Immobilization Can Improve the Stability of Hybridoma Antibody Productivity in Serum-Free Media

Gyun Min Lee and Bernhard O. Palsson*
Department of Chemical Engineering, University of Michigan, Herbert H. Dow Building, Ann Arbor, Michigan 48109

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Hybridoma cells (S3H5/γ2bA2) were cultivated in spinner flasks with 1% serum media and serum-free media. Monoclonal antibody productivity was maintained in 1% serum media. However, cells in serum-free media showed a decrease in antibody productivity, and it completely disappeared in IMDM-based low protein medium. This loss of antibody productivity was not observed when the cells were immobilized in alginate beads. In fact, immobilization enhanced the specific MAb productivity.

INTRODUCTION

Monoclonal antibodies (MAbs) are among the most important products derived from large-scale mammalian cell culture.[1-4] The rising demand for MAbs has triggered efforts to improve the economics of large-scale in vitro production of MAbs. The requirement for expensive serum in the growth media represents one of the major costs associated with large-scale cultivation.[5,6] Serum is a complex mixture of hormones, growth factors, nutrients, and other trace elements. In addition, the composition of serum is undefined, of variable quality, and often a source of microbial contamination. Serum also introduces proteins which may make product purification difficult. The requirement for serum has been extensively studied in order to limit its use in view of its high cost and limited supply. As a result, several varieties of serum-free (SF) media for hybridoma cell culture are now commercially available, and many hybridoma cell lines can be cultivated in SF media.[7,8] Alternatively, the amount of serum for cell growth may be reduced in high density culture. Serum requirements for cell growth (S3H5/γ2bA2) in a batch cultivation was reduced at high initial cell densities.[9]

However, it has been reported that some hybridoma cells lose their MAb productivity during long-term cultivation.[10-13] A murine hybridoma cell line, 167.4G5.3, lost MAb productivity in 1.25% serum medium over a time period of about 4 months, while the cells could maintain MAb productivity in 5% serum medium for the same period.[13] Cells in low serum medium may be more prone to lose MAb productivity, which is perhaps due to the more stressful environment that the lack of serum components creates as compared to high serum medium.

In this manuscript we will examine the hypothesis that the instability of the cell's MAb productivity may be overcome by immobilizing the cells in gel beads. Several lines of reasoning argue for such improved stability. First, the microenvironment created by immobilized cells may be more favorable than the environment found in the surrounding media.[14,15] The high local cell density in the gel beads could reduce the serum requirement for cell growth since the concentration of any autocrine growth factors may be high in the gel beads. Second, because cells in low serum or SF media are more shear sensitive than in high serum media, it may be necessary to protect cells from mechanical damage by immobilizing them in gel beads.[7,16,17] Third, immobilized cells may have limited cell division, and thus genetic drift or instability is less likely to occur.[7]

In the present study, we cultivated suspended cells in low serum and SF media for several months to assess cell growth and loss of MAb productivity. Cells immobilized in alginate beads were also cultivated in SF media to test whether immobilization can improve the stability of the cell's MAb productivity.

MATERIALS AND METHODS

Cell Line and Culture Maintenance

The murine hybridoma used was S3H5/γ2bA2 provided by Dr. Mark Kaminski from the University of Michigan Medical Center. The antibody produced by this cell line is γ2bA anti-idiotype antibody, directed against the 38C13. The fusion partner used was P3/x63/Ag8.653. The cell culture medium for stock cells was Iscove's modified Dulbecco's medium (IMDM, Sigma, St. Louis, MO) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco Laboratories, Grand Island, NY) and 100 units each of penicillin and streptomycin (Sigma). The cells were main-

* To whom all correspondence should be addressed.

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tained in T25 cm^2 plastic cell culture flask (Bellco Glass, Vineland, NJ) at 37°C in a humidified CO_2 incubator (VWR Scientific, San Francisco, CA). The cells were diluted 1:5 with fresh medium every other day.

Media

IMDM or RPMI 1640 media supplemented with 1% FBS were used as low serum media. For SF medium, Dulbecco's modified Eagle's medium and Ham's nutrient mixture F-12 (DME/F12, Sigma) supplemented with insulin/transferrin/sodium selenite (ITS, Sigma) and fatty acid free bovine serum albumin (BSA, ICN Biochemicals, Cleveland, OH) were used. The concentrations of insulin, transferrin, sodium selenite, and BSA are 5 μg/mL, 5 μg/mL, 5 ng/mL, and 1 g/L, respectively. Three different serum-free low protein (LP) media without BSA were also prepared by adding ITS supplements to DME/F12, RPMI 1640, and IMDM, respectively. The concentrations of ITS in serum-free LP media were the same as in SF medium with BSA.

Immobilization

Cells were entrapped in the gel beads of calcium alginate as follows. (1) Exponentially growing cells (cell density ≅ 2.0×10^6 cells/mL) were spun down at 1000 rpm for 10 min. After discarding the supernatant, cells were suspended in IMDM without serum and were spun down again. After discarding the supernatant, the cell pellet remaining at the bottom of the centrifuge tube was then resuspended in alginate solution [1.5% (w/v) in 0.85% (w/v) NaCl]. The final cell concentration was approximately 6.0×1.0^6 cells/mL 1.5% sodium alginate (Keltone LV, Kelco, Chicago, IL). A mixture of viable cells and alginate solution was packed in a syringe. (2) The mixture was extruded into 1.5% (w/v) $CaCl_2$ solution using an air-syringe droplet generator.[18] Uniform-sized gel beads (0.8–1.0 mm) were obtained in this manner. Gel-entrapped hybridoma cells were allowed to stand for 10 min in order to achieve proper gelation. Immobilized cells were washed 3 times with fresh media. Finally, the immobilized cells were transferred to 100-mL spinner flasks (Bellco Glass, Vineland, NJ).

Cell Culture

For free cell suspension culture, exponentially growing cells in IMDM medium with 10% FBS were inoculated into spinner flasks (Bellco) containing 50 mL of IMDM medium with 10% FBS at an initial density of 1×10^5 cells/mL. Cell culture was done in a repeated fed-batch mode. Cells were subjected to a stepping-down procedure from 10% to 1% serum IMDM media over 2 months in a suspension culture. Cells in the late exponential phase were passed at the dilution rate of 1:5. The working volume of the spinner flask was 50 mL. Cells were cultivated in 1% serum media and then passed to serum-free media with BSA and to serum-free LP media. For immobilized cell culture, immobilized cells were cultivated in IMDM medium with 10% FBS. After 2 weeks, the medium was switched to IMDM-based LP medium. After immobilized cells settled down, the supernatant was removed and fresh medium was added to the spinner flask at different dilution rates.

Analytical Methods

Cell growth was monitored by counting viable cells with a hemocytometer. The viable cell population was distinguished from dead cells by the trypan blue dye exclusion method. Specific growth rates were determined from the slope of the viable cell concentration vs. time during the exponential growth. The cell culture suspension was centrifuged and supernatant was aliquoted and kept frozen at −80°C. Glucose and lactate were measured using a glucose/lactate analyzer (Yellow Springs Instruments, Model 2000, Yellow Springs, OH). The total antibody, γ2b, was quantified using an enzyme-linked immunosorbent assay (ELISA), and the specific antibody production rate was calculated as described previously.[9] For cell count and flow cytometric sample preparation of immobilized cells, the gel beads containing cells were dissolved in isotonic citrate solution (3% aqueous sodium citrate diluted 1:1 in 0.9% NaCl and adjusted to pH 7.4).[19]

The amounts of intracellular antibody and DNA were quantified simultaneously using flow cytometry. Cells were fixed with ethanol for analysis of intracellular antibody and DNA. The procedures were modified from those described previously.[20–22] Then 1×10^6 cells were washed twice by centrifugation (5 min, 4°C, 200 *g*) in phosphate-buffered saline (PBS) and spun down again. After discarding supernatant, the cell pellet was resuspended in 70% ethanol and stored at 4°C. We did not use an internal standard for negative control since the control peak overlapped the peak of the nonproducer. Instead, samples accumulated within a month were stained and analyzed at the same time and conditions in order to avoid day-to-day variation in flow cytometer measurements. We did not see any significant decrease in intracellular antibody content during storage over a month. Ethanol-fixed cells were washed twice by centrifugation in PBS. Cells were resuspended and incubated at 4°C with 0.4 mL of a 1:30 dilution of fluorescein-isothiocyanate (FITC)-labeled goat antimouse IgG2b (heavy chain specific, Southern Biotechnology Associates, Birmingham, AL) for 30 min. After washing, cells were stained for DNA with propidium iodide (PI) over a concentration range of 10–20 μg/mL in PBS containing RNAse (Sigma). The cells were incubated for at least 20 min at 37°C. After centrifugation, cells were washed and resuspended in PBS. Prior to flow cytometric (FCM) analysis, cell suspensions were filtered through a 40-μm nylon-mesh filter. Green and red fluorescences were simultaneously monitored in a Coulter EPICS 751 flow cytometer (Coulter Corporation, Hialeah, FL). Gates were set on forward light scatter and red fluorescence to ensure

that only green fluorescence of live cells was included in the IgG2b histogram. A logarithmic amplifier was used to accumulate green fluorescence (Interacellular MAb content) data with a large dynamic range. To determine the correspondence between logarithmic channel number and relative linear fluorescence intensity, microspheres of four varying fluorescence intensities (EPICS intensity standard kit for calibration of fluorescence intensity, Coulter Corporation) were used.[23]

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of the supernatant of the cell culture solution was done under reducing conditions. Supernatant was concentrated 2.5 times using Centricon centrifugal microconcentrators (10,000 MW cutoff, Amicon, Beverly, MA). Mini-protean II dual slab cell (BioRad Laboratories, Richmond, CA) was used. Samples were heated to 100°C for 5 min before electrophoresis. The gel was run essentially as described by Laemmli[24] and consisted of a 10% running gel and a 3% stacking gel. Electrophoresis was performed at 150 V for about 1 h. For the determination of molecular weight, an SDS–PAGE low molecular weight standard (BioRad) was used. The gel was stained by 0.1% Comassie blue.

RESULTS

Cultivation of Suspended Cells in Low Serum and Serum-Free Media

Cell culture was performed using spinner flasks in a repeated fed-batch mode. Cells were cultivated in 1% serum media and then passed to SF followed by LP media. Cells in 1% serum media could grow well irrespective of the media type although cells in RPMI 1640 have higher specific growth rates than in IMDM (Fig. 1). The specific growth rates of cells in 1% serum media were in the range of 0.6–1.2 day^{-1}. Cells in SF media and LP media grew as fast as in 1% serum media for about 2 months. Cell viability in these media was maintained at over 90%. However, cells in IMDM-based LP medium did not grow well after five passes and cell viability dropped rapidly. Furthermore, cells in SF media started to lose MAb productivity. As shown in Figure 2, MAb productivity was maintained at about 0.13 pg/cell · h in 1% serum media, started to deteriorate in SF medium, and disappeared in IMDM-based LP medium.

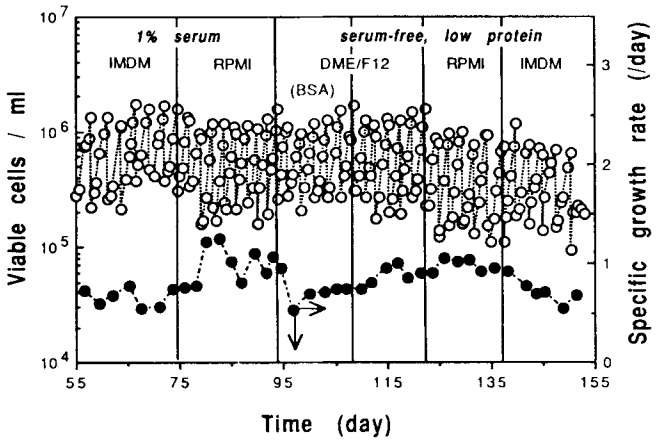

Figure 1. Cultivation of cells in suspension in low serum and serum-free media. The figure indicates how the media composition was changed with time.

The cell number vs. log green fluorescences obtained in the flow cytometer are plotted in Figure 3. The log green fluorescence indicates the amount of intracellular MAb.[12,22] A single population of cells was found in IMDM with 1% serum concentration. However, in SF media, two subpopulations appeared. One population had much lower fluorescent intensity than the other. As the passage number increased, the population with low internal antibody increased, and after about 15–20 passages, a single population of nonproducers was present. Three-dimensional plots of intracellular MAb, DNA, and cell population are shown in Figure 4. The population of nonproducers with low internal antibody content increased with passage number. It is likely that the low fluorescence intensity of the nonproducer is due to autofluorescence of cells or nonspecific binding of FITC-labeled goat antimouse IgG2b (heavy chain specific). When IgG1 producing cells were stained with FITC-labeled goat antimouse IgG2b, they had approximately the same intensity of fluorescence as nonproducers stained with FITC-labeled goat antimouse IgG2b (data not shown). The specific MAb production rate could be correlated with the fraction of the nonproducers evaluated from the relative peak area of the flow-cytometric data within the population (Fig. 5). A similar correlation between specific MAb productivity and the fraction of nonproducers was observed during adaptation to low serum containing medium.[13]

It is possible that cells may produce structurally altered MAb molecules which are not detected by ELISA. In the case of mouse myelomas, three types of synthesis variants have been observed: (1) light chain only producer, (2) heavy chain only producer, and (3) nonproducer of heavy and light chains.[25] Subclass switch mutants arise frequently in cultured mouse myeloma cells.[25,26] The SDS–PAGE shows that in this case the nonproducer does not produce any MAb molecules or parts thereof (Fig. 6). When nonproducers isolated from IMDM-based LP medium were cultivated in IMDM medium with 10% serum, they still did not produce MAb.

Cultivation of Immobilized Cells in IMDM-Based LP Medium

First, immobilized cells were cultivated in IMDM medium with 10% serum concentration. Since cell counts could not be obtained, we monitored the activity of the culture by following glucose and lactate concentrations. As shown in Figure 7(A), glucose consumption rate increased at the beginning of cultivation and then approached a constant value, probably because the cell density in the beads reaches a constant value. After the glucose consumption

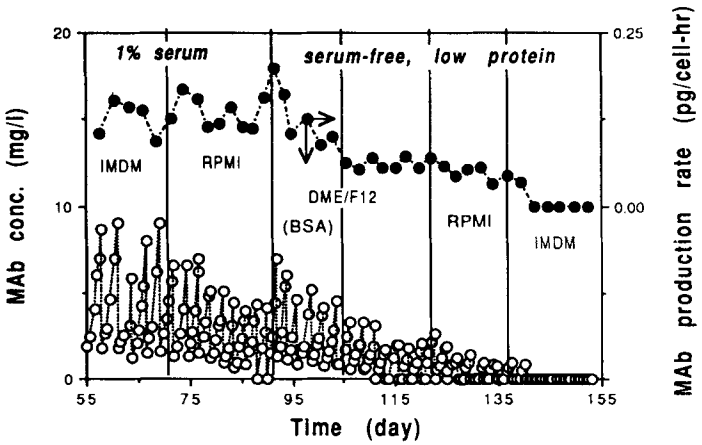

Figure 2. MAb production of suspended cells in low serum and serum-free media. The media changes are the same as in Figure 1.

rate became constant, the 10% serum medium was changed to IMDM-based LP medium. The immobilized cells in LP medium consumed glucose and produced lactate over a month following the media switch. Thus, immobilized cells were growing or at least maintaining viability much longer than cells grown in suspension in the same LP media, possibly due to protection from adverse shear forces.

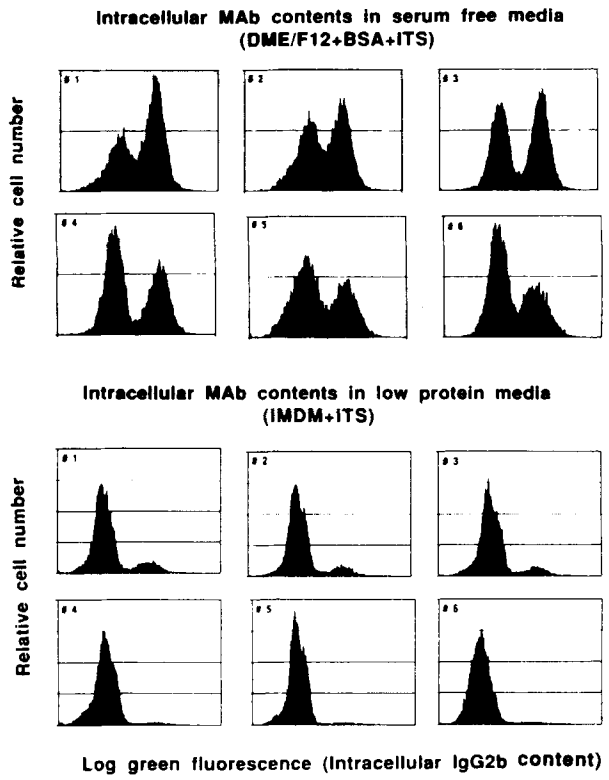

Figure 3. Intracellular MAb contents of cells in suspension in serum-free media as determined by flow cytometry. The numbers in the figures represent the passage number in the specific media. The top two rows show the changes in the intracellular antibody content following the change to DME/F12 + BSA + ITS media on day 92 in Figure 1. The bottom two rows show the same changes following the switch to IMDM + ITS media on day 137.

The MAb concentrations were measured to test whether immobilized cells in LP medium could maintain MAb productivity. As shown in Figure 7(B), immobilized cells in LP medium were producing the MAb over a month, indicating that immobilization can improve the stability of the cell's MAb-producing ability. Flow cytometry showed that immobilized cells contained only a single population with a high fluorescent intensity (Fig. 8).

The intracellular MAb content of suspended cells is compared with immobilized cells in Figure 8. The histogram to the left is from the free cells in IMDM-based LP media which completely lost their MAb productivity. The histogram in the middle is from free cells grown in IMDM medium with 10% serum concentration, which are the same cells as were used for immobilization. The histogram to the right is obtained from immobilized cells in LP medium. The fluorescence intensity of immobilized cells in LP media was about 3 times higher than that of free cells in 10% serum medium. The specific MAb productivity of immobilized cells in LP medium was determined to be about 0.4 pg/cell · h. Consequently, we found that immobilization not only improved the stability of the cells but also enhanced the cells' specific MAb productivity.

Cell cycle analysis using flow cytometry of immobilized cells was carried out to determine whether the immobilized cells in LP media were growing or only maintaining their viability (Fig. 9). About 14% of cells were in G2/M phases, which indicates that immobilized cells were dividing.

A plot of intracellular MAb content vs. DNA content of immobilized cells is shown in Figure 10. The intracellular MAb content appears to be constant throughout the cell cycle.

DISCUSSION

Cultivation of hybridoma cells that grow in SF or LP media will result in a significant reduction in media costs and

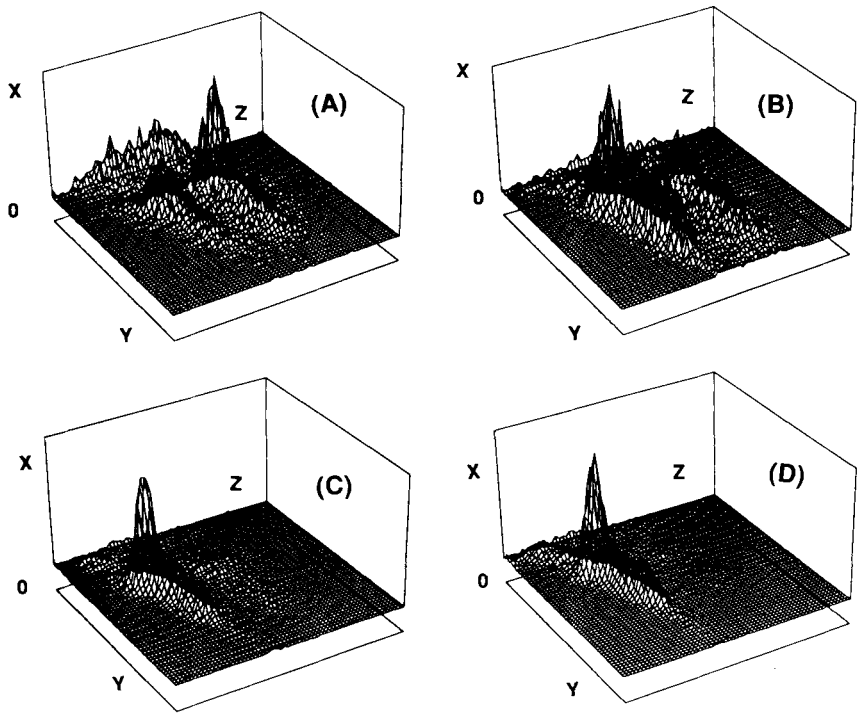

Figure 4. Three-dimensional plots of intracellular MAb (x axis), DNA (y axis), and cell population (z axis). (A) Cells at the first passage in DME/F12 + BSA + ITS SF medium. (B) Cells at the second passage in DME/F12 + BSA + ITS SF medium. (C) Cells at the fifth passage in IMDM + ITS LP medium. (D) Cells at the sixth passage in IMDM + ITS LP medium.

make MAb purification easier. Hybridoma cells (S3H5/γ2bA2) in SF media could grow in a repeated fed-batch mode in spinner flasks for about 2 months before they lost viability. Cells in the exponential phase had to be passed; otherwise, they exhibited either a 2–3-day lag phase before resuming growth or dying. This requirement may be due to the fact that cells in the decline phase are more shear sensitive than in the exponential phase and thus do not survive subcultures as well as cells passed in the exponential phase.[27] However, it is not always desirable to pass the cells in the exponential phase when MAb titer and efficiency of media utilization are considered. It has been noted that hybridoma cells secrete MAb throughout batch growth and a significant portion of MAb is produced during the stationary and decline phases.[27–29] The problem arising from shear sensitivity can be prevented by immobilizing cells in gel beads.

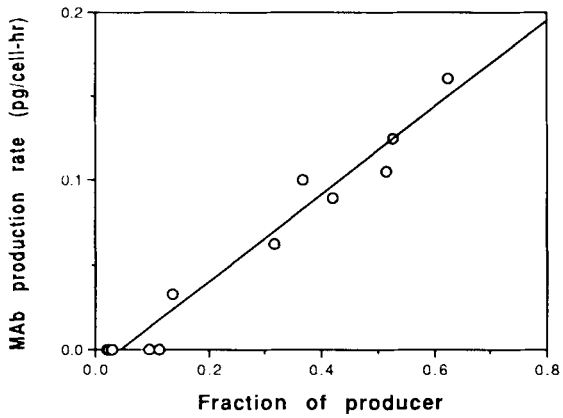

Figure 5. Correlation of the fraction of producers with specific MAb production rate.

If cells lose their MAb productivity in LP media, it is meaningless to cultivate these cells over a long period for continuous MAb production. It is unclear why cells in SF media lose their MAb productivity. Our data show that immobilization can improve the stability of cells' MAb productivity. This observation may result from the fact that the concentrations of autocrine growth factors inside gel beads are high due to the high local cell density in the gel beads (on the order of 10^7 cells/mL), and these factors may help cells maintain MAb productivity. One of the autocrine growth factors is interleukin 6 (IL-6), which is known to stimulate immunoglobulin synthesis in lymphoid cells.[30,31] In serum media, the role of autocrine growth factors may be negligible because serum may contain them in concentrations sufficient to mask any autocrine effects.

In addition to the improvement of stability of the cell's MAb productivity, immobilization enhanced the specific MAb productivity of cells. This observation may be due to

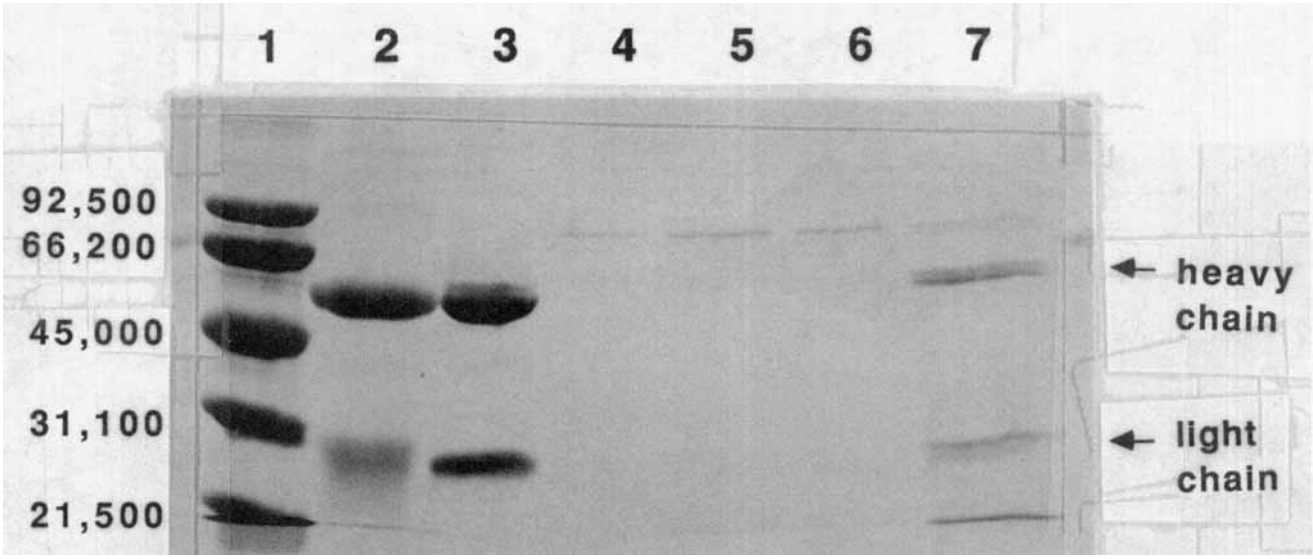

Figure 6. Sodium dodecyl sulfate–polyacrilamide gel electrophoresis analysis of supernatants of cell culture. Lane 1, low molecular weight standard; lane 2, purified IgG2b (southern Biotechnology); lane 3, purified IgG2b from S5A8/γ2b cells (mouse hybridoma); lane 4, IMDM-based LP medium; lane 5, cell culture supernatants in IMDM-based LP medium. Sample was taken at the fifth passage in this medium; lane 6, cell culture supernatants in IMDM-based LP medium. Sample was taken at the sixth passage in this medium; lane 7, cell culture supernatant from immobilized cells in IMDM-based LP medium (day 40).

the very low growth rate of immobilized cells. It has been reported that the specific MAb productivity is inversely proportional to the specific growth rate.[32–34] Immobilized cells in LP medium could divide, but their growth rate was almost zero when the cell density in the gel was saturated. Limiting the number of divisions would reduce the chances of somatic mutations that can change the monoclonality and possibly the efficiency of the antibody.[7]

In conclusion, the results presented here indicate that the efficient production of MAb can be achieved by a two-stage process. First, immobilized cells should be grown in serum containing media to reach high cell density in the beads. Second, the media should be switched to SFLP media for prolonged MAb production with slowly growing immobilized cells.

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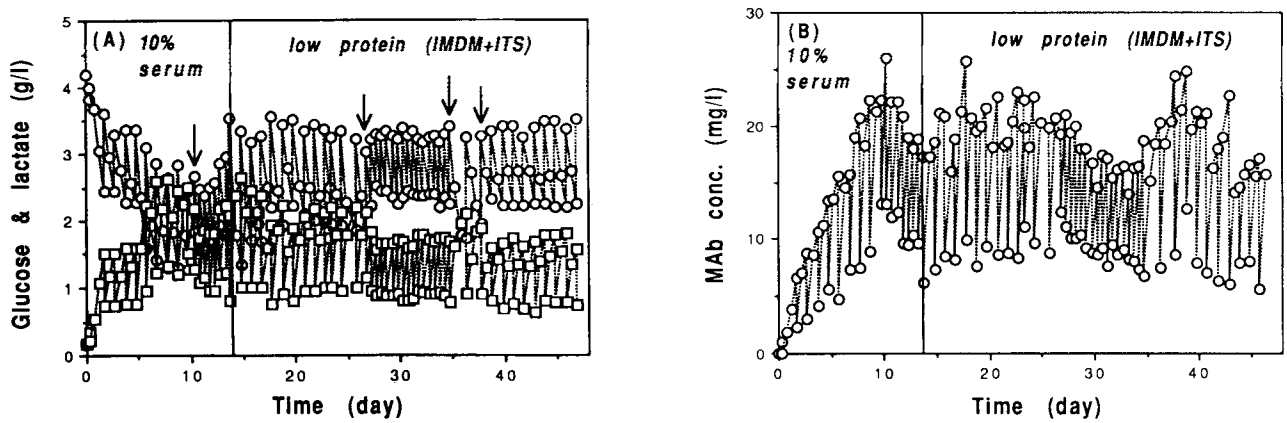

Figure 7. (A) Glucose and lactate concentrations in immobilized cell culture. Circles represent glucose concentration and squares represent lactate concentration. The arrows indicate the changes in the feeding schedule, which is as follows: days 0–10, 60 mL/24 h; days 10–14, 50 mL/12 h; days 14–26, 60 mL/24 h; days 26–34, 50 mL/12 h; days 34–37, 65 mL/36 h; days 34–, 60 mL/h. (B) MAb concentrations corresponding to panel A.

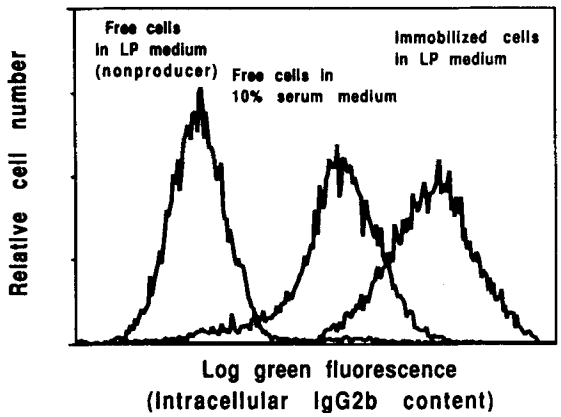

Figure 8. Comparison of intracellular MAb contents between free and immobilized cells.

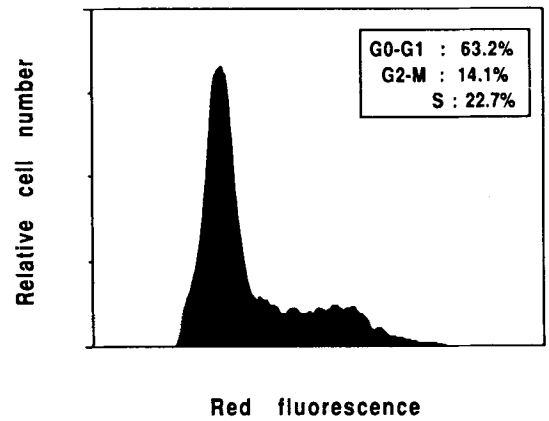

Figure 9. DNA histogram of immobilized cells in IMDM-based LP medium.

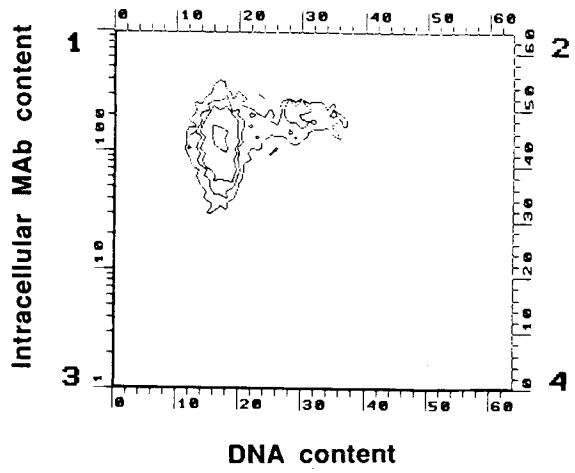

Figure 10. Cytogram (DNA content vs. intracellular MAb content) of immobilized cells in IMDM-based LP medium.

References

1. M. Ratafia, *Bio/Technology*, **5,** 692 (1987).
2. R. Spier, *Trends Biotechnol.*, **6,** 2 (1988).
3. W. Lebherz, in *Commercial Production of Monoclonal Antibodies*, S. S. Seaver, Ed. (Marcel Dekker, New York, 1987), p. 93.
4. A. Handa-Corrigan, *Bio/Technology*, **6,** 784 (1988).
5. B. Griffiths, *Develop. Biol. Stand.*, **66,** 155 (1987).
6. B. Griffiths, *Trends Biotechnol.*, **4,** 268 (1986).
7. E. Shacter, *Trends Biotechnol.*, **7,** 248 (1989).
8. M. Ratafia, *Bio/Technology*, **7,** 574 (1989).
9. G. M. Lee, T. K. Huard, and B. O. Palsson, *Hybridoma*, **8,** 369 (1989).
10. W. S. Hu, K. K. Frame, and S. Sen, "Loss of Productivity of Hybridoma Cells in Culture," paper presented at AIChE National Meeting, New York, NY, 1987.
11. K. K. Frame and W. S. Hu, *Biotechnol. Bioeng.*, **35,** 469 (1990).
12. C. A. Heath, G. L. Altschuler, G. Belfort, and R. Dilwith, "Biochemical and Flowcytometric Analysis of Hybridoma Growth and Antibody Production in Suspension and Entrapped Cultures," paper presented at ACS National Meeting, Los Angeles, CA, 1988.
13. S. S. Ozturk and B. O. Palsson, *Hybridoma* **9,** 167 (1990).
14. K. Nilsson, *Trends Bioechnol.*, **5,** 114 (1987).
15. J. N. Vournakis and P. W. Runstadler, *Bio/Technology*, **7,** 143 (1989).
16. G. M. Lee, J. M. Savinell, and B. O. Palsson, *Hybridoma*, **8,** 639 (1989).
17. H. Murakami, H. Masui, and G. Sato, in *Growth of Cells in Hormonally Defined Media*, G. Sato, A. Pardee, and D. Sinbasku, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), p. 711.
18. F. Lim, in *Biomedical Application of Microencapsulation*, F. Lim, Ed. (CRC Press, Boca Raton, FL, 1984), p. 137.
19. F. Lim, in *Upstream Processes: Equipment and Techniques*, A. Mizrahi, Ed. (Alan R. Liss, New York, 1988), p. 185.
20. C. V. Clevenger, K. D. Bauer, and A. L. Esptein, *Cytometry*, **6,** 208 (1985).
21. H. A. Crissman and J. A. Steinkamp, *Cytometry*, **3,** 84 (1982).
22. S. Sen, F. Srienc, and W. S. Hu, "Batch Kinetics of Hybridoma Cell Growth: A Flow Cytometric Study," paper presented at ACS National Meeting, New Orleans, LA, 1987.
23. K. A. Muirhead, T. C. Schmitt, and A. R. Muirhead, *Cytometry*, **4,** 251 (1983).
24. U. K. Laemmli, *Nature*, **227,** 680 (1970).
25. S. L. Morrison and M. D. Scharff, *CRC Crit. Rev. Immunol.*, **1,** 1 (1981).
26. C. A. Gritzmacher, *CRC Crit. Rev. Immunol.*, **9,** 173 (1989).
27. G. M. Lee, T. K. Huard, M. S. Kaminski, and B. O. Palsson, *Biotechnol. Lett.*, **10,** 625 (1988).
28. J. M. Renard, R. Spagnoli, C. Mazier, M. F. Salley, and E. Mandine, *Biotechnol. Lett.*, **10,** 91, (1988).
29. G. A. Walker, W. Davison, and C. A. Lambe, *Proc. 4th Eur. Congr. Biotechnol.*, **3,** 587 (1987).
30. L. A. Aarden, *Ann. N. Y. Acad. Sci.*, **557,** 192 (1989).
31. T. Hirano, K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunasawa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto, *Nature*, **324,** 73 (1986).
32. W. M. Miller, H. W. Blanch, and C. R. Wilke, *Biotechnol. Bioeng.*, **32,** 947 (1988).
33. W. M. Miller, C. R. Wilke, and H. W. Blanch, *J. Cell. Physiol.*, **132,** 524 (1987).
34. B. Maiorella, B. Howarth, A. Shauger, and D. Inlow, "Optimized pH Control for Antibody Production," paper presented at ACS National Meeting, Miami, FL, 1989.