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Letter to the Editor

SERUM-FREE MEDIUM FOR LONG-TERM GROWTH, FREEZING, CLONING AND FUSION OF MYELOMAS AND HYBRIDOMAS

Dear Editor:

A number of laboratories and companies have reported on the development of serum-free media for the growth of myelomas and hybridomas in tissue culture (1–4,6–9). Due to the different growth requirements of different cell lines, these serum-free media generally have not been broadly applicable. For example, HB101 (Hana Biologics) supported the growth of SP2/0 cells, SP2/0-derived hybridomas, human-human hybridomas, etc., but not NS-1 and NS-1-derived hybridomas (6); the SFH medium described by Kovar and Franek (4) was suitable for the growth of F0 myeloma cells and T3-03 cells (F0-derived hybridomas) but not X63-Ag8.653 cells and PGG-05 cells (X63-Ag8.653-derived hybridomas); the SSF medium of Chang et al. (1) supported five hybridoma cells but not P3X63Ag8 and 653 myeloma cells; the SFFD/ITES medium of Murakami et al. (7) supported MPC11- or SP2/0-derived hybridomas but not sufficiently NS-1-derived hybridomas; and the media of Cleveland et al. (2) supported P3X63-Ag8.653 myelomas and some of hybridomas but not M1/75.16.4 rat-mouse hybridomas. Our experimental findings indicated that DMI serum-free medium supported the growth of all tested cell lines which covered human, rat and mouse myelomas, rat-mouse heterohybridomas, mouse-mouse homohybridomas, K562 cells etc. (Table 1) (5). It was shown that successful growth of hybridoma in a serum-free medium in short-term culture did not mean successful adaptation to long-term cultivation in the serum-free medium (4). In the present study it was confirmed that DMI could be successfully used to long-term culture a variety of cell lines tested (Table 1). Although to date a number of serum-free media have been described, the cell densities in these media mostly were not superior to 10^6 cells/ml (1,4,6,8). The present results showed that the densities of all cell lines passaged in DMI were more than 130×10^4 cells/ml when the death rate was less than 10% (Table 1), and SP2/0 cells once reached a maximal density of 299×10^4 cells/ml. In addition, all tested 8 myeloma and hybridoma cell lines growing in DMI could be frozen in the absence of serum for storage in liquid nitrogen with quantitative serum-free cell recovery (data not shown). If the cells growing in the conventional medium containing 15% newborn calf serum (RPS15) were frozen with DMI for storage and, 2 weeks later, resurrected with RPS15 for reculture, cell recovery was similar to the conventional freezing and resurrection with 20% serum-supplemented medium (RPS20).

TABLE 1

LONG-TERM CULTIVATION AND DENSITIES OF MYELOMA AND HYBRIDOMA CELLS IN SERUM-FREE MEDIUM (DMI)

Cell Line	Passage Period (months)	Passage No.	Cell Density ($\times 10^4$/ml)	Death Rate of Cells (%)
Myeloma				
SP2/0	16	160	251	7.2
653	16	161	198	3.2
P3U1	9	88	193	6.7
983	9	87	152	5.3
Hybridoma				
41RF5	12	118	169	7.4
94D1	3	27	149	7.7
21E3	3	30	162	8.5
13A2	5	49	157	9.2
13F3	6	60	158	7.0
32B5	6	58	132	7.0
A2E5	3	28	139	5.1
Other				
K562	2	20		

TABLE 2

CLONING EFFICIENCIES OF MYELOMA AND HYBRIDOMA LINES FROM SINGLE CELL STAGE IN SERUM-FREE MEDIUM (DMI) AND 15% SERUM-SUPPLEMENTED MEDIUM (RPS15)

Cell Lines	No. of Wells Seeded	No. of Wells with Hybrids		No. of Clones in the Plates	
		DMI	RPS15	DMI	RPS15
Myeloma					
SP2/0	96	37	44	46	68
653	96	43	54	51	83
P3U1	96	26	50	28	63
983	96	35	37	51	49
Hybridoma					
41RF5	96	38	48	51	70
13F3	96	20	25	25	28

Monoclonal antibody (mAb) production at high titers is a major goal of hybridoma technology. The most important factor in determining the utility of serum-free media in hybridoma culture is whether mAb secretion is impaired by the removal of serum components. Our experimental results showed that the hybridoma cells cultured in DMI continued to secrete mAbs at levels similar to those obtained in RPS15 (data not shown).

Of evaluating the quantity of serum-free media, one is to test the ability of a serum-free medium to support the clonal growth from the single cell stage. McHugh et al. (6) demonstrated that the cloning efficiencies of SP2/0 and SP2/0-derived hybridomas in HB101 medium ranged from 1 to 3% as compared to 69–90% in serum-supplemented medium. Shacter (8) reported that the cloning efficiency of a hybridoma in SFM medium was 20% (9/48),

TABLE 3

CELL FUSIONS BETWEEN P3U1 MYELOMA PASSAGED IN SERUM-FREE MEDIUM (DMI) AND RODENT SPLEEN CELLS IN SERUM-FREE MEDIUM (DMI) AND 20% SERUM-SUPPLEMENTED MEDIUM (RPS20)

Exp. No.[a]	Medium	No. of Wells Seeded	No. of Wells With Hybrids	Fusion Frequency[b]
I	DMI	96	93	2.4
	RPS20	96	96	4.7
II	DMI	96	96	5.0
	RPS20	96	96	6.0

[a] Mouse-rat fusion (I) and mouse-mouse fusion (II); [b]average number of colonies per well.

whereas that obtained in the presence of 10% serum was 50% (48/96). Our data (Table 2) showed that the cloning efficiencies (number of wells with hybrids) obtained in DMI ranged from 21 to 45% as compared to 26 to 56% in RPS15, and that the number of clones grown in the plates varied from 26 to 53% in DMI as compared to 29 to 86% in RPS15.

The production of antibody-secreting hybridomas from the fusions of mouse myeloma cells with spleen cells in serum-free medium has a number of practical advantages (3). However, whether or not serum-free media developed specifically for parental myeloma lines and/or established hybridoma lines can be used in the cell fusion between immunized spleen cells and myeloma cell lines and subsequently in the isolation of hybridomas directly after the fusion is less clear. Kawamoto et al. (3) reported that the KSLM medium could be used in the cell fusion and then in the isolation of antibody-secreting hybridomas. When using NS-1 as parental cells, the fusion efficiencies ranged from 52 to 54% , while for NS-1-503 partner, the fusion efficiencies varied from 85 to 92%. Yabe et al. (9) indicated that the NYSF-404 medium was able to use for the selection of antibody-producing hybridomas after fusion of antigen-sensitized mouse spleen cells with myeloma cell lines P3-U1, Ag8.653 or NS-1. The result presented here has confirmed that DMI medium could be used in the cell fusions of immunized rat or mouse spleen cells with SP2/0 or P3U1 myelomas either passaged in DMI medium or obtained from the subcutaneous somatic neoplasm in BALB/c mice with a maximal fusion efficiency of 100% (Table 3) and in the isolation of antibody-secreting hybridomas (data not shown). Thus, it is concluded that DMI medium may be broadly applicable.

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