3 K chain radioimmune inhibition assay (Table 4). Therefore, antigen-binding assays have also been used, and the combination of both assays distinguishes between all three different types of chain loss events from NSI or P3-derived HLK cells (Table 4). The only other disadvantage is the necessity of preparing the anti-P3 serum and absorbing it with rat or mouse IgG.

Specific light chain secretion was also quantitated in this study. The total concentration of antibody was calculated from L and K chain data and is

TABLE 4

Combined use of K chain and antigen binding assays discriminates all classes of chain loss variants arising from HLK NSI-spleen hybrid cells.

Nature of chain loss	Intracellular synthesis	Ig secretion phenotype[c]	K chain assay inhibition	Antigen binding assay
None	HLK	HLK	+	+
−K	HL	HL	—	+
−L	HK	HK	+	—[b]
−H	LK	L[a]	—	—

[a] There may be rare cases in which H chain expression is required for L chain secretion; however this will not affect the radioimmunoassay results.
[b] There may be rare cases in which K chain complements antigen binding activity.
[c] Milstein et al. (1977).

quite high, 50—150 μg/ml of spent culture fluid depending on the cell line. Few detailed estimates of the antibody concentration secreted by myeloma hybrid cells into culture medium have previously been reported. Galfré et al. (1979) suggested 1—20 μg/ml, but do not indicate on what data this is based, while Andersson and Melchers (1978) reported 500 μg/ml using spleen-NSI hybrids. A rat-mouse hybrid formed using the S194/5.XXO.BU.1 nonsecreting myeloma line was found to secrete only 1—2 μg of rat antibody/ml (Trowbridge, 1978). Although this value was subject to some inaccuracy because inhibition of an anti-rat IgG rather than anti-rat Fab was assayed, it raises the possibility that the concentration of secreted antibody may depend on the nature of the myeloma fusion partner.

Because the M1 series of hybrid lines secrete rat L chains with species-specific markers and mouse myeloma K chains with V_L-specific markers, they are an excellent model system for studying the relative amounts of specific and myeloma light chains secreted by hybrid cells. The amounts of specific and myeloma light chains secreted by hybrid cells had not previously been quantitated. A number of the M1 lines studied here were found to have considerably greater quantities of L than K chain secretion, up to 12-fold more. This was not due to contamination of HLK cultures with HL variants, as shown by the finding of only 1 HL variant among 217 subclones of the M1/9.3.4 HLK line. Nor can it be accounted for by secretion of free L chains, since this is incompatible with the amounts of radioactive lysine (Springer et al., 1978b) and leucine (this study) incorporated into the H and L chains. Köhler and Shulman (1978) have described a hybrid cell synthesizing both IgM and IgG2b antibodies, in which the γ2b but not the μ chain exhibits preferential association for its homologous light chains. In some but not all of the lines studied here, preferential association of the H chain for its homologous L chain might occur, along with intracellular degradation (Milstein et al., 1977) of unassembled K chains. This could explain why some lines secreting more L than K, but

none secreting more K than L, were found in the present study. If so, this raises interesting question about the selection of H and L partners in normal cells. Another possible explanation for K and L inequality would be unequal synthesis. Subclones differing in their ratios of K and L secretion would provide an interesting model system. While it appears that M1/69.16.2 and M1/69.16.11 studied at a number of points in time differ in their ratios of L and K secretion, this requires further investigation because HK variants within the cultures could have contributed to the observed differences.

Stable secretion of active antibody is essential to the usefulness of myeloma-hybrid lines. Hengartner et al. (1978) have shown that loss of chain expression in mouse myeloma-mouse spleen cell hybrids is correlated with loss of a single copy of chromosome 12 (heavy chain) or 6 (kappa chain). The importance of regular recloning and activity screening in preventing overgrowth by inactive chain loss variants has previously been stressed (Springer et al., 1978b; Milstein et al., 1979). This type of instability was noted in this study during growth in tissue culture of the M1/69.16.11 HLK line. An increase in the ratio of K to L chains occurred after 6 months, and by 8 months all L chain secretion had disappeared, suggesting a faster-growing HK variant had arisen and become clonally dominant. However, no tendency to lose L chain was noted in the M1/69.16.11 HL variant subclone during growth for this same period of time. Köhler et al. (1977) have proposed that HL (or HK) lines are more resistant to light chain loss than HLK lines due to the toxicity of free normal H chains to the cells. This notion is also supported by work on the isolation of L chain loss variants in the MPC-11 myeloma line (Morrison, 1978). No variants secreting normal H chains in the absence of L chain were ever found, although L chain loss variants could be isolated from mutant cells secreting abnormal H chains. However, it should also be pointed out that rare instances of myeloma lines which can give rise to variants secreting normal H chains in the absence of L chains have been reported (Bailey et al., 1973; Morrison and Scharff, 1975).

The major advantage of HL over HLK lines is the homogeneity of the antibody. An increase in L chain secretion comparable to the loss in K chain secretion was seen in the HL variant lines studied here. Thus an equivalent amount of total antibody is secreted, but it is a pure population of bivalently active $H_2 L_2$ IgG rather than a mixture of $H_2 L_2$, $H_2 LK$, and $H_2 K_2$ molecules.

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Note added in proof. Recently, a modified solid-phase radioimmunoassay was successfully used for isolation of HL variants (M. Ho and T. Springer). Again, it was found that

an IgM HLK was more efficient than IgG HLKs. Soft microtiter plates were coated with a purified IgM HLK (0.4 μg/well). Cloned supernatants (50 μl) were added, then during shaking of the plate ^{125}I-anti-P3 Fab was added. Other procedures were as described (Tsu and Herzenberg, 1980). Hl variants gave no inhibition of binding of the ^{125}I-anti-P3 Fab to the wells.

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