

Efficient laboratory-scale production of monoclonal antibodies using membrane-based high-density cell culture technology

Mohamed Trebak, Jae Min Chong, Dorothee Herlyn, David W. Speicher *

The Wistar Institute, Room 151, 3601 Spruce Street, Philadelphia, PA 19104, USA

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Abstract

Monoclonal antibodies (MAbs) are important tools used in basic research as well as in the imaging and therapy of cancer. Many countries have limited the use of animals for large-scale production of MAbs, obliging laboratories to find efficient *in vitro* alternatives to ascites production. In this report we describe a protocol for laboratory-scale production of MAbs by culturing hybridoma cells in the two-chamber cell culture device CELLine 1000. This culture flask supports high cell densities (10^7 – 10^8 cells/ml) and generates high concentrations of MAbs (0.7–2.5 mg/ml). Two hybridomas producing MAbs directed against the gastrointestinal antigen GA733-2, GA733 MAb and CO17-1A MAb, were evaluated over culture periods of up to two months using several alternative conditions. Two different sets of conditions are reported; the first using serum-supplemented medium (20% v/v) and the second using serum-free medium (SFM). Average weekly yields of the purified MAbs in serum-supplemented medium were 24 mg and 33 mg, and in SFM were 21 mg and 17 mg for GA733 MAb and CO17-1A MAb, respectively. Experimental variables that can affect antibody production and economy include: nutrient medium and cell compartment medium compositions (cell line dependent), the proportion of the cell compartment medium harvested every 3 days (50% to 80% with 80% optimal) and the frequency of nutrient medium changes (3 to 9 days with 6 days as most cost effective). Protein-A Sepharose purification followed by antigen-specific affinity purification showed that MAbs obtained from serum-supplemented cultures contain less than 0.6% of bovine IgG contamination, while MAbs obtained from serum-free cultures contained no extraneous IgG. In addition, MAbs from both culture media were fully active (essentially 100%) as measured by their ability to bind to an antigen column. In contrast, the same MAbs purified from ascites using Protein-A-Sepharose typically contained a major portion of inactive IgG. This *in vitro* method for laboratory-scale production of MAbs (10 to 500 mg) proved to be simple, reproducible and cost effective. It represents a useful alternative to the *in vivo* production of MAbs in mice. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Monoclonal antibody production; Serum free media; CELLine high-density cell culture device; Affinity purification; Bovine Ig contamination; MAb activity

Abbreviations: DMEM, Dulbecco's modified eagle medium; ELISA, enzyme linked immunosorbent assay; FBS, fetal bovine serum; Ig, immunoglobulin; MAb, monoclonal antibody; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SFM, serum free medium

* Corresponding author. Tel.: +1-215-898-3972; fax: +1-215-898-0664; e-mail: speicher@wistar.upenn.edu

1. Introduction

Since the introduction of the hybridoma technology by Köhler and Milstein (1975), a variety of methodological approaches and technologies have been developed for large-scale production of MAbs. For a long time, the *in vivo* production in mice, by

ascites induction, was preferred for its cost effectiveness and high concentrations of MAbs produced. The growing ethical concern about MAbs production by ascites induction along with the improvement in cell culture equipment and techniques have led to an increased emphasis on *in vitro* methods, which are able to compete with the *in vivo* method both in capacity and cost effectiveness (for a recent review see De Geus and Hendriksen, 1998).

Conventional low cell density culture methods permit *in vitro* production of MAbs, which are released in the culture medium at concentrations between 1 and 100 $\mu\text{g}/\text{ml}$ (Falkenberg, 1998). The low concentration of MAbs in these cultures and the high degree of contamination with bovine serum proteins essential for the *in vitro* culture of hybridoma cells complicate purification methods. Contamination with bovine serum proteins, especially IgG can also limit the use of these MAbs in the field of clinical diagnosis and therapy, and in some basic research techniques that require MAbs of high purity and in a fully functional form.

In the past few years, effort has been invested in the design of high-density culture systems, leading to the development of various “bioreactors”. For example, hollow fiber systems can generate high yields of MAbs (100 mg/week on average) (Lowrey et al., 1994; Jackson et al., 1996; Kreutz et al., 1997) but only allow the production of one MAb at a time and suffer the disadvantages of expense, complexity, and being prone to contamination. Autoclavable tumbling chamber systems (Jaspert et al., 1995), modular minifermentors (Falkenberg et al., 1995) and packed-bed bioreactors containing glass cylinders (Moro et al., 1994) offer alternative possibilities for growing multiple hybridomas simultaneously and producing large quantities of MAbs (25 mg–1.5 g/week). However, the risk of contamination of these bioreactors with infectious agents can be substantial. In addition, such technology is typically limited to a few specialized laboratories with routine requirements for large quantities of MAbs since dedicated relatively expensive specialized equipment is needed.

In this study, we evaluated CELLline two-chamber culture flasks as a simple *in vitro* system to conveniently produce highly active MAbs. These specialized culture flasks only require standard cell culture equipment, involve conventional culture techniques,

provide high antibody concentrations (about 1 mg/ml), allow long-term use of one culture device for several weeks to several months, and permit the production of many MAbs in parallel. With a single cell culture device, we prepared more than 240 mg of a purified MAb in a two-month period. This MAb was shown to be fully active and highly pure, as assessed by affinity chromatography on an antigen column and by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. In addition, the production cost of the purified MAb did not exceed US\$5/mg. In summary, the CELLline culture flasks permit long-term culture of hybridoma cells in high density allowing abundant production of highly active and pure MAbs necessary for certain specific purposes.

2. Materials and methods

2.1. Media and reagents

The cell culture media used in this work were: (1) Dulbecco's Modified Eagle Medium (DMEM, 11995-073, Gibco BRL, Gaithersburg, MD) with 4.5 mg/l D-glucose, L-glutamine, 110 mg/l sodium pyruvate and pyridoxine hydrochloride; (2) Hybridoma-Serum-Free Medium (SFM, 12045-084, Gibco BRL) containing 20 mg/l of insulin and transferrin. Fetal Bovine Serum (FBS, S12450, Atlanta Biologicals, Norcross, GA) was de-complemented for 45 min at 57°C in a water bath before use. Versene 1/5000 (0.2 g EDTA/l, 15040-066) used during sub-culture to detach the hybridoma cells from T75 culture flasks was purchased from Gibco BRL. Recombinant GA733-2E (Strassburg et al., 1992) corresponding to the extracellular domain of GA733 was expressed in High Five insect cells and purified from the cell supernatants with a GA733 MAb-Sepharose affinity column. GA733 and CO17-1A MAbs were purified from ascitic fluids in our laboratory using Protein-A-Sepharose (CL-4B) purchased from Pharmacia Biotech, Uppsala, Sweden.

2.2. Hybridomas and culture conditions

The two hybridomas used in this work both produce IgG2a MAbs, and recognize the gastrointestinal

antigen GA733-2 with either high affinity (GA733 MAb, subsequently called “GA”; Herlyn et al., 1984) or moderate affinity (CO17-1A MAb, subsequently called “CO”; Herlyn et al., 1979; Koprowski et al., 1979; Powe et al., 1985). In vitro culture of hybridoma cells was performed using standard procedures in a 37°C incubator with an atmosphere containing 5% CO₂. Low-density cultures were performed in parallel with the high-density cultures under the same conditions. For low-density cultures, adherent hybridoma cells were washed twice with phosphate buffered saline (PBS, without calcium and magnesium), and detached using 1 ml of Versene solution. About 1.0×10^6 hybridoma cells were seeded in T75 flasks and cultured until confluence with medium renewal twice a week. For high-density cultures, about 2.6×10^7 cells in 15 ml of fresh culture medium were transferred to the cell chamber of a CELLLine 1000 flask (90-005, INTEGRA Biosciences, Ljamsville, MD). CELLLine 1000 is a membrane-based two-chamber flask where a 15 ml cell compartment is separated from an upper 1000 ml nutrient compartment by a 10 kDa molecu-

lar weight cut off (MWCO) membrane (Fig. 1). All media were antibiotic/antimycotic free. In general, cell compartment harvest and nutrient medium exchanges were done every three days. Briefly, 7.5 ml of mixed cell suspension from total cell chamber volume (15 ml) were removed and 7.5 ml of fresh pre-warmed culture medium were added to the 7.5 ml of cell suspension remaining in the cell compartment chamber. The harvested mixed cell suspension was centrifuged at $20,000 \times g$ for 20 min, the supernatant was removed, filtered with a 0.22 μm filter and stored at -20°C until purification; the cell pellet was discarded. The 1000 ml of nutrient compartment medium were replaced with fresh medium.

Two different culture conditions were evaluated with both hybridomas: (a) DMEM 20% FBS in the cell compartment and DMEM 5% FBS in the nutrient compartment; or (b) SFM in the cell compartment and a combination of 50% SFM and 50% DMEM 10% FBS in the nutrient compartment. Cell viability was routinely monitored by Trypan blue dye exclusion. The hybridomas were adapted to grow in SFM in five passages with increasing proportions

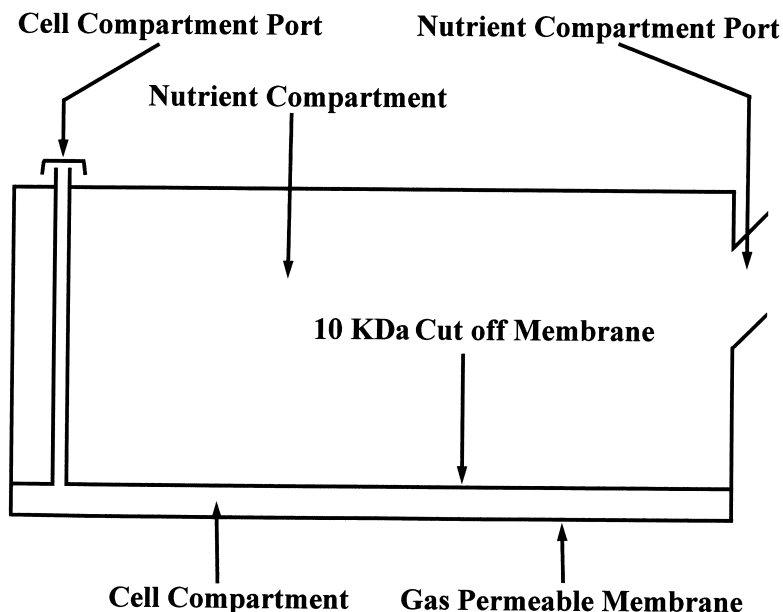


Fig. 1. Diagram of the CELLLine device. MAbs produced by the cells are retained within the small cell compartment volume while nutrients and other small molecules (< 10 kDa) pass across the semi-permeable membrane into and out of the cell compartment. The bottom surface of the cell compartment contains a gas permeable membrane across which oxygen and carbon dioxide rapidly diffuse, permitting high cell densities within this small cell compartment volume.

of SFM (25%, 50%, 75%, 87.5%, 100%) in the standard medium (DMEM 20% FBS) prior to large-scale culture in the two chamber flasks.

2.3. ELISA procedure

ELISA experiments were performed following standard procedures in 96 well Falcon Microtest III

non-tissue culture-treated polystyrene flexible plates (Becton Dickinson, Lincoln Park, NJ) coated with purified GA733-2E antigen and using Protein-A coupled to horseradish peroxidase (HRPO-Protein-A, 172-1018, Bio-Rad laboratories, Richmond, CA). CELLline culture supernatant that had been diluted 30-fold in PBS with 0.04% Triton X-100 was added in the first well and serial two fold dilutions were

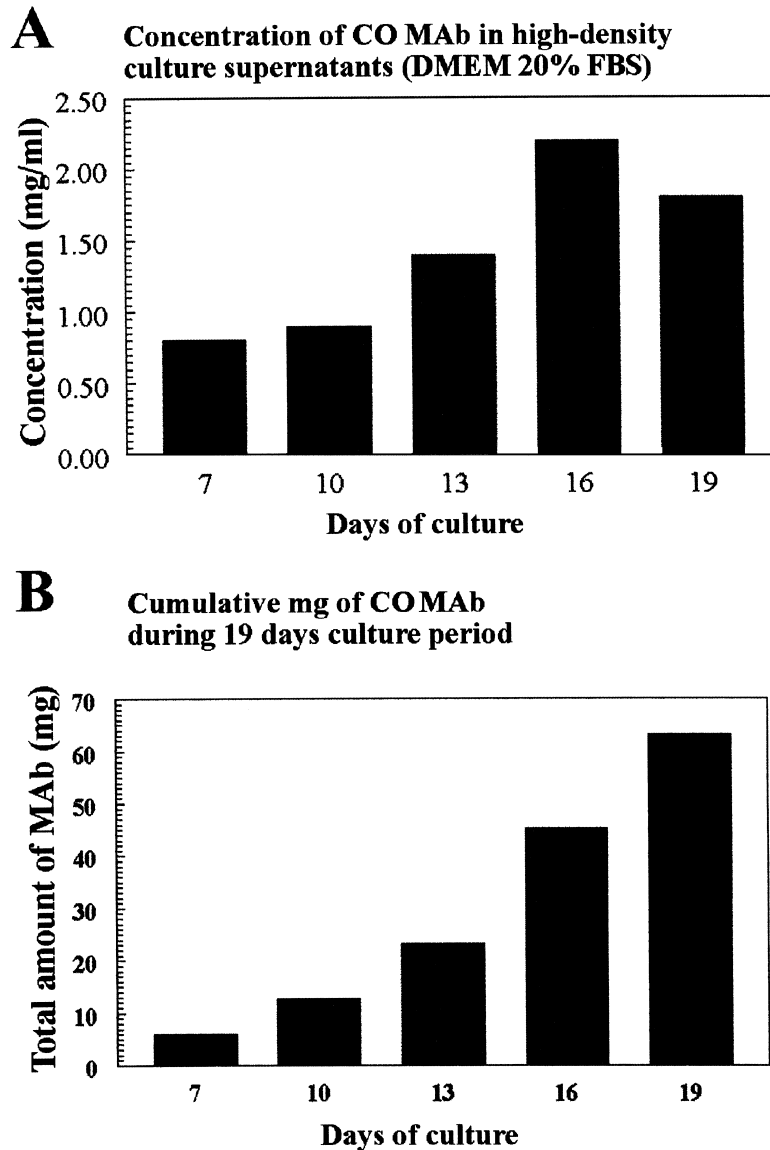


Fig. 2. (A) CO MAb concentration (determined by ELISA) in high-density culture supernatants (DMEM 20% FBS) from a single CELLline device. (B) Cumulative amount of CO MAb during the 19-day continuous culture period.

made. On each ELISA plate, samples of dilutions of the corresponding MAb that had been affinity purified on a specific antigen column were applied as an internal standard at known concentrations. From the colorimetric results obtained, a standard curve was plotted, and MAb concentrations in the culture supernatants were determined.

2.4. MAb purification and quantitation

IgG2a class GA and CO MAbs were purified using Protein-A-Sepharose column affinity chromatography. Bound fractions were eluted with 100 mM citric acid, pH 5.0. An additional purification on a GA733-2E-Sepharose column was performed to assess the proportion of the MAb pool that could bind to the specific antigen. Elution of the bound fraction from this column was accomplished with 100 mM triethylamine (TEA), pH 11.5. The GA and CO MAbs concentrations were determined by spectrophotometry at 280 nm using an extinction coefficient of $1.551 \text{ (mg/ml)}^{-1}$ using a 1-cm path length, calculated according to Pace et al. (1995).

2.5. SDS-PAGE

MAb purity was monitored before and after each purification step using 6% Tricine-SDS-PAGE under

non-reducing and reducing conditions according to Schagger and Von Jagow (1987). The protein bands were visualized by Coomassie blue R250.

3. Results

3.1. High yield MAb production using the membrane-based cell culture device, CELLline CL 1000

Two hybridomas secreting GA and CO MAbs directed against the colorectal carcinoma antigen GA733-2 were cultured using various conditions in the high density CELLline 1000 tissue culture devices. In the first series of experiments, hybridomas were cultured using DMEM with 20% FBS in the cell compartment and DMEM with 5% FBS in the nutrient compartment. Cell densities increased progressively and on day 13 reached 2.7×10^7 and 2.2×10^7 viable cells/ml for the CO and GA hybridomas, respectively. The first cell compartment harvest and nutrient medium renewal was on day 7.

The CO MAb concentration in the culture supernatants from the CELLline device, as determined by ELISA, ranged between 0.8 and 2.2 mg/ml (Fig. 2A). Over a 12-day period (days 7–19), approximately 64 mg of CO MAb were obtained (as deter-

Table 1

Concentration and weekly production of GA and CO MAbs in low density and high density cell culture systems with and without serum addition

| Antibodies | Culture type ^a | Medium | Number of weeks of production ^b | Purified MAb per ml of culture supernatant (mg/ml) ^c | Amount of MAb after purification (mg/week) ^d |
|------------|---------------------------|----------|--|---|---|
| GA MAb | Low-density | DMEM 20% | 16 | 0.046 | – |
| | | SFM | 6 | 0.025 | – |
| | High-density | DMEM 20% | 8 | 0.90 | 24.3 |
| | | SFM | 1 | 0.70 | 20.7 |
| CO MAb | Low-density | DMEM 20% | 16 | ND ^e | – |
| | | SFM | 6 | 0.026 | – |
| | High-density | DMEM 20% | 2 | 1.10 | 33.4 |
| | | SFM | 1 | 0.55 | 16.7 |

^aLow density-culture was performed in T75 tissue culture flasks in a total medium volume of 25 ml. High-density culture was carried out in the CELLline 1000 cell culture device (15 ml in the cell compartment and 1000 ml in the nutrient compartment).

^bDoes not include the first 7 days of culture required to reach high cell density.

^cValues based on total MAb recovered from the entire supernatant pool.

^dValues represent the average production per week (total mg purified/number of weeks of production). All values are based on MAbs recovered from a single CELLline cell culture device.

^eND: Not determined.

mined by ELISA) from a total volume of 60 ml (Fig. 2B). A total of 66.8 mg MAb were obtained after purification using Protein-A-Sepharose, which is in agreement with the amount estimated by ELISA. SDS-PAGE analysis showed essentially a 100% recovery of this MAb after Protein-A-Sepharose purifi-

cation (data not shown). The apparent 104% recovery reflects experimental uncertainty of the techniques used for estimating protein concentrations. Therefore, about 1.1 mg purified MAb was recovered per ml of culture supernatant, representing a production rate of about 33 mg/week (Table 1).

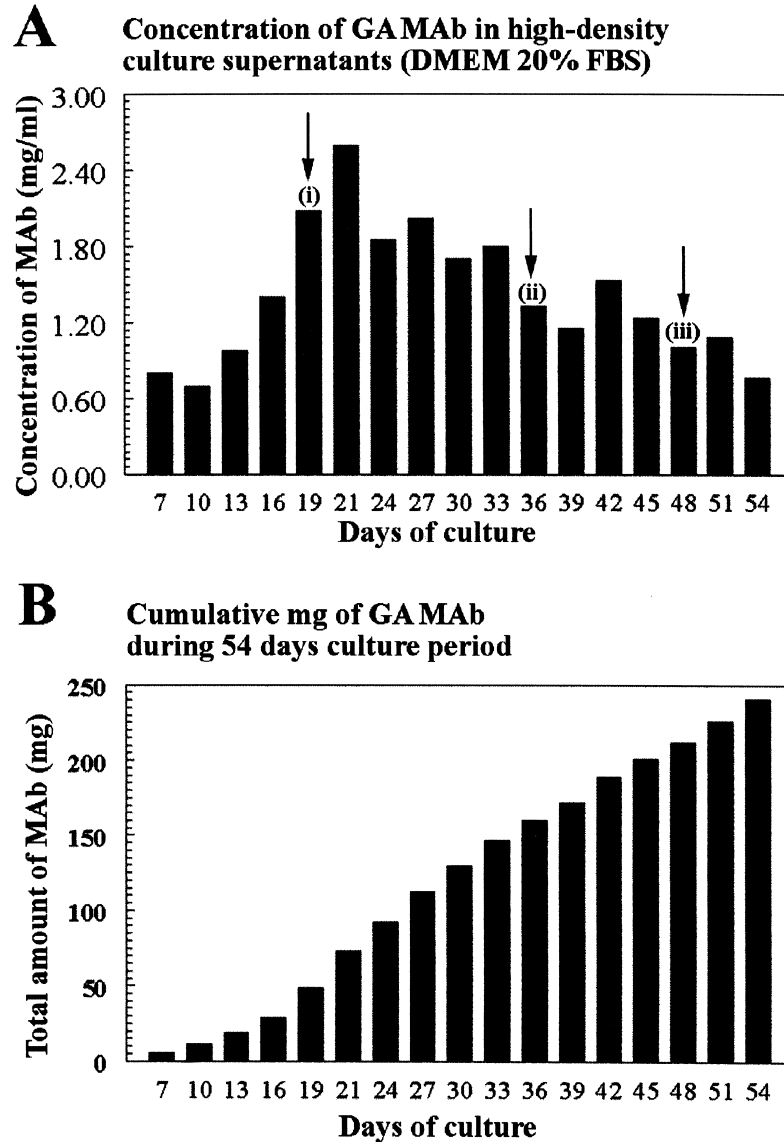


Fig. 3. (A) GA MAb concentration (determined by ELISA) in high-density culture supernatants (DMEM 20% FBS) from a single CELLline device. (i) Starting on day 19, 10–12 ml of the total cell compartment volume (15 ml) were taken during the harvest instead of 7.5 ml. (ii) Between days 36 and 45, nutrient medium was exchanged every 6 days (originally, nutrient medium was exchanged every 3 days). (iii) Between days 48 and 54, nutrient medium was exchanged every 9 days. (B) Cumulative amount of GA733 MAb during the 54 day continuous culture period.

The GA hybridoma was maintained over a culture period of two months to produce larger quantities of MAb and to explore culture method variations. The GA MAb ranged between 0.7 and 2.5 mg/ml (Fig. 3A). After 19 days of culture, a total of 50 mg (compared with 64 mg for CO MAb, see Fig. 2B)

had been produced in this single flask and after 2 months, a total of 241 mg had been produced (Fig. 3B). This corresponds to an estimated production rate of about 30 mg/week before purification (Fig. 3B). During the first 23 days of supernatant collection (days 7–30), a total of 85.6 mg was recovered

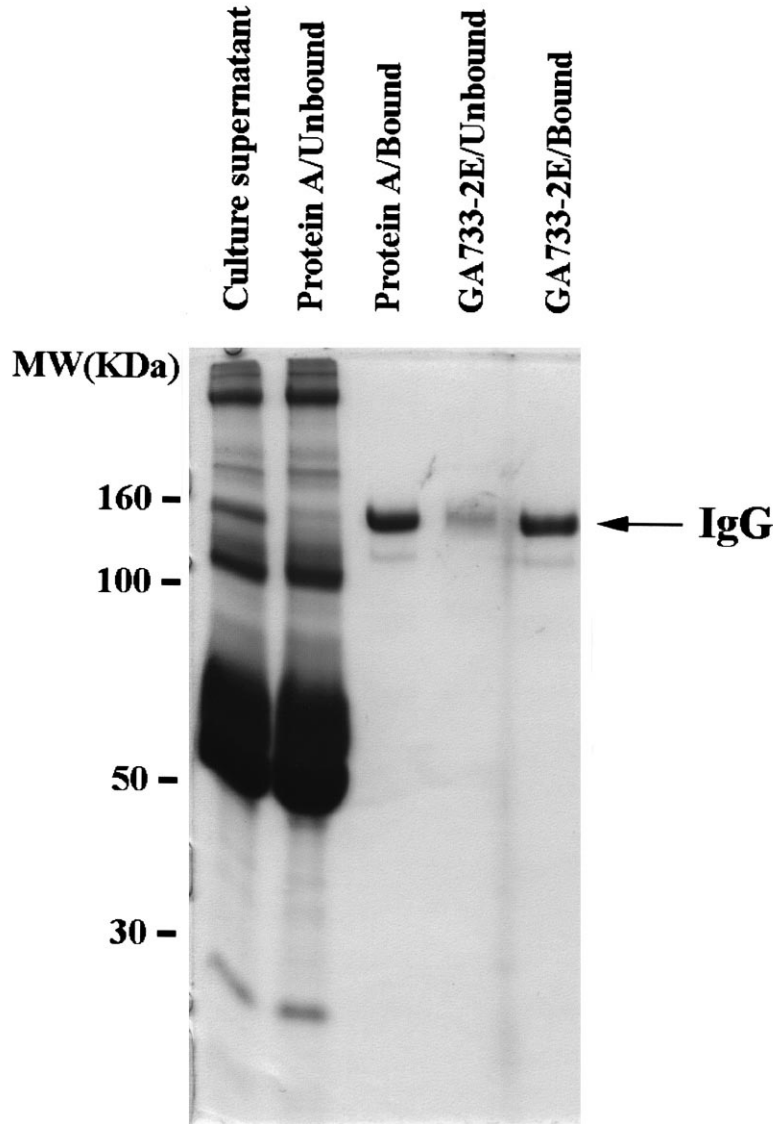


Fig. 4. SDS-PAGE analysis of fractions from Protein-A affinity purification and GA733-2E affinity purification of low-density cell culture supernatant of GA hybridoma cultured in DMEM 20% FBS. The Protein-A-bound fractions were eluted, pooled, dialyzed into PBS, and loaded onto the GA733-2E affinity column. The unbound fractions from this last purification represent 12% of the total amount of MAb loaded on to the column, which corresponds to the expected amount of non-specific bovine Ig contaminant.

Table 2

Comparison of quality, quantity and cost of GA MAb produced by different methods

| GA MAb production method ^a | Culture medium ^b | Estimated concentration range of different harvests by ELISA (mg/ml) | Purified MAb per ml of culture supernatant ^c (mg/ml) | Contamination (bovine Ig) | Activity ^d | Approximate cost (US\$/mg) |
|---------------------------------------|-----------------------------|--|---|---------------------------|-----------------------|----------------------------|
| Ascites | In vivo (mice) | 0.5–1.3 ^e | 0.1–0.3 | NA ^f | 22–73% | ND ^g |
| Low-density | DMEM 20% | 0.04–0.05 | 0.046 | 12% | 100% | 12–15 |
| High-density | DMEM 20% | 0.7–2.4 | 0.90 | 0.6% | 100% | 2–5 |
| Low-density | SFM | 0.025–0.040 | 0.025 | 0% | 100% | 16–21 |
| High-density | SFM | 0.8–1.7 | 0.70 | 0% | 100% | 3–6.8 |

^aAll high-density data are obtained with a single cell culture (CELLine) device.

^bFor DMEM 20% FBS, high-density results are based on an 8-week harvest period while the results of the SFM condition are based on a 1 week harvest period.

^cValues based on total MAb recovered from the entire supernatant pool.

^dActivity of MAbs was determined by the ability to bind the specific antigen in a GA733-2E affinity column.

^eConcentration based on total IgG recovery after Protein-A purification.

^fNA: not applicable; percentage of non-specific murine IgG in ascites fluid was not determined.

^gND: not determined.

after purification using Protein-A-Sepharose from 95 ml of pooled supernatants, which represented a final production rate of 24.3 mg/week (Table 1). Therefore, about 0.9 mg purified GA MAb was recovered per ml of culture supernatant (Table 1). High-density culture supernatants were also monitored before purification by analyzing aliquots of crude supernatants using 6% non-reducing SDS-gels (data not shown).

The frequency of nutrient medium exchange was one of the parameters evaluated during the long-term culture of the GA hybridoma. This initial evaluation showed that the best MAb yields were achieved by changing the medium every three days (Fig. 3A, days 7–33). However, exchanging the nutrient medium every 6 days (Fig. 3A, days 36–45) or every nine days (Fig. 3A, days 48–54) only moderately reduced weekly MAb production. Cell compartment harvests were performed every three days throughout the 2-month culture period. When the nutrient medium was renewed every 6 days and every 9 days, the GA MAb concentrations dropped to an average of 82% and 60% of the initial concentration, respectively (Fig. 3A). Hence, less frequent nutrient

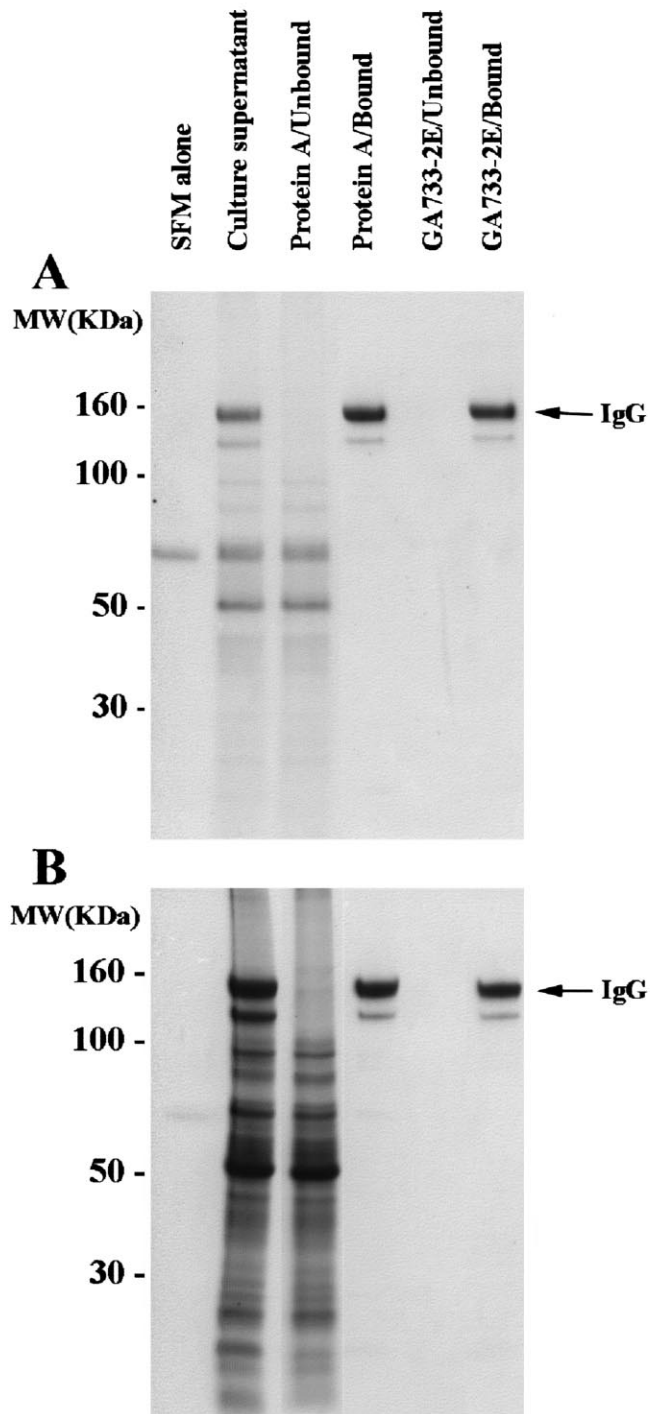
medium exchanges (every 6 days) should substantially reduce MAb production costs.

For comparative purposes, GA MAb was also purified from culture supernatants of hybridoma grown in regular low cell density tissue culture flasks (T75 Falcon, Becton Dickinson, Franklin Lakes, NJ) with DMEM 20% FBS. The concentration of GA MAb purified from this low-density culture was about 0.046 mg/ml; that is, at least 20-fold less concentrated than the same MAb purified from high-density culture (Table 1).

3.2. Assessment of bovine serum Ig contamination of MAbs

Standard methods for in vitro culture of hybridoma cells require the use of media supplemented with FBS (10–20%). Therefore, MAbs purified from these culture supernatants are usually contaminated by bovine serum proteins, especially bovine Ig that co-purify with the MAb during Protein-A affinity chromatography. Purification of FBS on a Protein-A-Sepharose column using the same conditions

Fig. 5. SDS-PAGE analysis of fractions from Protein-A affinity purification and GA733-2E affinity purification of low-density (A) and high-density (B) cell culture supernatants of GA733 hybridoma in SFM. The Protein-A-bound fractions were eluted, pooled, dialyzed into PBS, and loaded onto the GA733-2E affinity column. The unbound fractions from GA733-2E purification in both types of cell culture do not show any contamination at the Coomassie blue stain level.



showed approximately 0.0275 mg bovine Ig was recovered per ml of serum (data not shown). Based on this value, the amount of bovine Ig contamination in culture supernatants could be estimated.

We also directly evaluated the level of inactive Ig in low-density cell culture supernatants (culture in T75 flasks with DMEM 20% FBS) by rechromatographing Protein-A-purified GA MAb on a GA733-2E affinity purification column (Fig. 4). This two step purification showed that the inactive Ig contamination of GA733 MAb (Fig. 4, GA733-2E/unbound) in low-density culture supernatants was about 12% of the total MAb recovered after Protein-A column (Table 2) consistent with the amount of expected bovine IgG. As shown above, the concentration of MAbs obtained from high-density culture supernatants (CELLLine 1000 device) was at least 20-fold higher than that from low-density culture supernatants. Therefore, bovine Ig contamination in MAbs purified from the high-density culture supernatants was estimated to be lower than 0.6% (Table 2).

3.3. High-density hybridoma cell culture with SFM

The feasibility of using SFM in the two-chamber culture device was evaluated since it may be necessary to avoid even low level bovine Ig contamination for some MAb applications. GA and CO hybridomas adapted to SFM were grown in high-density CELLLine 1000 culture flask using SFM in the cell compartment and a 50% SFM + 50% DMEM 10% FBS in the nutrient compartment. In a 1-week culture period a total of 16.6 mg of CO MAb was recovered after Protein-A purification, corresponding to 0.55 mg purified MAb per ml of culture supernatant (Table 1). Similarly, about 0.7 mg purified GA MAb was recovered per ml of culture supernatant, with a production rate of 20.7 mg/week (Table 1). GA and CO MAbs yields estimated by ELISA were consistent with the values obtained after purification (data not shown). MAbs purified from SFM culture supernatants of hybridomas grown in parallel low-density culture flasks yielded 0.026 mg/ml (CO MAb) and 0.025 mg/ml (GA MAb) as shown in Table 1. These supernatants were 21- to 28-fold less concentrated than the same MAbs purified from high-density cell culture.

Protein-A purification followed by GA733-2E purification of SFM hybridoma supernatants from low-density (Fig. 5A) and high-density (Fig. 5B) cultures showed no detectable contamination of GA MAb with other proteins at the Coomassie blue stain level. The minor band at about 140 kDa is presumably a less extensively glycosylated form of the MAbs based on anti-mouse Ig immunoblots and MALDI mass spectrometry (data not shown). The GA MAb purified from these supernatants was essentially 100% active as shown by its ability to bind antigen during GA733-2E affinity purification (Fig. 5A and B). No IgG was detected in the GA733-2E-unbound fractions by spectrophotometry or SDS-PAGE analysis (Table 2). In contrast, the antigen binding activity of Protein-A-purified GA MAb from several batches of ascites fluid ranged between 22% and 73% using the same antigen column (Table 2).

Table 2 compares the results of all culture conditions evaluated in this study using the GA hybridoma, including low-density vs. high-density and serum-supplemented vs. SFM. MAb concentration ranges before (based on ELISA) and after purification (average based on recovery from entire culture supernatant pool) are also represented. The production costs (supplies and labor) using the high-density culture system proved to be much lower (5-fold on average) than the low-density culture method (Table 2). Similarly, the total time required for maintenance of a single two-chamber flask is far less than the time that would be required to produce and purify an analogous amount of MAb from low-density cultures.

4. Discussion

In this report, we describe the evaluation of the membrane-based cell culture device (CELLLine 1000) (reviewed by Marx, 1998) for high-density culture of hybridoma cells. To the best of our knowledge, this is the first study reporting the successful production of MAbs *in vitro* using these cell culture devices. Our data shows that this system allows efficient production of MAbs with high concentration, purity and activity. We have successfully used these preparations to make MAb-Sepharose affinity columns

and purify recombinant GA733 protein expressed in insect cells, as well as stoichiometry studies using isothermal titration calorimetry (manuscript in preparation).

For GA MAb, the use of SFM in conjunction with high-density cell culture eliminates completely non-specific Ig contamination with minimal effect on the yield or the concentration of MAb obtained. However, the serum-free culture of CO hybridoma produced about 50% less antibody/week than the serum-supplemented cultures. Some authors (Frame and Hu, 1990; Ozturc and Palsson, 1990) reported the loss of MAbs productivity during long-term culture of hybridomas adapted to SFM, due to the appearance of a non-producer cell population. Alternatively, we consistently observed a slower growth of the CO hybridoma in SFM compared with serum-supplemented medium that could explain the decrease in production of this MAb under these conditions. During the cell compartment harvest, cell pellets resuspended in fresh SFM and injected again into the culture may help maintain a high cell density (5 to 20×10^6 viable cells/ml compared with 0.5×10^6 viable cells/ml in conventional tissue culture flasks) and high MAb productivity during the course of the culture. Alternatively, further improvements in productivity in SFM might be achieved by recloning the hybridomas in SFM using limiting dilution and selecting surviving clones (Moro et al., 1994). Recently, Liu et al. (1998) successfully generated murine hybridomas by performing the fusion process in SFM supplemented by interleukin-6 (IL-6).

In terms of quality, the MAbs produced in the CELLline flask with SFM display high purity (specific MAb content), full activity (binding efficiency to the specific antigen) and integrity. This *in vitro* approach using SFM can be applied when a highly purified MAb, without any trace bovine IgG contamination, is needed for specific applications.

This two-chamber *in vitro* culture method compares very favorably with ascites production. Very high concentrations of MAbs (1–28 mg/ml) in ascitic fluid are often reported (Hendriksen and De Leeuw, 1998) but we typically observed MAb yields from ascites fluid in the 0.3 to 3 mg/ml range. Also, MAbs produced by ascites induction sometimes had reduced antigen-binding activity compared to MAbs produced by the *in vitro* method, presumably due to

contamination with irrelevant murine IgG. Parameters affecting the MAb productivity of the ascites induction method in laboratory animals were reviewed recently by Hendriksen and De Leeuw (1998).

The efficiency of the *in vitro* method described in this work can be further increased by tailoring the number of CELLline devices to the amount of MAb desired. This production method proved to be both economical and minimally time consuming. With an average of US\$3.5/mg, the costs of antibody production (supplies and labor) are significantly lower when compared with those published for other *in vitro* antibody production techniques such as dialysis tubing, spinner flask, roller bottle and hollow fiber (Hendriksen and De Leeuw, 1998). The CELLline devices are simple and can be handled as easily as regular tissue culture flasks. Unlike the minifermentor (Falkenberg et al., 1995) or the hollow fiber (Jackson et al., 1996; Kreutz et al., 1997) systems where special equipment is needed, the CELLline system is accessible to any laboratory possessing basic tissue culture equipment.

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