Lait (1994) 74, 127-137

Original article

Whey and β-lactoglobulin: 2 milk by-products which can replace fetal calf serum in mouse hybridoma cell culture

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(Received 29 September 1993; accepted 6 January 1994)

Summary — In previous papers we showed that bovine whey allows short-term hybridoma cell culture and that for long-term culture it was necessary to add 1% fetal calf serum. In this paper we are demonstrating firstly, how to substitute 1% fetal calf serum by 1% horse serum and show that the mixture, 9% bovine whey + 1% horse serum, gives similar cell growth results and antibody production results; secondly, we are demonstrating that this mixture, less expensive, could be lyophilized which allows to envisage industrially processed preparations; and finally, how in order to obtain a substitute which is better defined and still less expensive than fetal calf serum, one can replace bovine whey by its major protein, β-lactoglobulin, which has also been complemented with 1% horse serum.

whey / β-lactoglobulin / cell culture / hybridoma

Résumé — Lactosérum et β-lactoglobuline : 2 coproduits du lait qui peuvent remplacer le sérum de veau fœtal dans la culture de cellules d'hybridomes de souris. *Nous avons déjà montré que le lactosérum bovin permettait la culture des cellules d'hybridomes à court terme et que, par contre il était nécessaire, pour les cultures à long terme de lui ajouter 1% de sérum de veau fœtal. Dans ce travail, nous avons remplacé le sérum de veau fœtal par du sérum de cheval et montré que le mélange 9% lactosérum + 1% sérum de cheval, beaucoup moins cher que le sérum de veau fœtal, donnait des résultats similaires, tant en croissance qu'en production d'anticorps. De plus, nous avons montré que ce substitut pouvait être lyophilisé, ce qui permet d'envisager d'éventuelles préparations par des procédés industriels. Enfin, afin d'obtenir un substitut mieux défini et qui reste moins cher que le sérum de veau fœtal, nous avons remplacé le lactosérum par sa protéine majeure : la β-lactoglobuline, qui a été, elle aussi, complémentée par 1% de sérum de cheval.*

lactosérum / β-lactoglobuline / culture cellulaire / hybridomes

INTRODUCTION

Optimization of culture conditions, especially for media, has been the subject of numerous investigations.

The cost of media is the most crucial factor in determining unit production cost for mammalian cell cultures; fetal calf serum accounts for 80% of this cost. Moreover, its use is undesirable because of its high protein content which hinders purification and because it may carry the bovine spongiform encephalitis (EBS) prion. Many substitutes are now available and some authors have shown that bovine colostrum or milk can provide indispensable products for eucaryotic cell growth *in vitro* (Ramirez *et al,* 1990; Pakkanen *et al,* 1992). In our laboratory, we have previously demonstrated that bovine whey (BW), a dairy by-product, can also replace fetal calf serum in short-term hybridoma cell line cultures (Damerdji *et al,* 1988). However Legrand *et al* (1993) have shown that for long-term culture, 1% of fetal calf serum (FCS) must be added to bovine whey.

In addition, bovine whey is a mixture as complex as FCS and it contains numerous hormones, peptides, glucides, lipids, proteins, and minerals (Derouiche *et al,* 1990). Recently, proteose-peptones of bovine whey were revealed as mitogenic factors of the Mark 3 hybridoma (Mati *et al,* 1993).

The aim of the present work was: 1) to find a substitute for the FCS added at whey by-products which would be as efficient, in terms of cell proliferation, and less expensive than FCS; newborn calf serum (NBCS) and horse serum (HS) were tested; 2) to obtain a medium of better defined composition by replacing bovine whey with its principal constituant, β-lactoglobulin (β-Lg); 3) to check the conservation of different media tested for their eventual preparation by industrial processing.

MATERIALS AND METHODS

Cell line

The hybridoma used in this study was a murine-murine Mark 3 hybridoma, secreting IgG1 against rat kappa chain immunoglobulin. The line was kindly provided by Pr H Bazin (Brussels, Belgium).

The cells were routinely cultured in RPMI 1640 medium (T 121-10 Seromed Biochrom; Berlin, Germany) supplemented with 2 mmol/l glutamine (G 1517 Sigma, St Louis, USA) and 10% fetal calf serum (J Boy, Reims, France) for control culture. For the other cultures, FCS was replaced by different substitutes.

Before the experiments, cell and culture media substitutes were tested for the absence of mycoplasma according to the methods of the Pasteur Institute (Paris, France): DAPI (Russel *et al,* 1975), direct cultures (Aymard *et al,* 1982) and enzymatic test using adenosine phosphorylase (Bonissol *et al,* 1984).

Whey preparation

Bovine whey, a by-product of cheese-making, was kindly provided by Est-lait (Vigneulles-lès-Hattonchatel, France). Whey was filtered in our laboratory on ten-inch cartridges (Millipore Corporation, Bedford, USA) and by tangential ultrafiltration with two polysulfone membranes (Pellicon cut-off 10 000 Da). Then the retentate was heated for 1 h at 56°C, concentrated by tangential ultrafiltration to obtain a final concentration of about 20 g l^{-1} protein (Derouiche *et al,* 1990), sterilized by filtration on a 0.22-μm membrane (Millipack 200, Millipore) and stored at –20°C in sterile 500-ml flasks until the experiments. It was heated again for 1 h at 56°C immediately before use.

Horse serum preparation

Home-made horse serum (HMHS) was obtained from blood collected without coagulant. After centrifugation (25 min, 20°C, 2800 *g*), it was filtered

twice at 0.22 µm on a Millipack 200 filter and stored at –20°C in sterile 100-ml flasks until the experiments. It was heated for 1 h at 56°C before use.

Conservation of substitutes

Generally FCS or substitutes were conserved at –20°C. Lyophilisation was also tested: the BW + HS mixture had been lyophilized, stored 1 year at room temperature and rehydrated up to initial volume just before its use as medium supplement.

β-Lactoglobulin purification

The β-lactoglobulin variant A/B was obtained according to the method of Iung *et al* (1987) and Moulti-Mati *et al* (1991) from a pool of milk. The whey was dialyzed briefly against buffer 1 (Tris-HCl, 0.001 mol/l (pH 7.5), 0.02 mol/l NaCl) and the purification was performed in two steps. The first step was an anion exchange chromatography on DEAE Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden). β-Lg was eluted with buffer 2 (Tris-HCl 0.04 mol/l (pH 7.5), 0.25 mol/l NaCl). The second step was a gel filtration on Sephadex G100. β-Lg was eluted with a phosphate buffer 0.02 mol/l (pH 6.7), containing NaCl 0.1 mol/l. β-Lactoglobulin purity was checked by PAGE electrophoresis in a non-dissociation buffer as described by Hillier (1976) and by FPLC chromatography using an anion exchange column (Mono Q).

Cultures

Before the experiments, the cells were progressively adapted to the substitutes. The cells were cultured in 25-cm^2 flask in the presence of increasing amounts of substitutes for about 15 days. The control cells were cultured in a medium supplemented with 10% FCS.

At day 0, cells were inoculated at 20 10^4 cells/ml into spinner flasks containing 150 ml RPMI medium supplemented with substitutes and maintained under agitation (50 rpm) in CO_2 incubator.

All the results were compared to data obtained with control cultures performed in the same conditions but in the presence of 10% FCS. Daily cell density and living cell number of each flask were determined in triplicate using a hemacytometer after staining with 0.5% trypan blue solution.

Monoclonal antibody (MAb) measurement was carried out on the culture supernatants obtained after centrifugation (800 g, 5 min) of the cell suspension. The whole cells were seeded again in spinner flasks containing fresh medium. These cultures were carried out for 6 or 7 days, until the cell viability fell to 50%.

Antibody concentration determination

An ELISA test was performed in order to monitor the capacity of hybridomas to produce and secrete immunoglobulins: goat antimouse IgG antibody (Tago, Burlingame, USA) was absorbed onto plastic plates (3912, Microtest 96 wells, Becton Dickinson Labware) overnight at 4°C. After removing the solution and rinsing with PBS (pH 7.3), BSA (20 mg/ml, Gibco Cergy-Pontoise, France) was added to each well for 2 h at 37°C. Samples to test were added at different dilutions and incubated for 2 h at 37°C. The wells were washed with PBS-Tween (1:1000 v/v) and antimouse IgG and IgM peroxidase-conjugated antibodies (Tago) were added in each well and incubated for 1 h at 37°C. Then, each well was washed with a pH 6 phosphate buffer and an enzyme substrate solution was added. The reaction was stopped after 10 min and absorbance was measured at 450 nm on an ELISA reader (Vietech). Each MAb concentration was measured in triplicate. The results were expressed in mg/10^4 cells and were noted as 'specific secretions'.

Assays

Endotoxins were determined using the *Limulus* amebocyte lysate (LAL) gel-clot method (Levin and Bang, 1964) using the kit from IBF Biotechnics (Villeneuve-la-Garenne, France). The amebocyte lysate proteins coagulate in presence of lipopolysaccharides of Gram-negative bacteria.

The substitute sterility was controlled using the techniques of the Pharmacopée Française,

1985 with a nutritive broth and trypticase soja gelose.

The search for animal viruses was performed by the Laboratoire National de Pathologie Bovine (Lyon, France).

RESULTS

Substitution for FCS

Newborn calf serum (NBCS)

In this experiment, the RPMI 1640 medium was supplemented either with 10% FCS or with 10% NBCS. After adaptation, the Mark 3 hybridoma cells were cultivated for 6 days in spinner flasks. Figure 1A shows that the cell proliferations were quite similar in both media, but the MAb-specific secretion was lower in medium supplemented with NBCS after day 2. So, we discarded this serum although it is less expensive than FCS.

Horse serum

Then, two horse sera were tested, one commercially available (Boehringer Mannheim France SA, Meylan, France) and one home-made horse serum. As previously, the cells were grown in spinner flasks in medium containing either 10% FCS or 10% HS or HMHS.

Results differed according to the origin of the horse serum (fig 2A). With commercialized horse serum, cell proliferation was lower compared to control culture, whereas with HMHS, at day 4, the maximum cell count was 30% higher than in control culture (424 10^4 cells *versus* 320 10^4). The decrease in cell proliferation was slightly faster with horse sera than with FCS. The MAb-specific production was similar with both horse sera and with FCS (fig 2B). Since the home-made horse serum was cheap, it was used to substitute FCS in the following experiments.

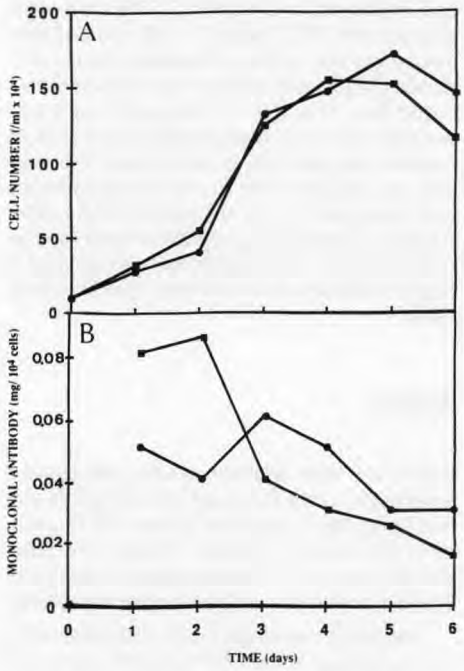

Fig 1. Comparison of cell proliferation (**A**) and monoclonal antibody production (**B**) in presence of fetal calf serum or newborn calf serum: hybridoma cells Mark 3 were seeded at 20 10^4 ml^{-1} in RPMI 1640 + 2 mmol/l glutamine + 10% FCS (●) or 10% NBCS (■). Cellular viability was estimated by trypan blue staining. Each data point represents the mean of three measurements. Monoclonal antibody concentration was measured by ELISA. The experiment was performed in duplicate.
Comparaison de la prolifération cellulaire (A) et de la production d'anticorps monoclonaux (B) en présence de sérum de veau fœtal (SVF) et de sérum de veau nouveau-né (SVNN) : les cellules d'hybridomes Mark 3 ont été ensemencées à 20 x 10^4 cellules/ml en RPMI 1640 + 2 mmol/l de glutamine + 10% de SVF (●) ou 10% de SVNN (■). La viabilité cellulaire a été estimée par coloration au bleu trypan. Chaque point représente la moyenne de 3 mesures. La concentration en anticorps a été mesurée par une technique ELISA. L'expérience a été réalisée 2 fois.

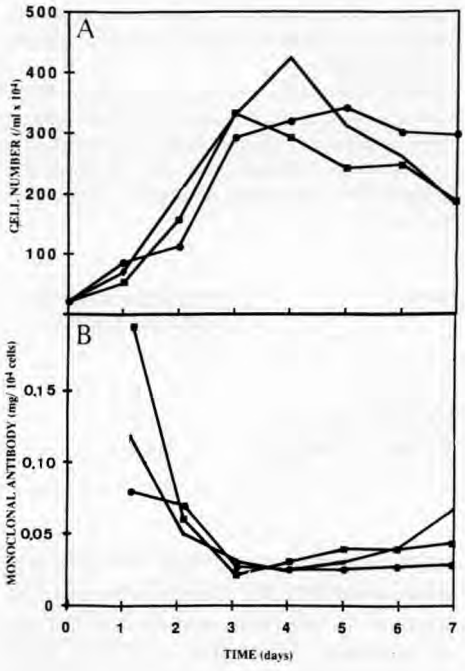

Fig 2. Comparaison of cell proliferation (**A**) and monoclonal antibody production (**B**) in presence of fetal calf serum or horse serum: hybridoma cells Mark 3 were seeded at 20 10^4 ml^{-1} in RPMI 1640 + 2 mmol/l glutamine + 10% FCS (●), 10% Boehringer horse serum (■) or 10% home-made horse serum (HMHS) (—). Cellular viability was estimated by trypan blue staining. Each data point represents the mean of three measurements. Monoclonal antibody concentration was measured by ELISA. The experiment was performed in duplicate.

Comparaison de la prolifération cellulaire (A) et de la production d'anticorps monoclonaux (B) en présence de sérum de veau fœtal (SVF) et de sérum de cheval (SC) : les cellules d'hybridomes Mark 3 ont été ensemencées à 20 x 10^4 cellules/ml en RPMI 1640 + 2 mmol/l de glutamine + 10% de SVF (●), 10% de SC Boehringer (■), ou 10% de SC «fabrication maison» (SCFM) (—). La viabilité cellulaire a été estimée par coloration au bleu trypan. Chaque point représente la moyenne de 3 mesures. La concentration en anticorps a été mesurée par une technique ELISA. L'expérience a été réalisée 2 fois.

Cell growth and antibody production with supplemented wheys

We have repeatedly shown that medium containing 9% bovine whey +1% FCS allows growth similar to that obtained with 10% FCS and an up to 20% increase in antibody cumulative production (Legrand *et al,* 1993). Previous experiments have shown that replacing FCS by horse serum allows good cell proliferation. So three comparative cultures were carried out in spinner flasks: one with 10% FCS, a second with 9% BW + 1% FCS and a third with 9% BW +1% HMHS.

The best cell proliferation was obtained with 9% BW + 1% FCS, although 9% BW +1% HS also gave a good proliferation (fig 3A). The secretions/cell count ratios were better for the cells cultured in the presence of whey especially when it was supplemented with HMHS (fig 3B). This observation confirmed the results obtained in other experiments (Legrand *et al,* 1993).

However, the horse serum used in this experiment was preserved for 1 year at –20°C. Therefore another experiment under the same conditions was started using fresh horse serum and we obtained the same results. The cell proliferations were similar in the three media, with nevertheless a small deficit in BW + 1% HMHS cell count (fig 4A). The MAb-specific productions were higher in whey throughout the duration of the culture, whether it was supplemented with FCS or with HS (fig 4B) and the mixture of whey and HMHS gave a 15 mg/l increase in MAb-cumulated production compared to the control medium (table I).

Lyophilized bovine whey

Therefore, whey added to FCS or HS permitted cell growth and MAb production. Nevertheless, we have observed a decrease in its activity after 6 months of storage at

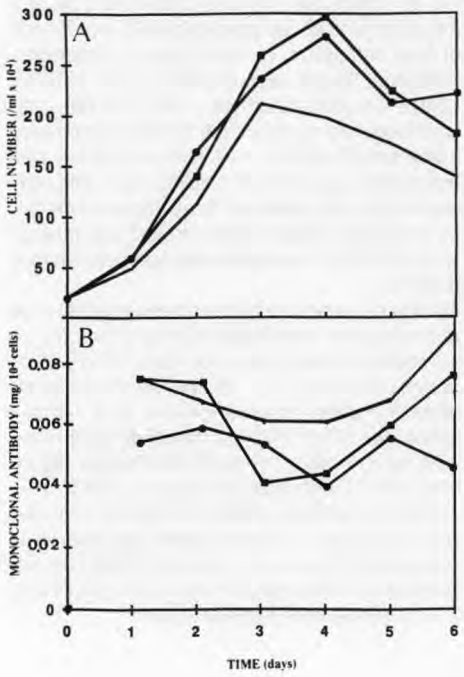

Fig 3. Comparison of cell proliferation (**A**) and monoclonal antibody production (**B**) in presence of fetal calf serum or whey supplemented: hybridoma cells Mark 3 were seeded at 20 10^4 ml^{-1} in RPMI 1640 + 2 mmol/l glutamine + 10% FCS (●), 9% BW + 1% FCS (■) or 9% BW + 1% HMHS (—). Cellular viability was estimated by trypan blue staining. Each data point represents the mean of three measurements. Monoclonal antibody concentration was measured by ELISA in triplicate.

Comparaison de la prolifération cellulaire (A) et de la production d'anticorps monoclonaux (B) en présence de sérum de veau fœtal (SVF) ou de lactosérum supplémenté. Les cellules d'hybridomes Mark 3 ont été ensemencées à 20 x 10^4 cellules/ml en RPMI 1640 + 2 mmol/l de glutamine + 10% de SVF (●), 9% de lactosérum + 1% SVF (■) ou 9% de lactosérum + 1% SCFM (—). La viabilité cellulaire a été estimée par coloration au bleu trypan. Chaque point représente la moyenne de 3 mesures. La concentration en anticorps a été mesurée par une technique ELISA. L'expérience a été réalisée 3 fois.

Table I. Comparison of monoclonal antibody production cumulated during 7 days of culture in three media.

Comparaison des productions d'anticorps monoclonaux cumulées durant 7 j de culture dans 3 milieux : 10% sérum de veau fœtal, 9% lactosérum + 1% sérum de veau fœtal et 9% lactosérum + 1% sérum de cheval «fabrication maison».

Media	*Monoclonal antibody production (mg)*
10% FCS	18.6
9% BW + 1% FCS	27.3
9% BW + 1% HMHS	33.7

–20°C (Legrand *et al,* 1993). Similarly, previous experiments showed that HS lost some of its mitogenic activity during cryoconservation. As the BW + HMHS mixture appeared promising for MAb productivity we studied the mitogenic effect of the lyophilized mixture (bovine whey + 1% HMHS).

As shown in figure 5A, cell proliferations in the three media (FCS, cryoconserved BW + HMHS, lyophilized BW + HMHS) were very similar with however a better result for the lyophilized BW + HMHS compared to BW + HS, frozen for 2 months at –20°C.

As in our previous results, MAb-specific production was slightly superior in presence of frozen whey and similar in presence of lyophilized whey (fig 5B). As lyophilization allows a much better long-term conservation, we have currently used the lyophilized substitute for supplementation of our culture media.

β-Lactoglobulin substitution for whey

Bovine whey has been shown to be a good substitute for FCS, but it remains (as FCS) an undefined composition medium. β-Lg is

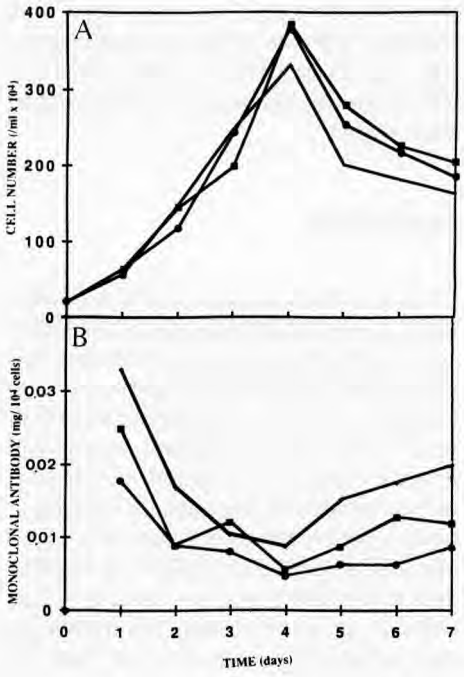

Fig 4. Comparison of cell proliferation (**A**) and monoclonal antibody production (**B**) in presence of fetal calf serum or whey supplemented: hybridoma cells Mark 3 were seeded at 20 10^4 ml^{-1} in RPMI 1640 + 2 mmol/l glutamine + 10% FCS (●), 9% BW + 1% FCS (■) or 9% BW + 1% fresh HMHS (—). Cellular viability was estimated by trypan blue staining. Each data point represents the mean of three measurements. Monoclonal antibody concentration was measured by ELISA in triplicate.

Comparaison de la prolifération cellulaire (A) et de la production d'anticorps monoclonaux (B) en présence de sérum de veau fœtal (SVF) ou de lactosérum supplémenté. Les cellules d'hybridomes Mark 3 ont été ensemencées à 20 x 10^4 cellules/ml en RPMI 1640 + 2 mmol/l de glutamine + 10% de SVF (●), 9% de lactosérum + 1% SVF (■) ou 9% de lactosérum + 1% SCFM frais (—). La viabilité cellulaire a été estimée par coloration au bleu trypan. Chaque point représente la moyenne de 3 mesures. La concentration en anticorps a été mesurée par une technique ELISA. L'expérience a été réalisée 3 fois.

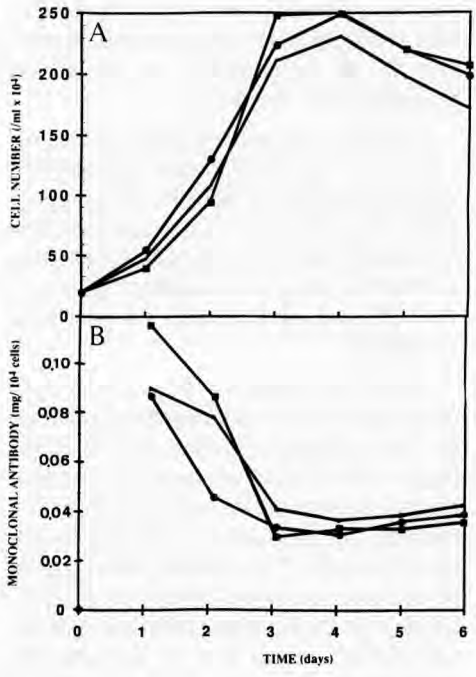

Fig 5. Comparison of cell proliferation (**A**) and monoclonal antibody production (**B**) in presence of fetal calf serum or whey supplemented: hybridoma cells Mark 3 were seeded at 20 10^4 ml^{-1} in RPMI 1640 + 2 mmol/l glutamine + 10% FCS (●), lyophilized 9% BW + 1% HMHS (■) or cryoconserved 9% BW + 1% HMHS (—). Cellular viability was estimated by trypan blue staining. Each data point represents the mean of three measurements. Monoclonal antibody concentration was measured by ELISA. The experiment was performed in duplicate.

Comparaison de la prolifération cellulaire (A) et de la production d'anticorps monoclonaux (B) en présence de sérum de veau fœtal (SVF) ou de lactosérum supplémenté. Les cellules d'hybridomes Mark 3 ont été ensemencées à 20 x 10^4 cellules/ml en RPMI 1640 + 2 mmol/l de glutamine + 10% de SVF (●), 9% de lactosérum lyophilisé + 1% SVF (■) ou 9% de lactosérum congelé + 1% SCFM frais (—). La viabilité cellulaire a été estimée par coloration au bleu trypan. Chaque point représente la moyenne de 3 mesures. La concentration en anticorps a été mesurée par une technique ELISA. L'expérience a été réalisée 2 fois.

the major protein of whey (2.7 g/l); Moulti-Mati *et al* (1991) demonstrated that in short runs, 2.7 g/l β-Lg + 1% FCS may be used as a substitute for 10% FCS.

In order to determine if HMHS, in the presence of 2.7 g/l β-Lg, could replace FCS, we compared cell growth and MAb production in three cultures in 7-day spinners. The first culture was performed with RPMI 1640 + 10% FCS, the second with 2.7 g/l β-Lg + 1% FCS and the third with 2.7 g/l β-Lg + 1% HMHS.

Figure 6A shows that β-Lg + FCS induced a slight lag-time cell growth and decreased cell proliferation. On the other hand, the β-Lg + HMHS medium permitted a more regular growth curve with maximum cell density: 20% above that of the FCS reference medium. The specific MAb productions were somewhat difficult to interpret because of the important differences of cellular growth. However, if we consider the cumulative antibody production during the whole of the culture, they were similar in the three media (table II). Therefore, this medium, with better defined composition, was interesting for the hybridoma cell culture. However, it was more expensive than bovine whey.

DISCUSSION

From a biotechnological and economical point of view, the processing costs of monoclonal antibody production should be as low as possible. Some efforts have been made to reduce or even completely eliminate FCS as a nutritional supplement. Kouar and Franck (1986) and Mariani *et al* (1991) demonstrated that hybridomas adapted to grow in serum-free media retain their capacity, affinity and yields, similar to those obtained using traditional culture conditions. But, while for short-term culture the hybridoma cells adapted to low serum levels grew better, a long-term exposure to low-serum or to serum-free media resulted in a loss of

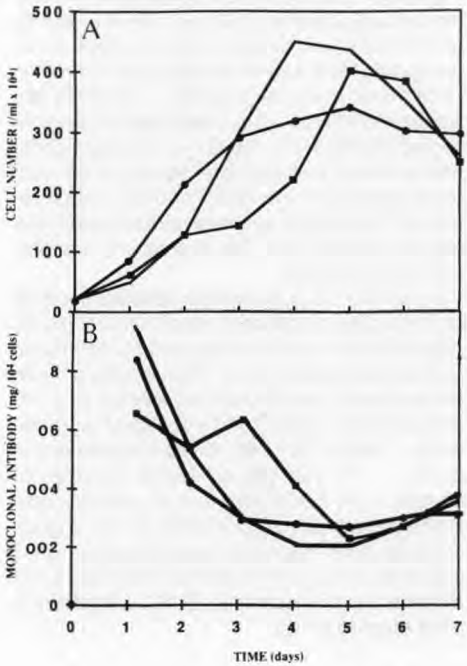

Fig 6. Comparison of cell proliferation (**A**) and monoclonal antibody production (**B**) : hybridoma cells Mark 3 were seeded at 20 10^4 ml^{-1} in RPMI 1640 + 2 mmol/l glutamine + 10% FCS (●), 2.7 g l^{-1}. β-Lg + 1% FCS (■) or 2.7 g l^{-1}. β-Lg + 1% HMHS (—). Cellular viability was estimated by trypan blue staining. Each data point represents the mean of three measurements. Monoclonal antibody concentration was measured by ELISA. The experiment was performed in duplicate.
Comparaison de la prolifération cellulaire (A) et de la production d'anticorps monoclonaux (B). Les cellules d'hybridomes Mark 3 ont été ensemencées à 20 x 10^4 cellules/ml en RPMI 1640 + 2 mmol/l de glutamine + 10% de SVF (●), 2,7 g/l de β-Lg + 1% SVF (■) ou 2,7 g/l β-Lg de lactosérum congelé + 1% SCFM frais (—). La viabilité cellulaire a été estimée par coloration au bleu trypan. Chaque point représente la moyenne de 3 mesures. La concentration en anticorps a été mesurée par une technique ELISA. L'expérience a été réalisée 2 fois.

Table II. Comparison of monoclonal antibody production cumulated during 7 days of culture in three media.
Comparaison des productions d'anticorps monoclonaux cumulées durant 7 j de culture dans 3 milieux : 10% sérum de veau fœtal, 2,7 g/l β-Lg + 1% sérum de veau fœtal et 2,7 g/l β-Lg + 1% sérum de cheval «fabrication maison».

Media	*Monoclonal antibody secretion (mg)*
10% FCS	8.2
β-Lg + 1% FCS	7.9
β-Lg + 1% HMHS	8.0

specific antibody productivity (Ozturk and Palsson, 1990, 1991). Perhaps the diminution of various hormones and growth factors is involved.

We and others have used cheap biological fluids to replace FCS. Murakami *et al* (1988) replaced serum by egg yolk lipoproteins in a wide variety of mammalian cell lines. The lipoproteins supported cell growth when used with insulin, transferrin, ethanolamine and selenite. Pakkanen *et al* (1992) showed that bovine colostrum ultrafiltrate provides an alternative to serum for production of monoclonal antibodies. However, the high-protein content and the presence of some insoluble sediments make colostrum difficult to handle although ultrafiltration resolved a part of these problems. This product, secreted during the first day after calving, remains available in lower quantities than whey.

We have chosen a substitute (9% bovine whey and 1% FCS or 1% HMHS) whose total protein concentration was lower than that brought by FCS (20 g/l *versus* 50 g/l). This substitute yielded similar cell concentrations and an increase of antibody production up to 20% compared to that obtained in medium supplemented with 10% FCS. It contains hormones, growth factors, metal and trace elements, such as Zn, Fe, Cu and L carnitine, an amino acid essential for cell proliferation as demonstrated by Derouiche *et al* (1990).

Nevertheless, 1% of serum remains necessary for good cell growth and secretion. Therefore, the substitution of 1% FCS by 1% HS is an attractive alternative: indeed, because of its specific alimentation, the horse is not susceptible to contract bovine spongiform encephalitis prion. Moreover, to our knowledge, no report describes identification of this prion in horse serum. It was desirable to replace the FCS in media by the preparation BW + HS in order to decrease the risk of infection agent in the final cell culture product. In addition, the toxins analyzed by LAL test (approved by the FDA in 1977), are present in negligible amounts in the mixture BW + HS.

Faulkner *et al* (1987) demonstrated that horse serum gave similar levels of growth promotion as FCS. Our results showed that it would be worthwhile to lyophilize BW + HS. This allows to diminish the problems of standardization of culture conditions, conservation and storage volumes.

The results obtained with β-Lg show that the mitogenic activity of whey is widely linked to β-Lg presence. In addition to its well-known nutriment function, its mitogenic activity is due to its capacity to transport hydrophobic components and minerals (Baumy and Brulé, 1988).

Spiecker-Polet and Polet (1981) have observed that β-Lg binds molecules such as fatty acids, triglycerides, vitamin A and metals such as Zn, Fe, Cu, whereas the long-term culture of murine hybridomas was not possible in medium supplemented with only 1% FCS (Moulti-Mati *et al*, 1991). The synergy of the mixture β-Lg + 1% FCS (or 1% HS) allows interesting results in growth and secretion.

Functional evaluation of each component is difficult and we can assume the existence of some unknown interactions. We nevertheless have with β-Lg a much better defined medium which is interesting even though its preparation requires some preliminary treatments of whey. It is worth noting that the purification steps are relatively simple and the product obtained has a good percentage of purity and an acceptable yield (Moulti-Mati *et al,* 1991). The novel combination of additives in β-Lg medium proved to be highly advantageous for antibody purification. These results have been checked with different hybrid cell lines established in our laboratory. We have demonstrated that some murine hybridomas can be grown and secreted in media complemented by cheap by-products of milk, similar to the parental lines grown in 10% FCS. Then it will be very interesting to study the substitution of 1% animal sera by a minimum number of hormones, other mitogenic molecules, perhaps selenite which is absent in BW or other components employed to varying degrees by others.

ACKNOWLEDGMENTS

This work was supported by grants from the University of Nancy I, Lorraine Region and Europeen Contrat (Biotechnology Action Program n° 0129 F). The authors thank Pr H Bazin for providing the hybridoma cell line used in this study and JM Girardet for his good advice.

REFERENCES

Aymard M, Bouvet C, Bossard S, Calvet A (1982) Diagnostic and epidemiology of acute respiratory due to *Mycoplasma pneumoniae. Rev Inst Pasteur* (Lyon) oct 42-44

Baumy JJ, Brulé G (1988) Binding of bivalent cations to α-lactalbumin and β-lactoglobulin: effect of pH and ionic strength. *Lait* 68, 33-48

Bonissol C, Traincard F, Stoiljkovic B, Hosli P (1984) Adenosine phosphorylase activity as a technique for detection of mycoplasmas in biological media. *Ann Microbiol (Inst Pasteur)* 135A, 63-72

Damerdji O, Derouiche F, Legrand C, Capiaumont J, Bour JM, Maugras M, Belleville F, Nabet P (1988) Utilization of whey fractions as a substitute for fetal calf serum in culture media. *Biotechnol Tech* 2, 253-258

Derouiche F, Legrand C, Bour JM, Capiaumont J, Gelot MA, Dousset B, Belleville F, Nabet P, Linden G (1990) Biochemical aspects of a whey fraction capable of promoting hybridoma proliferation. Comparison with fetal calf serum. *Lait* 70, 313-324

Faulkner J, Ling NR, Shakespere M (1987) Comparisons between serum supplements used in tissue culture. *Int Biotechnol Lab* 10, 8-16

Hillier RM (1976) The quantitative measurement of whey proteins using polyacrylamide-gel electrophoresis. *J Dairy Res* 43, 259-261

Iung C, Paquet D, Linden G (1987) Isolement de la β-lactoglobuline. Mise au point d'une méthode préparative rapide. *Sci Aliments* 7 (n° hors série VIII), 167-176

Kouar J, Franck F (1986) Serum-free medium for hybridoma and parental myeloma cultivation. *Methods Enzymol* 121, 277-285

Legrand C, Capiaumont J, Belleville F, Nabet P (1993) Comparison of the metabolism of hybridoma cells cultured in media supplemented with whey or fetal calf serum. *Biotechnol Progress,* in press

Levin J, Bang FB (1964) The role of endotoxin in the extracellular coagulation of limulus blood. *Bull Johns Hopkins Hosp* 115, 265-274

Mariani E, Mariani AR, Monaco MCG, Lalli E, Vitale M, Facchini A (1991) Commercial serum-free media: hybridoma growth and monoclonal antibody production. *J Immunol Methods* 145, 175-183

Mati A, Moulti-Mati F, Girardet JM, Fokou E, Belleville-Nabet F, Nabet P, Linden G (1993) Mitogenic activity of hydrophobic fractions of proteose peptone from cow', eves' and goats' milk measured with Mark 3 hybridoma culture. *J Dairy Res* 60, 443-448

Moulti-Mati F, Mati A, Capiaumont J, Belleville F, Linden G, Nabet P (1991) Rôle de la β-lactoglobuline dans l'activité proliférative du lactosérum. *Lait* 71, 543-553

Murakami H, Okazaki Y, Yamada K, Omura H (1988) Egg yolk lipoprotein, a new supplement for the growth of mammalian cells in serum-free medium. *Cytotechnology* 1, 159-169

Ozturk SS, Palsson BO (1990) Loss of antibody production during long-term cultivation of a hybridoma cell line in low serum and serum free media. *Hybridoma* 9, 167-175

Ozturk SS, Palsson BO (1991) Physiological changes during the adaptation of hybridoma cells to low serum and serum-free media. *Biotechnol Bioeng* 37, 35-46

Pakkanen R, Kanttinen A, Satama L, Aalto J (1992) Bovine colostrum fraction as a serum substitute for the cultivation of mouse hybridomas. *Appl Microbiol Biotechnol* 37, 451-456

Ramirez OT, Sureshkumar GK, Mutharasan R (1990) Bovine colostrum or milk as a serum substitute for the cultivation of a mouse hybridoma. *Biotechnol Bioeng* 35, 882-889

Russel WC, Newman C, Williamson DH (1975) A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasma and viruses. *Nature* 253, 461-462

Spieker-Polet E, Polet H (1981) Requirement of combination of a satured and unsatured free fatty acid and a fatty acid carrier protein for *in vitro* growth of lymphocytes. *J Immunol* 126, 949-954