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Commercial serum-free media: hybridoma growth and monoclonal antibody production

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Various growth factors, hormones and proteins present in serum are presumed to be responsible for its growth-stimulating activity in culture media. However, synthetic or serum substitute media supporting cell growth are advantageous when it is necessary to standardize culture conditions, particularly when cell products are used. In this study we have evaluated and compared the effects of some commercially available serum substitute media (10% NU Serum, 10% BMS, 2% Ultroser HY, 1% ITS + Premix, 1% Nutridoma, Ultradoma, FEB100, DCCM1 and DCCM2) on growth and immunoglobulin production in different hybridoma cell lines. Six different hybrids were studied: OKT3, OKT4 and OKT8 (producing monoclonal antibodies against CD3, CD4 and CD8 molecules), HB43 and HB57 (producing monoclonal antibodies against human IgG and IgM), and CRL 8019 (producing monoclonal antibodies against high-molecular weight CEA). Several parameters were evaluated, such as viability, doubling time, DNA synthesis, monoclonal antibody production and cell cycle under different culture conditions. Since not all of the hybridomas grew equally well in the same serum substitute media, one synthetic medium cannot be used for all the lines. We found that the serum-free media that best supported hybridoma growth were Nutridoma, DCCM1, DCCM2, NU Serum and FEB100.

Key words: Serum-free medium; Hybridoma cell lines; Serum-free culture; Monoclonal antibody

Introduction

Serum is considered to be an essential component of any culture medium supporting an optimal rate of cell proliferation, since it provides essential unidentified nutrients which cannot be included in common media formulations (Rizzino et al., 1979; Shacter, 1989). It would be advantageous to replace serum with a limited number of defined molecules for studies on cell proliferation and differentiation.

Serum substitute media designed for serum-free culture of animal cells eliminate the complex and variable effects of serum on cell growth. These substitutes are complicated mixtures of salts, amino acids, vitamins, glucose and various compounds such as nucleic acid and lipid precursors or antioxidative substances. They are supplemented with hormones (insulin, growth factors, steroids), binding proteins (transferrin, albumin) and trace elements (Barnes and Stato 1980; Barnes, 1987; Shacter, 1989).

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Different established hybrid cell lines have been reported to proliferate and produce antibodies in chemically defined serum-free media specifically developed for this purpose by individual laboratories (Tharakan et al., 1986; Murakami, 1989; Schneider, 1989). This method of culture facilitates studies requiring the absence of serum proteins and endogenous serum substances, such as hormones or natural antibodies.

Hybridoma cultivation in serum-free media also makes purification of monoclonal antibodies relatively easy and in some cases they can be applied directly or chemically coupled with other molecules without further purification.

As these media are not easily prepared in many laboratories and different growth supplements may be required for various cell lines, serum-free media have not been widely adopted.

This work aimed to evaluate and compare the growth supporting capacity of commercially available serum substitute media. We investigated the effect of decreasing concentrations of serum and of 'serum-free' substitute media on the in vitro culture conditions of six different murine hybrid cell lines.

Viability, doubling time and proliferative capacity of hybridoma cells, cell cycle and monoclonal antibody production were compared under different culture conditions.

Materials and methods

Hybrid cell lines

The following hybrid cell lines were used:

OKT3 (CRL 8001), OKT4 (CRL 8002), OKT8 (CRL 8014) (anti-human T lymphocytes) produced by fusion with the P3x63Ag8U1 mouse myeloma line;

141OKG7 (HB43) (anti-human gamma heavy chain) developed by fusion with the Sp2/0-Ag14 cells;

DA4-4 (HB57) (anti-human μ heavy chain), 1116NS-3d (CRL8019) (anti-high-molecular weight CEA) derived by fusion with the P3x63Ag-8.653 line.

All the lines were obtained from the American Type Culture Collection, Rockville, MD, U.S.A.

Medium formulations

RPMI 1640 (Flow, U.S.A.) was prepared with 2 g/l $NaHCO_3$ (Carlo Erba, Italy), 25 mM Hepes buffer (Sigma, U.S.A.), 4 mM L-glutamine (Sigma U.S.A.) and 100 IU/ml penicillin + 100 μg/ml streptomycin (Gibco, U.S.A.). This standard formulation was used to prepare the different growth media with the addition of fetal calf serum (FCS) or serum substitutes. Heat-inactivated FCS (Gibco, U.S.A) was added at different concentrations ranging from 20 to 2.5%.

The following serum substitute media were commercially available and were added at the concentrations recommended by the manufacturer as follows: 10% NU-serum (Flow, U.S.A), 10% BMS (Biochrom, U.S.A.), 2% Ultroser-HY (Gibco, U.S.A), 1% ITS + Premix (Flow, U.S.A), 1% Nutridoma HS (Boehringer-Mannheim, F.R. G.).

The other growth media used were complete formulations and were supplied in their final form: FEB-100 (Biochrom, U.S.A.) based on RPMI medium, Ultradoma (Whittaker Bioproducts, U.S.A.), DCCM1 and DCCM2 (Biological Industries, Israel) of unknown formulation. All serum-free media were supplemented with 4 mM L-glutamine and the antibiotics as described above.

Growth conditions

Hybridoma cell lines were grown in flat bottom 24-well plates (Falcon, U.S.A.) at 37°C in a 5% CO_2 humidified atmosphere. Every 2–3 days cells were routinely subcultured by direct dilution in fresh medium and seeded at a density of 1×10^5 viable cells/ml in a volume of 2 ml/well. Cell counts and viability were determined by the eosin Y dye exclusion test.

In a first series of experiments we evaluated the effect of decreasing FCS concentrations. The different hybrid cell lines were initially cultured in RPMI 1640 medium containing 20% FCS, the cells were subsequently fed with medium containing decreasing amounts of FCS (10%, 5%, 2.5%) and were allowed about 1 week at a given percentage of FCS before decreasing the FCS once again.

In a second series of parallel experiments the hybrid cell lines were sequentially adapted to

serum-free culture conditions by gradually reducing the amount of FCS in the medium from 10% to 5%, then to 2.5% and to 0% and by increasing the amount of serum-free media. Then the cell numbers were allowed to double at least 3 times prior to transfer to the next lower serum concentration. The effect of the serum substitute media was determined after the entire adaptation period (3 weeks) and compared with growth in RPMI 1640 supplemented with 10% FCS.

Doubling time

The doubling time (d.t.) (h) was calculated on the hybrid cells in exponential growth, from the expression derived by Goding (1986):

$$\text{d.t.} = \frac{0.693\ t}{\log_e \frac{n}{n_0}}$$

where t = elapsed time, n_0 = starting number of cells, n = final number of cells and e = 2.7183.

Proliferative capacity

The mean proliferative capacity of the hybridoma was evaluated by calculating [^{3}H]thymidine uptake after incubation of 20,000 cells/well using a 200 μl reaction volume in triplicate with 0.4 μCi [^{3}H]thymidine (Amersham International, U.K.) (specific activity 25 Ci/mM) over the last 18 h of 4-day culture (Facchini et al., 1987). The results were expressed as increment in DNA synthesis which represented the dpm ratio between the cells grown in serum substitute media and those grown in 10% FCS.

Cell cycle

The cell cycle was evaluated on hybrid cells fixed with 70% ethanol at 4°C, washed twice and resuspended in 1 ml phosphate buffered saline (PBS). Thereafter the cells were incubated with 50 μg/ml propidium iodide (Sigma, U.S.A.) for 30 min at 4°C and analysed by flow cytometry with a FACStar Plus instrument (Becton Dickinson, U.S.A.), fitted with a 5-W argon laser (Coherent, U.S.A.); excitation at 488 nm was 200 mW and PI fluorescence was collected through a 585/42 BP filter. The percentage of hybrid cells in the different phases of the cell cycle was determined using the sum of broadened rectangles algorithm (Baish et al., 1982).

Monoclonal antibody production

Monoclonal antibody production was monitored in culture supernatants harvested 48 h after initiating fresh cultures. The supernatants were stored at −20°C until testing.

The specificity and affinity of monoclonal antibodies against OKT3, OKT4, OKT8 antigenic determinants in the hybridoma culture supernatant were evaluated by indirect immunofluorescence on peripheral blood lymphocytes (PBL). Briefly, PBL separated by density gradient were incubated with hybrid culture supernatants, undiluted and diluted 1/100 for 30 min at 4°C followed by FITC-conjugated rabbit-anti-mouse antiserum (DAKO, Denmark) (1/20) for 30 min, at 4°C. Cells were then analysed by flow cytometry as described (Mariani et al., 1990).

Fluorescence intensity was recorded on a log scale. Both the percentage of positive cells and the mean fluorescence channel were considered for analysis. Commercial monoclonal antibodies positive for CD3, CD4 and CD8 and tissue culture medium were used as controls.

The concentration of immunoglobulins (Ig) produced by HB43 and HB57 hybrid cell lines was determined by an enzyme-linked immunoassay: a 96-well plate (Flow, U.S.A.) was coated overnight with 100 μl supernatant diluted 1/800 in sodium bicarbonate, then blocked with 150 μl 5% dry milk in PBS-Tween 20 (rinse buffer) for 1 h at room temperature. After washing with PBS-Tween, the plate was incubated with 100 μl rabbit-anti-mouse antiserum (DAKO, Denmark) diluted 1/800 in rinse buffer for 90 min at 37°C. Then the plate was washed and peroxidase-conjugated swine anti-rabbit antiserum (DAKO, Denmark) diluted 1/1000 in rinse buffer was added for 90 min at 37°C. After 3 washes with PBS-Tween, 75 μl 1 mg/ml *o*-phenylendiamine (Sigma, U.S.A.) diluted in phosphate buffer + 0.05% H_2O_2 were added for 30 min at room temperature: the reaction was blocked with 2N H_2SO_4 and absorbance at 492 nm was read with a BIORAD 2550 EIA Reader (Bio-Rad, U.S.A.). The immunoglobulin content of the supernatants was quantitated using an IgG standard (OKDR,

Sc2 clone, 200 μg/ml; Ortho, U.S.A.) and was expressed as μg Ig/10^6 hybrid cells in 48 h. Tissue culture medium was used as negative control.

Score and statistical analysis

The hybrids were graded by assigning a score from 1 to 10 to each of the media examined (included 10% FCS), giving 10 to the best and 1 to the worst result obtained for viability, doubling time and proliferative capacity. Statistical analysis for viability, doubling time, cell cycle and monoclonal antibody production was performed using Student's *t*-test for independent and paired data.

Results

Effect of decreasing concentrations of FCS on viability and doubling time

Doubling time (Fig. 1) and percentage of viable cells (Fig. 2) were very similar both for the 20% and 10% FCS culture conditions. Hybrid cell survival was possible at 5% FCS but was associated with a longer doubling time (Fig. 1) and a lower viability (Fig. 2) than at 10% FCS. At 2.5% FCS, the hybridoma generally did not survive for more than a week (data not shown).

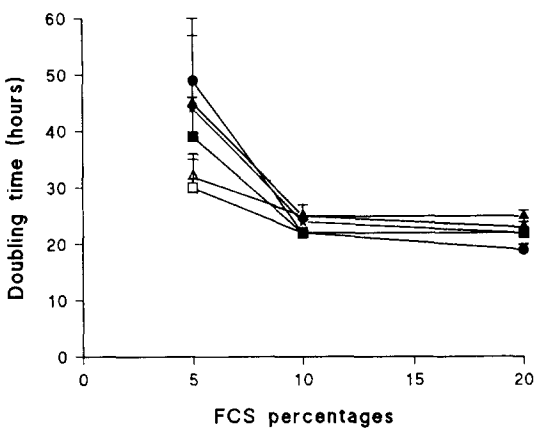

Fig. 1. Doubling time (h) of hybridomas in serum-supplemented medium. Cultures were set up in 24-well plates at 1×10^5 cells/ml RPMI 1640 medium supplemented with 20%, 10% and 5% FCS. Each point represents the mean + SE of 10 observations. OKT3 (●), OKT4 (■), OKT8 (▲), HB43 (★), HB57 (□) and CRL8019 (△). The difference between 10% and 5% FCS was significant with *P* ranging from 0.05 to 0.01 with the exception of HB43 and HB57 lines in which the difference was not significant.

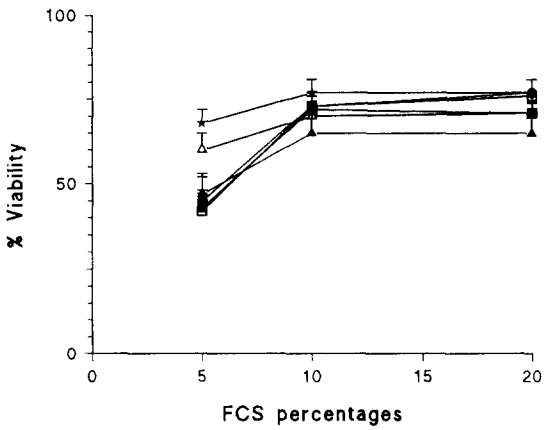

Fig. 2. Viability of hybridomas in serum-supplemented medium. Cultures were set up in 24-well plates at 1×10^5 cells/ml RPMI 1640 medium supplemented with 20%, 10% and 5% FCS and tested on the third day after seeding cultures. Viability was determined by the eosin Y exclusion test and each point represents the mean percentages + SE of 10 observations. OKT3 (●), OKT4 (■), OKT8 (▲), HB43 (★), HB57 (□) and CRL8019 (△). The difference between 10% and 5% FCS was significant with *P* ranging from 0.05 to 0.001 with the exception of HB43 and CRL8019 lines in which the difference was not significant.

Viability and doubling time in serum-free media conditions

In preliminary experiments not reported here we observed that the OKT3, OKT4 and HB43 hybridomas needed a 3-week period of adaptation to new culture conditions before their behaviour resembled the parental hybrid grown in FCS. Other lines (OKT8, HB57 and CRL8019) were able to proliferate rapidly as soon as the medium was changed completely although their fusion line (P3x63Ag8.653) has been described as a slow growing myeloma (Kovar and Franek 1986).

This behaviour was also verified for the media presented as adaptation-free by the manufacturer. Accordingly, we decided to unify culture conditions and to expose all the hybrids tested to a sequential adaptation to each serum-free substitute by gradually reducing the amount of FCS.

Cell viability was determined with hybridoma cells sequentially adapted to serum-free culture conditions and then followed for 6–10 weeks (Table I). In general, differences in cell viability

TABLE I

VIABILITY OF HYBRIDOMAS IN SERUM-FREE AND SERUM-SUPPLEMENTED MEDIA

Cultures were set up in 24-well plates at 1×10^5 cells/ml and tested on the third day after seeding cultures. For statistical analysis the different serum substitute media were compared with RPMI 1640 + 10% FCS medium. Viability was determined by the eosin Y exclusion test. Results are expressed as percentage (mean ± SE) of at least 18 observations.

Serum-substitute media	Hybridoma cell lines					
	OKT3	OKT4	OKT8	HB43	HB57	CRL8019
FCS 10%	73 ± 4	72 ± 4	74 ± 4	77 ± 4	70 ± 2	70 ± 4
NU-serum 10%	67 ± 4	56 ± 3 [b]	60 ± 5	60 ± 2 [b]	53 ± 8	64 ± 2
BMS 10%	62 ± 3	67 ± 3	< 50	< 50	[a]	60 ± 3
Ultroser-HY 2%	nd	< 50	< 50	< 50	[a]	< 50
ITS + premix 1%	nd	71 ± 5	< 50	80 ± 4	63 ± 5	< 50
Nutridoma HS 1%	59 ± 4 [b]	63 ± 4	71 ± 5	nd	nd	69 ± 4
Ultradoma 100%	nd	63 ± 6	< 50	[a]	[a]	< 50
FEB100 100%	65 ± 4	64 ± 5	66 ± 4	nd	nd	74 ± 2
DCCM1 100%	72 ± 3	68 ± 3	64 ± 3	nd	nd	76 ± 3
DCCM2 100%	74 ± 3	69 ± 3	65 ± 4	nd	nd	67 ± 4

[a] Died in the two weeks following adaptation.
[b] $P < 0.03$.
nd, not done.

between 10% FCS and serum-free media culture conditions were seen in all hybridoma tested without a clear association with a particular serum-free medium.

Determination of doubling time (Table II) showed broad variability, with some clones able to grow efficiently in almost all serum-free media tested (OKT3 and OKT4) and others having difficulty achieving optimal culture conditions (OKT8, CRL8019, HB43, HB57) (Table II).

Proliferative capacity in serum-free conditions

The proliferative capacity of 2×10^4 hybrid cells in serum-substitute media cultures (after the

TABLE II

DOUBLING TIME OF HYBRIDOMAS IN SERUM-FREE AND SERUM-SUPPLEMENTED MEDIA

Cultures were set up in 24-well plates at 1×10^5 cells/ml and tested on the third day after seeding cultures. For statistical analysis the different serum-substitute media were compared with RPMI 1640 + 10% FCS medium. Results are expressed as h (mean ± SE) of at least 18 observations.

Serum-substitute media	Hybridoma cell lines					
	OKT3	OKT4	OKT8	HB43	HB57	CRL8019
FCS 10%	20 ± 1.5	23 ± 2.2	25 ± 1.9	25 ± 1.6	25 ± 2.5	21 ± 1.6
NU-serum 10%	29 ± 2.6 [b]	30 ± 4.5	29 ± 2.5	27 ± 2.6	24 ± 2.5	23 ± 3.3
BMS 10%	24 ± 2.1	> 48	> 48	> 48	[a]	> 48
Ultroser-HY 2%	nd	> 48	> 48	> 48	[a]	> 48
ITS + premix 1%	nd	32 ± 4.4	> 48	26 ± 8.6	33 ± 6.4	> 48
Nutridoma HS 1%	23 ± 2.2	23 ± 1.7	22 ± 2.8	nd	nd	25 ± 1.6
Ultradoma 100%	nd	43 ± 2.7 [b]	> 48	[a]	[a]	> 48
FEB100 100%	28 ± 3.4	28 ± 3.0	28 ± 3.5	nd	nd	25 ± 3.7
DCCM1 100%	21 ± 1.8	23 ± 1.4	33 ± 1.8 [b]	nd	nd	23 ± 1.5
DCCM2 100%	24 ± 1.5	24 ± 1.1	34 ± 3.0 [b]	nd	nd	27 ± 2.1 [b]

[a] Died in the two weeks following adaptation.
[b] $P < 0.01$.
nd, not done.

adaptation period) was evaluated only in OKT3, OKT4, OKT8 and CRL8019 hybrid cell lines grown in the serum-free media with a doubling time under 48 h (Fig. 3).

All the serum-free media tested reported a proliferation rate similar or even higher than the values obtained in 10% FCS. Although HB43 and HB57 hybridomas demonstrated poor adaptability to serum-free culture conditions, a good proliferative capacity was obtained when NU serum and ITS + premix were used, (> of 10% FCS, data not shown).

Cell cycle in decreasing FCS and serum- free media conditions

No difference in the percentage of hybrid cells entering the G_1, S, G_2M phases of the cell cycle were found between 20% and 10% FCS, whereas few cells were seen to enter the G_2M phase with 5% FCS (data not shown).

Cell cycle analysis of hybrid cell lines growing in serum-free media with a doubling time similar to that obtained in cultures with 10% FCS also showed a similar or even higher percentage of cells entering the G_2M phase (Table III). On the other hand, the hybridomas with a doubling time

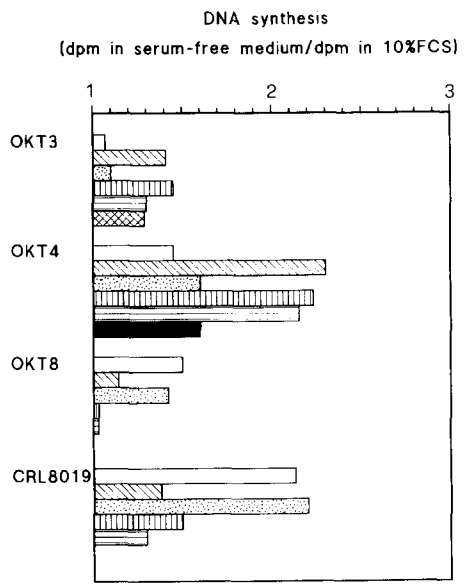

Fig. 3. DNA synthesis in serum-free conditions. DNA synthesis was evaluated only in lines grown with a doubling time shorter than 48 h. Results are expressed as increase of DNA synthesis found in cells cultured in serum substitute medium compared with 10% FCS. DNA synthesis = dpm in serum free medium/dpm in 10%FCS. Nu-serum (open bar), Nutridoma (hatched bar), Feb100 (stippled bar), DCCM1 (vertically striped bar), DCCM2 (horizontally striped bar), BMS (cross-hatched bar) and Ultradoma (solid bar).

TABLE III

CELL CYCLE IN SERUM-FREE CONDITIONS

Results are expressed as percentage of the cells present in the different cell cycle phases. Mean ± S.E.

Serum-substitute media	Phases	OKT3	OKT4	OKT8	CRL8019
FCS 10%	G_1	52.4 ± 3.6	39.8 ± 3.1	46.4 ± 7.0	49.4 ± 3.0
	S	37.8 ± 4.8	50.2 ± 3.5	43.9 ± 5.7	43.9 ± 5.7
	G_2M	8.2 ± 2.3	8.5 ± 1.5	8.7 ± 2.6	8.7 ± 2.6
NU serum	G_1	40.3 ± 4.3	41.2 ± 3.8	47.0 ± 4.3	39.2 ± 3.2
	S	38.8 ± 4.5	36.5 ± 2.7	26.6 ± 3.4	39.0 ± 4.8
	G_2M	17.9 ± 2.1	16.0 ± 3.0	16.6 ± 2.8	15.0 ± 2.5
Nutridoma HS	G_1	45.2 ± 7.7	38.4 ± 4.5	40.9 ± 3.9	41.9 ± 3.4
	S	48.3 ± 4.3	48.6 ± 4.3	46.6 ± 4.4	47.0 ± 3.4
	G_2M	11.2 ± 1.1	10.3 ± 0.5	11.8 ± 2.3	8.9 ± 1.7
FEB100	G_1	nd	28.6	46.4 ± 3.8	45.2 ± 2.2
	S	nd	49.1	41.7 ± 4.4	46.1 ± 2.2
	G_2M	nd	22.8	9.4 ± 2.1	7.4 ± 2.6
DCCM1	G_1	58.3	49.5	42.6	45.7
	S	28.3	38.7	39.8	43
	G_2M	7.0	11.6	11.6	11.1
DCCM2	G_1	57.1	53.5	43.1	53
	S	28.5	29.3	50.3	34.3
	G_2M	5.6	10.8	4.8	9

nd, not done.

TABLE IV

MONOCLONAL ANTIBODY PRODUCTION: TITRATION OF SUPERNATANT FROM HYBRIDOMAS

Results are expressed as mean ± SE.

	OKT3		OKT4		OKT8	
	Undiluted	1/100	Undiluted	1/100	Undiluted	1/100
% Positive lymphocytes						
FCS	61 ± 11	64 ± 4	41 ± 1	40 ± 1	27 ± 2	29 ± 6
NU serum	60 ± 7	54 ± 8	33 ± 13	32 ± 11	19 ± 2	21 ± 3
Nutridoma HS	62 ± 3	68 ± 8	42 ± 2	42 ± 3	25 ± 3	29 ± 4
FEB100	60	45	43	39	21 ± 5	27 ± 1
Peak fluorescence (channel number)						
FCS	674 ± 30	561 ± 30	452 ± 30	477 ± 16	630 ± 6	528 ± 18
NU serum	654 ± 23	511 ± 18	484 ± 7	475 ± 14	650 ± 18	396 ± 51
Nutridoma HS	671 ± 10	563 ± 38	446 ± 16	479 ± 6	574 ± 15	537 ± 20
FEB100	621	429	452	453	622 ± 11	556 ± 9

over 48 h were also associated with low percentages of cells in G_2M phase (data not shown).

Monoclonal antibody production in serum-free media conditions

The production of monoclonal antibodies by hybridomas growing continuously in serum-free media was tested on OKT3, OKT4, OKT8, HB43 and HB57 culture supernatants collected from growing hybrid cell lines.

The percentages of the lymphocytes recognized by the OKT3, OKT4, OKT8 monoclonal antibodies were similar between serum-free media and 10% FCS, culture conditions and dilution failed to influence this rough titration (Table IV). Furthermore the peak fluorescence (channel number) used as an indicator of binding affinity (Table IV) showed a similar distribution throughout.

Our results indicate that the HB43 and HB57 cell lines adapted to grow in serum-free media, produced similar amounts (about 28–36 μg per 10^6 cells per 48 h) of monoclonal antibodies in the different types of media tested throughout the experiment, with the sole exception of ITS + premix which in the case of the HB43 line gave a 70% higher production than that obtained in 10% FCS medium (46 μg per 10^6 cells per 48 h).

Score analysis

The score analysis for all the hybrids placed the media in the following decreasing order from best to worst: for viability: DCCM1, DCCM2, FEB100, ITS + premix, NU serum, Nutridoma, BMS, Ultradoma, Ultroser HY and for doubling time and proliferative capacity: Nutridoma, DCCM1, NU serum, FEB100, DCCM2, ITS + premix, BMS, Ultradoma, Ultroser HY.

Discussion

In this study we have evaluated and compared the growth supporting capacity of some commercially available serum substitute media (NU-serum, BMS, Ultroser HY, ITS + premix, Nutridoma HS, FEB100, Ultradoma, DCCM1, DCCM2) on the in vitro culture conditions of 6 different murine hybridomas (OKT3, OKT4, OKT8, HB43, HB57, CRL8019). Our results show that the hybrid cell lines tested can be successfully cultured in 5 out of 9 serum-free media tested.

Based on doubling time and pooling of data for all 6 different hybrids with each serum-substitute medium, the serum-free media that best supported growth of the hybrid cell lines tested were: Nutridoma, DCCM1, NU serum, FEB100, DCCM2 with no difference with FCS, while ITS + premix, BMS, Ultroser HY and Ultradoma gave the worst result.

Doubling time was chosen because it seemed to be the most informative parameter indicating optimal hybrid cell culture conditions. In fact the score placed the media in the same order for doubling time and proliferative capacity, while

for viability, the lower surviving percentages found in some cases were not always correlated with a parallel increase in doubling time. Therefore, determination of viability alone is not a predictive parameter for the evaluation of the growth supporting capacity of a serum substitute medium.

Since growth of murine hybridomas derived from various myeloma fusion partners, differs in dependence on individual components of serum-free media, we analysed the compositions of the various media tested.

Evaluation of the critical function of each component is difficult due to the poor or non-existent information on media formulations supplied by certain manufacturers.

DCCM1 and DCCM2 have a completely unknown composition whereas Nutridoma, FEB100 and ITS + premix are similar for the presence of insulin, transferrin, lipids and BSA. ITS + premix, FEB100 and also Ultroser HY contain selenium, while ethanolamine is present only in FEB100 and glucose, amino acids and vitamins only in Nutridoma. NU serum is a rich serum substitute which, in addition to the above substances, contains growth factors, hormones and FCS corresponding to a final concentration of 2.5% in culture medium but which proved unable to support cell growth when used alone.

If we compare cell viability in FEB100 and Nutridoma our data are partially in agreement with other reports demonstrating the essential role of ethanolamine for cell viability (Murakami et al., 1982; Kovar and Franek, 1986) but this was evident only for the OKT3, OKT8 and CRL8019 hybrid lines. On the other hand, hydrocortisone and 17β-estradiol present only in NU serum did not show any influence (Kovar and Franek, 1986).

Of the four media unable to support sufficient growth, Ultradoma has an unknown composition. BMS lacks the fundamental substances known to support cell proliferation (insulin, transferrin, selenium and lipids) and the results are as expected. Ultroser HY and ITS + premix are exceptions. They have a composition similar to FEB100, but they were unable to support cell viability over 50% and doubling times shorter than 48 h. We can only suggest that the different components were not present in sufficient amounts.

In conclusion, we have demonstrated that some murine hybridomas can be grown in commercial serum substitute media. Using the culture conditions adopted, most of the hybrid lines maintained viability percentages, doubling times and proliferative capacities similar to the parental lines grown in 10% FCS, but it is unlikely that one serum-free medium would support the growth of all hybrid cell lines. We can assume the existence of some unknown limiting nutrients. Furthermore, our and other experiments (Kovar and Franek, 1986) have demostrated that hybridomas adapted to grow in serum-free media retain their capacity to synthetise monoclonal antibodies with the specifity, affinity and yields similar to those obtained using traditional culture conditions.

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References

Baish, H., Beck, H.P., Christensen, I.J., Hartmann, N.R., Fried, J., Dean, P.N., Gray, J.W., Jett, J.H., Johnston, D.A., White, R.A., Nicolini, C., Zeitz, S. and Watson, J.V. (1982) A comparison of mathematical methods for the analysis of DNA histograms obtained by flow cytometry. Cell Tissue Kinet. 15, 235.

Barnes, D. (1987) Serum free animal cell culture. Biotechniques 5, 534.

Barnes, D. and Stato, G. (1980) Methods for growth of cultured cells in serum-free medium. Anal. Biochem. 102, 255.

Facchini, A., Mariani, E., Mariani, A.R., Papa, S. and Manzoli, F.A. (1987) Increased number of circulating Leu11c+ (CD16) large granular lymphocytes and decreased NK activity during human ageing. Clin. Exp. Immunol. 68, 340.

Goding, J.W. (1986) Monoclonal Antibodies: Principle and Practice. Academic Press, New York, p. 69.

Kovar, J. and Franek, F. (1986) Serum-free medium for hybridoma and parental myeloma cell cultivation. Methods Enzymol. 121, 277.

Mariani, E., Roda, P., Mariani, A.R., Vitale, M., Degrassi, A., Papa, S. and Facchini, A. (1990) Age-associated changes in $CD8^+$ and $CD16^+$ reactivity: clonal analysis. Clin. Exp. Immunol. 81, 479.

Murakami, H. (1989) In: A. Mizrahi (Ed.), Advances in Biotechnology Processes, Vol. 11. Alan R. Liss, New York, p. 107.

Murakami, H., Masui, H., Sato, G.H., Sveoka, N., Chow, T.P. and Kano-Svekoda, T. (1982) Growth of hybridoma cells in serum-free medium: ethanolamine is an essential component. Proc. Natl. Acad. Sci. U.S.A. 79, 1158.

Rizzino, A., Rizzino, H. and Sato, G. (1989) Defined media and the determination of nutritional and hormonal requirements of mammalian cells in culture. Nutr. Rev. 37, 369.

Schneider, Y.J. (1989) Optimisation of hybridoma cell growth and monoclonal antibody secretion in a chemically defined, serum- and protein-free culture medium. J. Immunol. Methods 116, 65.

Shacter, E. (1989) Serum-free media for bulk culture of hybridoma cells and the preparation of monoclonal antibodies. Tibtech 7, 248.

Tharakan, P., Lucas, A. and Chau, P. (1986) Hybridoma growth and antibody secretion in serum-supplemented and low protein serum-free media. J. Immunol. Methods 94, 225.