Molecular Immunology Vol. 19, No. 11, pp. 1415–1423, 1982.
Printed in Great Britain.

0161-5890/82/111415-09$03.00/0

INDUCTION OF 19S IgM SECRETION IN A MURINE PRE-B CELL LINE, 70Z/3, BY CELL HYBRIDIZATION WITH NON-SECRETING MYELOMA CELLS*

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(*First received* 25 *August* 1981; *accepted in revised form* 5 *March* 1982)

Abstract—Somatic cell hybrids were prepared between the TK-deficient variant of murine pre-B cell line, 70Z/3, cells and the HGPRT-deficient variant of non-secreting myeloma cells. Several hybrid clones which secreted IgM but did not express surface IgM were isolated. LPS stimulation did not induce the expression of surface IgM. SDS–PAGE analysis indicated that the IgM secreted by one of the hybrid clones was a 19S pentamer and that the size of its μ-chains was the same as that of μ-chains from MOPC 104E myeloma IgM. Two-dimensional gel electrophoresis of biosynthetically labeled immunoglobulin showed that the same pattern was obtained with κ-chains from two hybrid clones and from the LPS-induced 70Z/3 cells. The result showed that cell hybridization could induce L-chain synthesis in the pre-B cell line. Anti-idiotypic antibody against the secreted IgM was prepared and it was shown that the surface IgM expressed on all LPS-stimulated 70Z/3 cells bore the same idiotype. Those results indicated that the specificity commitment has already occurred in 70Z/3 cells.

INTRODUCTION

Sensitive immunofluorescent techniques have been employed to demonstrate small amounts of intracellular IgM in the first identifiable members of the B-cell lineage, termed pre-B cells (Raff *et al.*, 1976). It has recently been reported that light (L) chains can not be detected in pre-B cells using immunofluorescent techniques (Levitt & Cooper, 1980), while the presence of μ-chains within these cells can be easily demonstrated. An asynchronous onset of H- and L-chain synthesis in pre-B cells has been confirmed in fetal liver hybridomas (Burrows *et al.*, 1979) and in murine pre-B cell lines transformed with Abelson leukemia virus (Boss *et al.*, 1979; Siden & Baltimore, 1979), both of which synthesize only μ-chains. It has also been shown that malignant cells from patients with pre-B cell leukemia contain intracellular μ-chains without L-chains (Vogler *et al.*, 1981). However, the physiological significance of an asynchronous onset of immunoglobulin chain synthesis in the generation of antibody diversity is not known.

The murine pre-B cell like cell line, 70Z/3, has been shown to contain intracellular μ-chains but no L-chains (Paige *et al.*, 1978). LPS stimulation of these cells induced *de novo* synthesis of κ-chains and the expression of surface IgM (Paige *et al.*, 1978; Sakaguchi *et al.*, 1980). Several investigators have shown that hybridization of pre-B or B-cells with non-secreting myeloma cells is capable of inducing immunoglobulin production (Riley *et al.*, 1981; Laskov *et al.*, 1979; Schwaber & Rosen, 1978; Levy & Dilley, 1978). In the present study, the secretion of IgM by (70Z/3 × P_3U_1) hybrid cells was investigated in an attempt to determine whether the parent 70Z/3 cells are already committed to a particular L-chain specificity.

MATERIALS AND METHODS

Cell lines

The murine pre-B cell like tumor cell line, 70Z/3, was originally obtained from Dr C.

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§ Abbreviations: LPS, lipopolysaccharide; BudR, bromodeoxyuridine; HAT, hypoxanthine, aminopterin, thymidine; PEG, polyethylene glycol; DMSO, dimethylsulfoxide; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TK, thymidine kinase; HGPRT, hypoxanthine guanine phosphoribosyl transferase; BPB, bromophenolblue; NEPHGE, non-equilibrium pH gel electrophoresis.

Paige (Sloan-Kettering Institute for Cancer Research, Rye, New York). The characterization of the cells has been described by Paige *et al.* (1978) and by us (Sakaguchi *et al.*, 1980). 70Z/3 (BudR^R) was a mutant of 70Z/3 cells that could proliferate in the presence of 30 μg/ml of 5-BudR, but could not proliferate in a selective HAT medium containing hypoxanthine, aminopterin and thymidine. P3/X63-Ag8 (X63) is an 8-azaguanine-resistant mutant of MOPC 21 cells secreting IgG (γ_1, κ) as described by Köhler & Milstein (1975). P3/X63-Ag8U$_1$ (P$_3$U$_1$) is a mutant of X63 cells that was provided by Dr Scharff (Albert Einstein College of Medicine, New York) and secreted neither γ_1- nor κ-chains. Tumor cell lines and hybrid cell lines were maintained in RPMI 1640 medium containing 10% fetal calf serum (FCS, Centaurus Biol. Corp.), 5 m*M* glutamine, antibiotics and 5×10^{-5} *M* 2-mercaptoethanol (2-ME).

Somatic cell hybridization

70Z/3 (BudR^R) cells were hybridized with P$_3$U$_1$ cells with PEG-4000 (Wako Pure Chem. Ind.). Cell pellets of 1×10^7 70Z/3 (BudR^R) cells and 5×10^7 P$_3$U$_1$ cells were resuspended in 0.5 ml of 45% PEG-4000 and 15% DMSO in Dulbecco's modified Eagle's medium. After 1 min at 37°C, the cell suspension was gradually diluted to 10 ml over a period of 5 min with pre-warmed serum-free MEM. The cells were then centrifuged down, resuspended in the culture medium with murine thymocytes as feeder cells and dispensed into 84 wells in a Falcon tissue culture plate (Falcon No. 3042). After 24 hr, a half-volume of the culture medium (100 μl) was replaced with the HAT medium and the medium was changed every second day. Aminopterin was omitted from the HAT medium after 14 days and hybrid clones were grown in ordinary tissue culture conditions after 3–4 weeks. Cloning of hybrid cells was performed in 0.3% soft agar (Difco, Detroit, Michigan) containing McCoy's complete medium as described by Kincade *et al.* (1978).

Karyotype analysis

Karyotypes of hybrid cells were analysed by the method of Klinger (1972).

Measurement of secreted IgM

The amount of IgM secreted into the culture medium was measured by a solid-phase radioimmunoassay as described elsewhere (Kuritani *et al.*, 1979). Monospecific anti-IgM was prepared by immunization of rabbits with purified MOPC 104E protein and the antiserum was rendered monospecific by absorption with X5563 myeloma protein (γ_{2a}, λ)–Sepharose.

Detection of surface IgM

Surface IgM was detected by direct immunofluorescence with FITC-labeled F(ab')$_2$ fragments of rabbit anti-IgM.

SDS–PAGE analysis of IgM secreted by hybridomas

Two million hybrid cells were suspended in 2 ml of the culture medium containing 50 μCi of a ^{14}C-amino acid mixture (112–501 μCi/μmole specific activity, New England Nuclear, Boston, Massachusetts). After 8 hr incubation at 37°C, the culture supernatant was recovered, dialysed extensively against PBS and then incubated with anti-IgM antiserum for 18 hr at 4°C. Antigen–antibody complexes were precipitated with *Staphylococcus aureus* Cowan I (Calbiochem. Behring Corp., La Jolla, California) according to the method of Kessler (1975). Precipitates were washed 3 times with a 0.05% NP solution (0.05% NP-40, 50 m*M* Tris–HCl, pH 7.4, 150 m*M* NaCl, 5 m*M* EDTA and 0.02% NaN$_3$) and were analysed by SDS–PAGE in a reduced or non-reduced condition. In the reduced condition, the sample was suspended in a buffer containing 2-ME (60 m*M* Tris–HCl, pH 6.8, 5% 2-ME and 2% SDS), boiled and analysed on an 8% polyacrylamide gel according to the method of Laemmli (1970). In the non-reducing condition, the sample was suspended in a buffer without 2-ME (2% SDS and 10 *M* urea) and analysed on a 2% polyacrylamide gel containing 0.5% agarose as described by Dingman & Peacock (1968). As markers, IgM (MOPC 104E), the μ-chain from MOPC 104E, the γ-chain from X63 myeloma protein and the L-chain from MOPC 104E were employed.

Two-dimensional (2D) gel electrophoresis of biosynthetically labeled μ- and κ-chains

Biosynthetic labeling of IgM and IgG$_1$ was performed by incubating 4×10^6 hybrid cells, X63 myeloma cells or 4×10^7 70Z/3 cells, which had been stimulated with 5 μg/ml LPS for 20 hr, with 200 μCi [^{35}S]methionine for 6 hr. Labeled cells were lysed and immunoprecipitated with anti-μ-chain or anti-γ_1-chain

antibody and *S. aureus* Cowan I according to the method described by Siden & Baltimore (1979) with some modification. 2D gel electrophoresis was performed as follows. First-dimension electrophoresis was performed using the NEPHGE methods described by O'Farrell *et al.* (1977). Approximately 10^5 cpm of radiolabeled samples were applied onto disk gels that contained 9.2 *M* urea, 3.8% acrylamide, 2% NP-40, and 2% ampholine (pH 3.5–10). After electrophoresis at 3000 V-hr, gels were incubated with an SDS buffer that contained 0.0625 *M* Tris–HCl, pH 6.8, 10% glycerol, 5% 2-ME and 2.3% SDS. After incubation for 1 hr, gels were placed horizontally across the 9% SDS–polyacrylamide slab gels sealed with 1% agarose and run in the normal way. After electrophoresis, gels were treated with ENHANCE (New England Nuclear) for autofluorography, dried and exposed to Kodak AR film for 2–7 days at −80°C.

Purification of monoclonal IgM and IgG_1 and separation of H- and L-chains

Ascites was collected from BDF_1 mice bearing hybridomas $G_{10}B_4$ or B_4B_3. The concentration of IgM in ascites was approximately 1 mg/ml and it showed a monoclonal band in an agar gel electrophoresis. IgM was isolated by 50% ammonium sulfate precipitation, gel filtration on a Sephadex G-200 column and zone electrophoresis as described previously (Onoue *et al.*, 1967). Separation of H- and L-chains was carried out by a method described previously (Kishimoto & Onoue, 1971). Briefly, IgM was reduced with 5 m*M* dithiothreitol for 2 hr at room temperature and then alkylated with 20 m*M* monoiodoacetamide for 30 min at 4°C. Reduced and alkylated IgM was dialysed against 1 *M* propionic acid and applied to a column of Sephadex G-100 equilibrated with 1 *M* propionic acid. IgG_1 myeloma protein was isolated from ascites of BALB/c mice bearing X63 myeloma cells. IgG_1 was purified by 33% ammonium sulfate precipitation and DEAE-cellulose chromatography with a gradient from 0.005 to 0.2 *M* phosphate buffer, pH 8.0. The separation of H- and L-chains was exactly the same as that employed for IgM.

Preparation of anti-idiotypic antibody against monoclonal IgM secreted by a hybrid clone

Rabbits were immunized with purified IgM (0.5 mg) from a hybrid clone, $G_{10}B_4$, included in complete Freund's adjuvant and boosted 3 times. Antiserum was sequentially absorbed by using a Sepharose column conjugated with BDF_1 mouse serum, X63 (γ_1, κ) protein and MOPC 104E (μ, λ) protein The $F(ab')_2$ fragment of anti-idiotypic antibody, prepared by peptic digestion, was isolated by gel filtration on Sephadex G-150 and protein A–Sepharose. The biotin-conjugated $F(ab')_2$ fragment of anti-idiotypic antibody was prepared by coupling the specificially purified $F(ab')_2$ fragment with biotin-*N*-hydroxysuccinimide ester according to the method of Heitzmann & Richards (1974). Surface staining was carried out by employing biotin-labeled anti-idiotypic antibody and fluorescein-conjugated avidin (Vector Laboratories Inc., California).

RESULTS

Establishment of IgM secreting hybridomas between pre-B tumor cells (70Z/3) and myeloma cells (P_3U_1)

Somatic cell hybrids were prepared between the TK-deficient variant of 70Z/3 cells and the HGPRT-deficient variant of the myeloma cells, P_3U_1, which have lost the ability to secrete both myeloma γ_1- and κ-chains. Hybridomas obtained were cloned in soft agar containing complete McCoy's medium and seven hybrid clones were isolated. In 5 of these clones described in Table 1, the number of chromosomes was between 108 and 130. None of the clones expressed surface IgM when incubated with the FITC-labeled $F(ab')_2$ fragment of anti-IgM. The presence of cytoplasmic IgM in four clones was shown by intracellular staining with FITC–anti-IgM and these clones secreted IgM into culture supernatants when measured by a radioimmunoassay. Secretion of IgM by these four hybridomas was a stable property and they continued to produce IgM over an 18-month period in a condition of continuous growth. Previous studies showed that LPS stimulation induced surface IgM expression associated with *de novo* synthesis of a κ-chain in 70Z/3 cells (Sakaguchi *et al.*, 1980). However, LPS stimulation did not induce any surface IgM expression in hybrid cells and did not augment or inhibit IgM secretion of hybrid cells as shown in Table 2. IgG was not detected either on the cell surface or in the cytoplasm and LPS stimulation did not induce IgG expression (data not shown).

Table 1. Characterization of hybrid clones of pre-B tumor cells (70Z/3) and non-secreting myeloma cells (P_3U_1)

	Chromosome	% of sIgM-positive cells	cIgM	Secretion of IgM[a] (μg/ml)
70Z/3 (BudR^R)	40 – 45	0.8 – 2.0	faint (+)	(–)
P_3U_1	62 – 69	0	(–)	(–)
B_2A_6	108 – 130	< 1.0	(+)[b]	8.9
B_4B_3			(+)	13.6
E_7D_2			(+)	6.6
F_1D_2			(–)[b]	< 0.1
$G_{10}B_4$			(+)	8.4

[a]1 × 10^6 hybrid cells/ml were cultured for 24 hr and the amount of IgM in culture supernatants was measured by a solid-phase radioimmunoassay.
[b](+) = more than 90% of cells were brilliantly stained with FITC-labeled anti-IgM.
(–) = less than 1% of cells were stained.

Table 2. Effect of LPS stimulation on IgM production of hybrid clones

Clones	IgM in supernatants (μg/ml)[a] LPS (μg/ml) 0	1	10	50
B_2A_6	8.9	8.9	8.0	9.2
B_4B_3	13.6	ND	11.5	ND[b]
E_7D_2	6.6	6.5	6.6	ND
F_1D_2	<0.1	<0.1	<0.1	ND
$G_{10}B_4$	8.4	7.4	7.2	8.2

[a]1 × 10^6 hybrid cells/ml were cultured for 24 hr and the amount of IgM in culture supernatants was measured.
[b]Not done.

Characterization of IgM secreted by hybrid cells

Analysis of IgM secreted by hybrid cells was carried out by SDS–PAGE with biosynthetically labeled proteins. Hybrid cells were pulsed with a ^{14}C-amino acid mixture for 8 hr and secreted IgM present in culture supernatants was precipitated with anti-IgM. Specific immunoprecipitates were electrophoresed under both non-reducing and reducing conditions. As shown in Fig. 1, IgM secreted by hybrid cells migrated under non-reducing conditions to exactly the same position as that of the MOPC 104E protein, which was detected by staining with Coomassie Brilliant Blue R-250, showing that 19S pentameric IgM was secreted by the hybrid cells. When secreted IgM was electrophoresed under reducing conditions, two bands corresponding to μ- and κ-chains were observed and the size of the μ-chain derived from the secreted IgM was exactly the same as that of the μ-chain of MOPC 104E protein (Fig. 2).

In order to determine whether the L-chains of IgM secreted by the hybrid clones were derived from 70Z/3 cells of myeloma cells, patterns of 2D gel electrophoresis of the biosynthetically labeled κ-chains were compared. Two hybrid clones and LPS-induced 70Z/3 cells were biosynthetically labeled with [^{35}S]methionine and cell lysates were precipitated with anti-μ antibody. As shown in Fig. 3(A)–(C), κ-chains from two hybrid clones, $G_{10}B_4$ and B_2A_6, and from LPS-induced 70Z/3

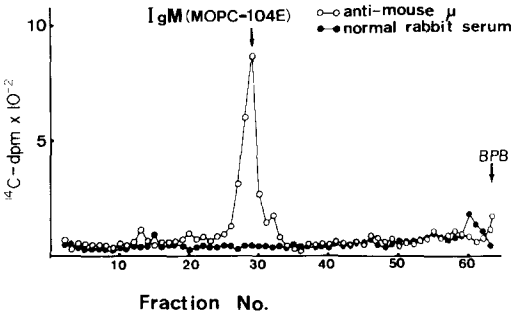

Fig. 1. SDS–PAGE analysis of non-reduced IgM secreted from hybrid clone B_2A_6. 2 × 10^6 cells in 2 ml of medium containing 50 μCi of a ^{14}C-amino acid mixture were cultured for 8 hr and radiolabeled IgM in the culture supernatant was precipitated with rabbit anti-mouse IgM (○—○) or with normal rabbit serum (●—●). IgM from MOPC 104E cells was employed as a marker and BPB was the front position of the gel.

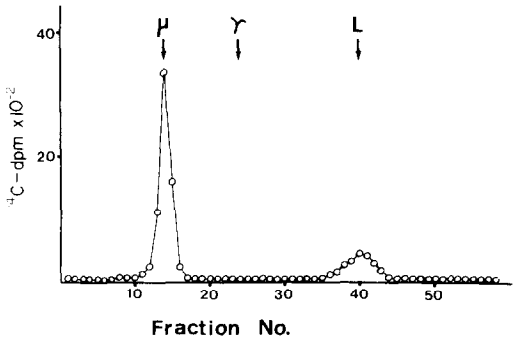

Fig. 2. SDS–PAGE analysis of clone B_2A_6 IgM in a reduced condition. μ- and L-chains from MOPC 104E IgM and γ-chains from X63 myeloma IgG_1 were employed as markers.

cells, showed exactly the same pattern in 2D gel electrophoresis. The amount of L-chains synthesized by LPS-stimulated 70Z/3 cells was so small that a long exposure was required for the detection of L-chain spots on the film [Fig. 3(A)]. As many non-specific spots appeared around the μ-chain spots because of long exposure [Fig. 3(A)], μ-chain patterns could not be compared between Fig. 3(A) and Fig. 3(B) and (C). Biosynthetically labeled κ-chains from X63 myeloma cells, which were precipitated with anti-γ_1 antibody, migrated to the entirely different position from that of κ-chains from hybrid clones or 70Z/3 cells as shown in Fig. 3(D). These results clearly showed that the L-chains of the IgM secreted by the hybrid cells were derived from the 70Z/3 genome.

Analysis of the idiotype of the secreted IgM

Anti-idiotypic antibody against the IgM secreted from a hybrid clone, $G_{10}B_4$, was prepared as described in Materials and Methods. The specificity of affinity-purified anti-idiotypic antibody was studied by an inhibition radioimmunoassay. As shown in Figs 4 and 5, binding of the anti-idiotypic antibody with radiolabeled IgM from a clone, $G_{10}B_4$, was inhibited by cold IgM from $G_{10}B_4$ or from the other clone, B_4B_3, but IgM from MOPC 104E or from IgM myeloma, W3469 (μ, κ), did not inhibit the binding, even when the concentration of IgM employed was 100 times higher than that of IgM from $G_{10}B_4$ required for complete inhibition. As shown in Fig. 5, the isolated μ-chains but not the κ-chains partially inhibited the binding of radiolabeled IgM from $G_{10}B_4$. However, the concentration of the μ-chains required for the comparable inhibition was about 500 times higher than that of the intact IgM.

By utilizing the anti-idiotypic antibody, the question of whether or not the surface IgM induced in LPS-stimulated 70Z/3 cells bore the same idiotype was examined. As shown in Table 3, the percentage of cells stained with the FITC-labeled $F(ab')_2$ fragment of the anti-idiotypic antibody in LPS-stimulated 70Z/3 cells was exactly the same as that obtained by staining with FITC-labeled anti-IgM. The same results were observed in two variants of 70Z/3 cells, i.e. the BudR-resistant, TK-deficient variant used for the hybridization and an LPS-resistant variant, which could continuously proliferate in the presence of 10 μg/ml of LPS with constant expression of surface IgM. In order to assess the non-specific binding of antibodies to 70Z/3 cells, rabbit anti-T15 idiotypic antibody was employed as a control. Less than 1% of LPS-stimulated 70Z/3 cells were stained with rabbit anti-T15 antibody, excluding the possibility that the staining with the anti-idiotypic antibody was non-specific. On the other hand, no WEHI 231 cells were stained with the anti-idiotypic antibody, while more than 70% of cells expressed surface IgM and less than 1% of BDF_1 spleen cells were stained by this antibody, confirming the specificity of the anti-idiotypic antibody. These results together with the results of 2D gel electrophoresis showed that two hybrid clones and LPS-induced 70Z/3 cells produced IgM molecules with μ- and κ-chains of the same specificity and indicated that the specificity commitment of both μ- and κ-chains has already occurred in a pre-B cell like cell line, 70Z/3.

DISCUSSION

The present study showed the induction of 19S IgM in 70Z/3 cells by hybridization with non-secreting myeloma cells. It has been shown by Paige *et al.* (1978) that 70Z/3 cells have intracellular μ-chains but no L-chains and that they neither express any surface IgM nor secrete any IgM molecules. LPS-stimulation of 70Z/3 cells induced an increase of κ-chain-specific messenger RNA and an expression of surface IgM (Sakaguchi *et al.*, 1980), but the stimulation did not induce any secretion of 7S or 19S IgM. Since patterns of 2D gel electrophoresis of the biosynthetically labeled κ-chains from 2 hybrid clones and LPS-induced 70Z/3 cells were the same but were different

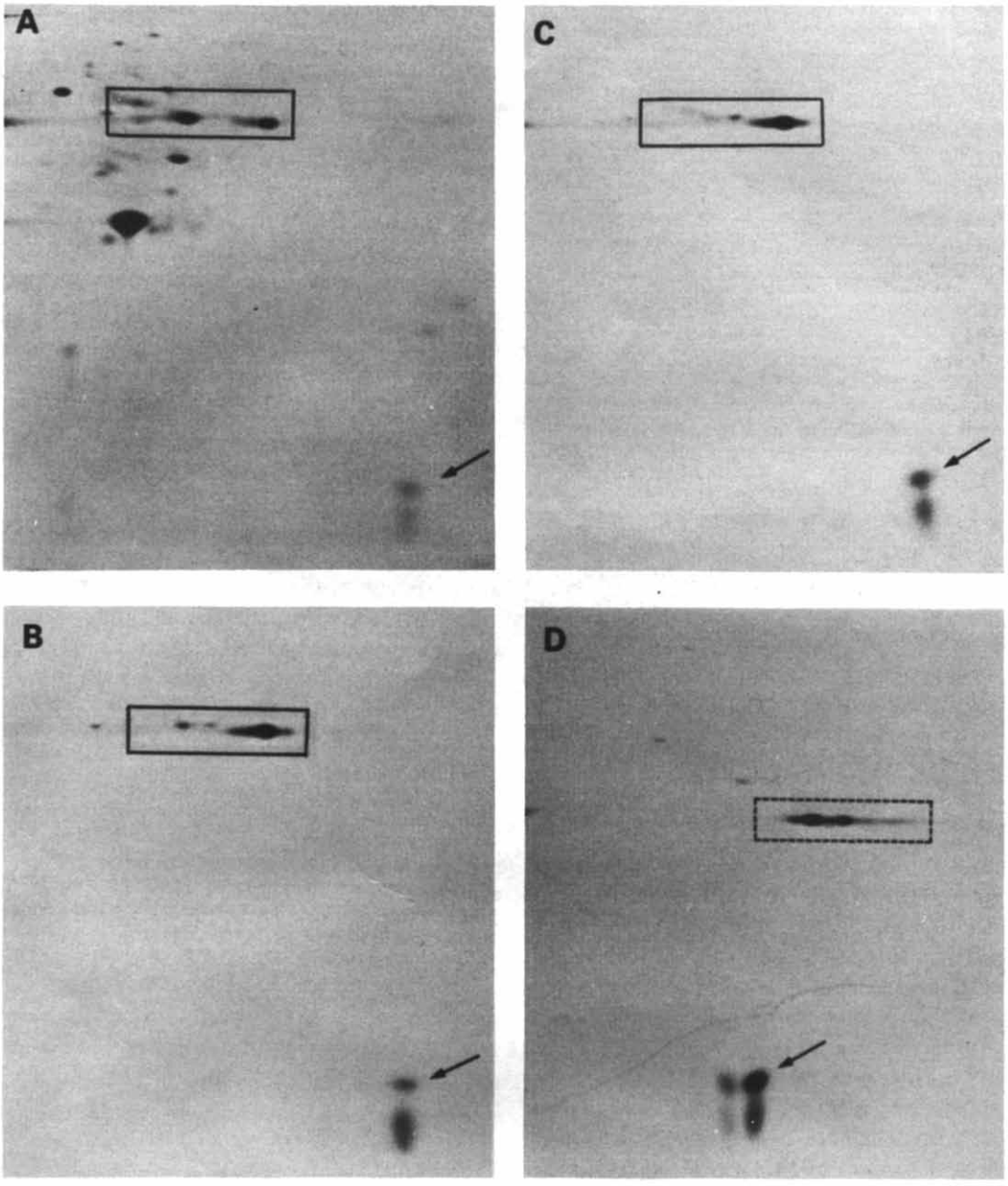

Fig. 3. Patterns of 2D gel electrophoresis of biosynthetically labeled IgM from two hybrid clones and LPS-induced 70Z/3 cells, and IgG from X63 cells. 4×10^7 70Z/3 cells (A), which had been stimulated with 5 μg/ml LPS for 20 hr or 4×10^6 hybrid clones, B_2A_6 (B), $G_{10}B_4$(C), or X63 myeloma cells (D), were labeled with [^{35}S]methionine, and then the lysates were precipitated with anti-μ or anti-γ_1 antibody. All gels are shown with the basic side on the right and decreasing mol. wt from top to bottom. Alignment of gels, (A)–(D), was done so as to adjust the origin of electrophoresis. μ-Chains (□), γ-chains (⬚), and κ-chains (arrow) are indicated in each figure.

from that of X63 cells, κ-chains produced by the hybridomas were derived not from the myeloma (P_3U_1) genome but from the 70Z/3 genome. Thus, not only LPS stimulation but also hybridization with non-secreting myeloma cells can induce the synthesis of L-chains in 70Z/3 cells.

Induction of the secretion of 19S IgM suggested that J-chain synthesis was also induced in hybrid cells, although the presence of J-chains was not directly demonstrated in SDS–PAGE (Fig. 2). Raschke *et al.* (1979) showed the secretion of pentameric IgM in a fusion between a non-secreting undifferentiated B-cell lymphoma and an IgG-secreting myeloma. The B-lymphoma (WEHI 231) employed

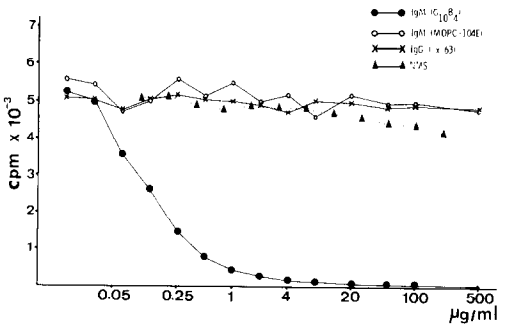

Fig. 4. Inhibition radioimmunoassay for the study of the specificity of the anti-idiotypic antibody. Microtiter plate (Cook Laboratories No. 1-220-24) was coated with IgG fraction of the anti-idiotypic antibody and the binding of ^{125}I-labeled IgM (2.8 × 10^9 cpm/mg) purified from ascites of the mice bearing a hybrid clone ($G_{10}B_4$) was competitively inhibited by varying concentrations of IgM from a hybrid clone ($G_{10}B_4$) (●——●), IgM from MOPC 104E (○——○), γ_1 myeloma protein from X63 (×——×) and normal BDF_1 mouse serum (▲——▲). The concentration of the serum was expressed as micrograms of total serum protein.

did not express J-chains whereas the plasmacytoma MPC11 synthesized but did not secrete J-chains. Thus, they suggested that the capacity of the MPC11 cells to synthesize J-chains was one of the factors that promoted the secretion of 19S IgM by hybrid cells. Although it is not known whether P_3U_1 cells have the capacity to synthesize J-chains, a similar situation might account for the present findings.

Cell hybridization did not induce the expression of surface IgM on 70Z/3 cells, although it induced the secretion of 19S IgM. As had been shown by Williams *et al.* (1978), μ-chains from membrane-bound IgM (μ_m) have an extra *C*-terminal segment which is not found in μ-chains isolated from secreted IgM (μ_s) and the synthesis of μ_s may be controlled by the alternative processing of Cμ-containing transcripts (Early *et al.*, 1980). Four distinct μ-mRNA components ranging in size from 2.1 to 3.0 Kb have been observed in 70Z/3 cells (Perry & Kelley, 1979). The authors suggested that two of these components, 2.4 and 2.7 Kb, coded for μ_s and μ_m, and the other two components, 2.1 and 3.0 Kb, coded for intracellular μ-chains. In the B-cell line WEHI 231, which expresses surface IgM, two kinds of mRNA, 2.4 and 2.7 Kb, were found, while IgM-secreting myeloma cells had only a single μ-mRNA of 2.4 Kb. The hybridization of 70Z/3 cells induced IgM secretion but not expression of surface IgM and, as shown in SDS–PAGE, μ-chains from hybridomas migrated at the same rate as MOPC 104E μ-chains, indicating that the hybridoma μ-chains were secreted-type μ-chains (μ_s). It is reasonable to speculate that cell hybridization with myeloma cells may induce the change of the pattern of the processing of μ-mRNA in 70Z/3 cells, i.e. from 2.7 to 2.4 Kb or from 3.0 to 2.4 Kb, although it is not

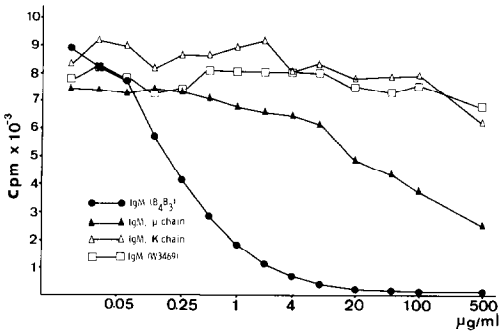

Fig. 5. Inhibition of the binding of ^{125}I-labeled IgM ($G_{10}B_4$) to the anti-idiotypic antibody by the addition of varying concentrations of IgM from a hybrid clone (B_4B_3) (●——●), μ-chains from B_4B_3 (▲——▲), κ-chains from B_4B_3 (△——△) and IgM from a myeloma [W3469 (μ, κ)] (□——□).

Table 3. Expression of the same idiotype on all LPS-stimulated 70Z/3 cells as that of IgM secreted from the hybrid clone

		% of sIgM (+) cell		% of sId (+) cell[a]	
		LPS(−)	LPS(+)	LPS(−)	LPS(+)
70Z/3[b]	Exp. 1	1.2	17.6	0.8	17.6
	Exp. 2	3.2	23.8	2.6	24.3
70Z/3[b]	Exp. 1	2.0	20.7	0.5	16.3
(BudR[R])	Exp. 2	1.2	26.6	0.8	27.9
70Z/3.12[c]		1.6	45.2	1.7	50.6
WEHI 231		74.8	ND	1.3	ND
Spleen cells (BDF_1)		22.8	ND	0.5	ND

[a]Cells were stained with biotin-conjugated anti-idiotypic antibody and FITC-labeled avidin.
[b]Cells were stimulated with 1 μg/ml LPS for 16 hr.
[c]Cells were stimulated with 5 μg/ml LPS for 16 hr.

known whether the enzyme responsible for the processing of mRNA for μ_s is activated in 70Z/3 cells or is derived from myeloma cells.

Cell hybridization of pre-B cells in murine fetal liver and non-secreting myeloma cells has been done by Burrows *et al.* (1979). In their experiments, however, only μ-chain synthesis was observed and L-chain synthesis of IgM secretion was not induced. Maki *et al.* (1980) studied the rearrangement of immunoglobulin L and H-chain genes in the DNA of these fetal liver hybridomas and 70Z/3 cells. No rearrangement of L-chain genes was observed in fetal liver hybridomas, but rearrangement of κ L-chain genes was apparent in 70Z/3 cells. Their result on DNA rearrangement coincided with the results on the induction of L-chain synthesis in 70Z/3 cell hybrids. Thus, in 70Z/3 cells, rearrangement of κ-chain genes has apparently occurred and IgM secretion was observed in hybridomas, whilst fetal liver hybridomas do not appear to have rearranged L-chain genes and L-chain synthesis was not observed in hybridomas. These results suggest that the hybridization of pre-B cells with myeloma cells may not be able to induce rearrangement of immunoglobulin genes but could affect transcriptional regulation and induce synthesis of κ-chains in 70Z/3 cells. However, a recent experiment done by Riley *et al.* (1981) showed the suggestive evidence that hybridization of the pre-B cell line 18–81, with variant myeloma cells, which did not express an L-chain, could induce L-chain gene rearrangement and expression of a κ-chain.

An asynchronous onset of immunoglobulin chain synthesis in the B-cell lineage has been clearly shown not only in fetal liver hybridomas (Burrows *et al.*, 1979), but in murine pre-B cells (Levitt & Cooper, 1980) and in murine cell lines transformed with Abelson leukemia virus (Boss *et al.*, 1979; Siden & Baltimore, 1979). The physiological significance of the onset of synthesis of μ-chains prior to L-chains in pre-B cells is not known, but it is conceivable that an asynchronous onset may play a role in the generation of diversity of B-cell clones, i.e. a single stem cell can give rise to many pre-B cells, each expressing a different V_H-gene and each member of the clonal progeny selecting a different V_L-gene. The present study indicated that those processes in the expansion of clonal diversity were entirely independent of antigenic stimulation and antigen-specificity of B-cells was committed prior to the expression of surface IgM. The fact that all 70Z/3 cells expressed surface IgM with the same idiotype and that μ- and κ-chains from hybrid clones and LPS-stimulated 70Z/3 cells were the same showed that the selection of V_H- as well as V_L-genes has already occurred and that clonal specificity has been decided in 70Z/3 cells which do not express surface IgM. The result is consistent with the report of Maki *et al.* (1980) which showed rearrangement of κ-chain genes in 70Z/3 cells.

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