The Influence of Temperature on a Mouse–Mouse Hybridoma Growth and Monoclonal Antibody Production

G.K. Sureshkumar and R. Mutharasan*
Department of Chemical Engineering, Drexel University, Philadelphia, Pennsylvania 19104

Accepted for publication June 26, 1990

INTRODUCTION

Monoclonal antibodies (MAb) are responsible to a large extent for the tremendous advancement in immunological research in the 1980's and they show great promise for applications in medicine. Hybridomas are used for in vitro production of MAb. Enhancement in MAb yield can be effected through various cultivation strategies such as nutrient supplementation[1,2] and using different propagation methods.[3] The most common strategy used to enhance MAb yield is to keep cells viable for extended time periods.[1,4–6] Consequently, factors that influence viability of cells in culture have been examined by several investigators.[1–3,5–7] Alternatively, cultivation variables that affect MAb productivity may be manipulated to enhance MAb yield. We have investigated the manipulation of cultivation temperature as a variable to enhance MAb productivity.

The normal temperature for cell cultivation is 37°C. However, temperature higher than the normal has been shown to enhance DNA responses in lymphocytes.[8] Temperature has also been reported to affect the kinetics of immunological responses in channel catfish leucocytes.[9] Thus it can be expected that changing the culture temperature may affect MAb productivity. This study investigates the effect of culture temperature on growth, MAb production, glucose consumption and lactate production of a mouse–mouse hybridoma cell line.

The effect of cultivation temperature on the viability of cells and MAb productivity has not been studied extensively. The only study[7] on temperature effects on hybridomas showed that temperatures lower than 37°C increased the time that the cells remained viable but decreased MAb production. The aim of that study was to determine conditions that potentially could prolong the time that the cells remained viable. Thus, high inoculum concentrations (1.5×10^6 cell/mL) were chosen[7] which obviated cell growth characterization. In contrast, we examine the entire growth phase and MAb production at various temperatures in this study, in order to investigate the effect of temperature on MAb productivity.

* To whom all correspondence should be addressed.

MATERIALS AND METHODS

A mouse–mouse hybridoma 14-4-4S (ATCC No. HB-32) which produces a cytotoxic monoclonal antibody ($IgG_{2a}\kappa$) reactive to $I\text{-}E_k/C_k$ determinants[10] was used in this study. The medium consisted of Dulbecco's Modified Eagle's Medium (Hazelton Research Products) containing 10% fetal calf serum (Sigma Chemical Co., St. Louis, MO), glucose (4 g/L), $NaHCO_3$ (2.2 g/L), oxaloacetic acid (13.2 mg/L), crystalline insulin (0.8 mg/L), sodium pyruvate (5.5 mg/L), 1% 100× nonessential amino acid solution (Sigma Chemical Co., St. Louis, MO) and 1% antibiotic antimycotic solution (Sigma Chemical Co., St. Louis, MO).

Experiments were carried out in triplicate or duplicate, in 75-cm^2 T-flasks placed in a CO_2 incubator. The temperatures used were 29, 33, 35, 37, 39, and 42°C. Cell counts were done by a hemacytometer and viability was determined by trypan blue exclusion. One to two samples were taken daily and frozen for later analysis of MAb, glucose, and lactate. MAb concentrations were measured by Enzyme Linked Immuno Sorbent Assay (ELISA)[11] as described elsewhere[5] using automatic microplate washer and reader (Flow Laboratories, Inc.). Glucose and lactate concentrations were determined enzymatically using glucose and lactate analyzers (Yellow Springs Instrument Co.) Glutamine was measured using an enzymatic assay (Boehringer Mannheim).

RESULTS AND DISCUSSION

Maximum Cell Concentration and Growth Rate

The growth profiles presented in Figure 1 show that maximum viable cell concentration ($X_{v\max}$) depends on the cultivation temperature. $X_{v\max}$ at 37°C was 3.2×10^6 cell/mL which compared well with results reported

Biotechnology and Bioengineering, Vol. 37, Pp. 292–295 (1991)

CCC 0006-3592/91/030292-04$04.00

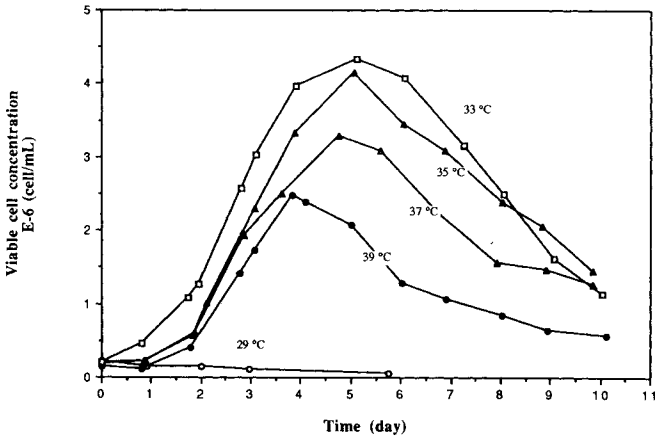

Figure 1. Growth profiles of HB-32 at various temperatures.

earlier for the same cell line.[5] The value of $X_{v\max}$ obtained at 33°C was about 1×10^6 cell/mL higher than that at 37°C. Cultivation at a temperature higher than 37°C yielded a lower value of $X_{v\max}$, 2.5×10^6 cell/mL, at 39°C. An initial decrease in the cell concentration after inoculation was observed at 39°C. However, no growth was observed at 29 or 42°C (data not shown).

Variation of maximum specific growth rate (μ) with temperature, presented in Table I, shows that μ was relatively constant in the temperature range of 35 to 39°C. Parameter μ was calculated from a plot of logarithm of viable cell concentration versus time, as the slope of the line in the exponential growth phase. The value of μ at 35°C (0.045 h^{-1}) and 39°C (0.048 h^{-1}) were not very different from the value at 37°C (0.045 h^{-1}), but, at 33°C it was found to be lower (0.034 h^{-1}). Thus it can be seen that cell growth occurs over a range of temperatures, with growth rate nearly constant between 35 and 39°C.

It has been reported that glutamine can be a limiting nutrient in mammalian cell culture[12-14] and is most often found to be depleted when maximum cell concentration is reached.[14,15] Glutamine was found to be depleted when the maximum cell concentration was reached in the experiments carried out at 33 and 39°C. Since glutamine degradation is a function of temperature,[16] the higher $X_{v\max}$ obtained at lower temperatures could also be due to glutamine availability resulting from slower degradation of glutamine. Taking experiments at 37°C as the base case, it can be estimated that glutamine degradation at 33°C would be lower by about 22%, while at 39°C it would be about 11% higher. However, since glucose uptake rate was found to vary with temperature (presented in the next section), it is reasonable to suggest that glutamine uptake may have also varied with temperature. Therefore, further experimentation is necessary to decouple the effects of temperature, glutamine degradation, and glutamine uptake rate on $X_{v\max}$ for the temperature range considered.

Glucose Consumption and Lactate Production

Specific rates of glucose consumption (q_G) and lactate production (q_L) in the early exponential phase are presented in Table I. The value of q_G at 37°C (1.0×10^{-7} μM/cell h) compared favorably with values reported in the literature for other cell lines.[13,17,18] As temperature was increased the specific rates of glucose uptake and lactate production increased. The value of q_G increased from 0.6×10^{-7} μM/cell h at 33°C to 1.4×10^{-7} μM/cell h at 39°C, an increase of almost 130% over a temperature range of 6°C. This trend was similar to the one reported in the literature.[7] The value of q_L also increased from 0.8×10^{-7} to 4.3×10^{-7} μM/cell h, an increase by about 440%, as the temperature was increased from 33 to 39°C. These results support the hypothesis that the rate of cellular metabolism increases with temperature, which was also indicated by Reuveny et al.[7] The amount of lactate produced per unit amount of glucose consumed, lactate yield ($Y_{L/G}$), on a mole/mole basis also increased from 1.3 at 33°C to 3.1 at 39°C. This indicates that as the temperature was increased more glucose was routed to produce lactate at the end of glycolysis rather than being routed to TCA cycle which is energetically more productive. A value of $Y_{L/G}$ higher than the theoretical maximum of 2 could have resulted from lactate production from other nutrients.

MAb Production

It has been suggested[19] that environmental stress increases antibody production by hybridomas. Increase in temperature poses an environmental stress and thus can be expected to increase MAb production. MAb formation at various temperatures, given in Figure 2, shows that MAb profiles and maximum MAb concentration vary with temperature. The maximum MAb concentration obtained at 39°C was 28 mg/L, which was lower than that obtained at the other temperatures (see also

Table I. Variation of specific rates of glucose uptake (q_G), lactate production (q_L), and lactate yield ($Y_{L/G}$) of HB-32 with temperature.

Temperature (°C)	Specific growth rate, μ (h^{-1})	Specific glucose uptake rate, q_G ($\times 10^7$) (μM/cell h)	Specific lactate production rate, q_L ($\times 10^7$) (μM/cell h)	Lactate yield $Y_{L/G}$ (mol/mol)
33	0.034	0.6	0.8	1.3
35	0.045	0.8	1.5	1.9
37	0.045	1.0	2.1	2.1
39	0.048	1.4	4.3	3.1

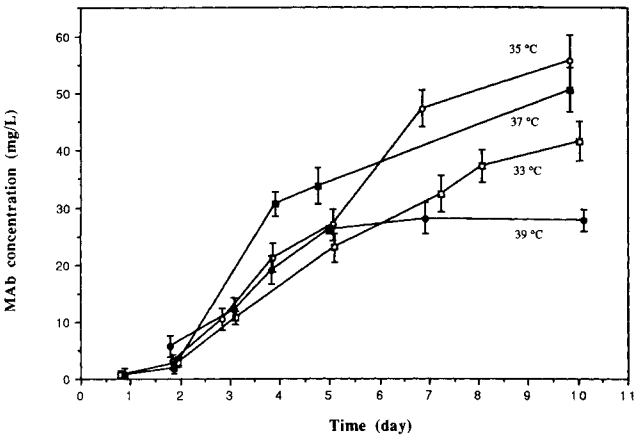

Figure 2. MAb profiles at various temperatures.

Table II). The Viability Index (V_I) is defined as

$$V_I = \int_0^t X_v dt$$

where X_v is viable cell concentration and t is time, has been found to be a better parameter for correlating antibody production by hybridomas.[1,4,6] Thus MAb concentration obtained can be expressed as

$$[\text{MAb}] = q_{\text{MAb}} V_I$$

where q_{MAb} is the specific MAb production rate. From a plot of MAb concentration vs. V_I, q_{MAb} was found to be a constant at a particular temperature, whereas it was found to increase with temperature in the temperature range studied (33 to 39°C in Table II). This increasing trend was similar to that found by Reuveny et al.[7] for a temperature range from 28 to 37°C for a different cell line.

It is interesting to note that although the Viability Index at 39°C was much lower (by a factor of 2.4) than at 33°C, the specific MAb production rate at 39°C was higher (Table II). Also, it has been shown[5] that at a particular temperature the specific MAb production rate is not dependent on the amount of limiting nutrient present in the medium. Thus it can be said that specific MAb production rate is related to the temperature at which the cells are grown. The increase in specific MAb production rate with temperature was concurrent with increase in level of cellular metabolism indicated by increases in specific rates of glucose uptake and lactate production.

Temperature Shift Experiment

Total MAb concentration produced during a batch culture can be seen to be a function of (1) specific MAb production rate which reflects on productivity by a production unit (viable cell) and (2) Viability Index which is a measure of the number of production units (viable cells) available over the entire time of culture. Maximization of MAb production can be achieved by enhancing both of these factors. It was shown earlier that as temperature was increased q_{MAb} increased whereas $X_{v\max}$ and V_I decreased. Therefore, one possible strategy for improving MAb yield is to grow cells at a suboptimal temperature until $X_{v\max}$ is attained and then to shift to a higher temperature. Higher cell concentrations could be achieved at the lower temperature which would contribute to an increase in V_I and shifting to a higher temperature would increase specific MAb production rate by the cells.

An experiment was carried out in which HB-32 cells were grown at 33°C until $X_{v\max}$ was achieved and then the temperature was increased to 39°C. To recall, maximum $X_{v\max}$ was obtained at 33°C and maximum q_{MAb} was obtained at 39°C in the cultures grown at a single temperature. Growth curve and MAb profile from the culture during which the temperature was shifted, is presented in Figure 3. The total MAb concentration was comparable (52 mg/L vs. 56 and 51 mg/L) to those obtained at 35 and 37°C despite the fact that Viability Index was much lower. As shown in Table II, V_I obtained in the temperature shift experiment was only 58% and 74% of that obtained at 35 and 37°C, respectively. The Viability Index was lower in the temperature shift experiment because viability decreased sharply after the temperature shift was effected. Therefore, if cells can be maintained viable for longer periods of time, temperature shift strategy can be used to improve MAb production. This may be achieved by a gradual increase in temperature after $X_{v\max}$ is attained, instead of a sudden increase, or by other means such as nutrient supplementation.

Table II. Variation of Viability Index (V_I), MAb concentration, and specific MAb production rate (q_{MAb}) of HB-32 with temperature.

Temperature (°C)	Viability Index, V_I (day 10) ($\times 10^{-6}$) (cell h/mL)	MAb concentration (day 10) (mg/L)	Specific MAb production rate, q_{MAb} ($\times 10^{9}$) (mg/cell h)
29[a]	16	3 ±1	—
33	616	41 ±3	0.07
35	534	56 ±5	0.11
37	420	51 ±4	0.12
39	261	28 ±2	0.15
33/39	312	52 ±3	—

[a] experiment was discontinued on day 6.

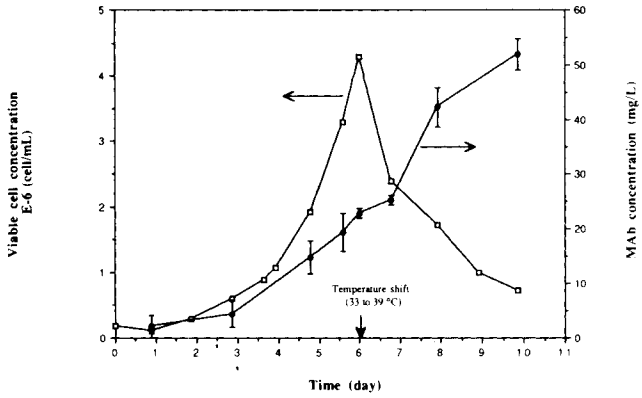

Figure 3. Growth response and MAb profile from the temperature shift experiment.

CONCLUSIONS

Cultivation temperature significantly affects specific MAb production rate, maximum specific growth rate, specific rates of glucose uptake, and lactate production of HB-32 cells. Within the range of examined temperatures permissive to growth, the specific MAb production rate increased with temperature. The specific MAb production rate at 33°C was 43% of that observed at 39°C. Maximum specific growth rate at 33°C was about 76% of the value found at other temperatures. Cells did not proliferate at 29 and 42°C. When the temperature was raised from 33 to 39°C, specific rate of glucose consumption increased by a factor of 2.3 and specific rate of lactate production increased by a factor of 5.4. Also the amount of lactate produced for every unit of glucose consumed increased by a factor of 2.4. These indicate that the level of cellular metabolism increased with temperature.

The authors would like to thank O.T. Ramirez for his helpful suggestions.

References

1. Y.T. Luan, R. Mutharasan, and W. E. Magee, *Biotechnol. Lett.*, **9,** 691 (1987).
2. O.T. Ramirez, R. Mutharasan and W. E. Magee, *Biotechnol. Techniques,* **1,** 245 (1987).
3. S. Reuveny, D. Velez, F. Riske, J. D. Macmillan, and L. Miller, *Dev. Biol. Stand.,* **60,** 185 (1985).
4. J. M. Renard, R. Spagnoli, C. Mazier, M. F. Salles, and E. Mandine, *Biotechnol. Lett.,* **10,** 91 (1988).
5. O.T. Ramirez, G. K. Sureshkumar, and R. Mutharasan, *Biotechnol. Bioeng.,* **35,** 882 (1990).
6. G. M. Lee, T. K. Huard, M. S. Kaminski, and B. O. Palsson, *Biotechnol. Lett.,* **10,** 625 (1988).
7. S. Reuveny, D. Velez, J. D. Macmillan and L. Miller, *J. Immunol. Methods,* **86,** 53 (1986).
8. J. B. Smith, R. P. Knowlton and S. S. Agarwal, *J. Immunol.,* **121,** 691 (1978).
9. N.W. Miller and L.W. Clem, *J. Immunol.,* **133,** 2356 (1984).
10. K. Ozato, N. Mayer and D. H. Sachs, *J. Immunol.,* **124,** 533 (1980).
11. J. Bosworth, A. Brimfield, J. Naylor and K. Hunter, *J. Immunol. Methods,* **62,** 331 (1983).
12. M.W. Glacken, R. J. Fleishaker and A. J. Sinskey, *Biotechnol. Bioeng.,* **28,** 1376 (1986).
13. W. M. Miller, C. R. Wilke, and H.W. Blanch, *Biotechnol. Bioeng.,* **33,** 477 (1989).
14. S. S. Ozturk and B. O. Palsson, *Biotechnol. Prog.,* to appear.
15. O.T. Ramirez and R. Mutharasan, *Biotechnol. Bioeng.,* to appear.
16. G. L. Tritsch and G. E. Moore, *Exp. Cell Res.,* **28,** 360 (1962).
17. M. Dalili and D. F. Ollis, *Biotechnol. Bioeng.,* **33,** 984 (1989).
18. M.W. Glacken, E. Adema, and A. J. Sinskey, *Biotechnol. Bioeng.,* **32,** 491 (1988).
19. W. M. Miller, H.W. Blanch, and C. R. Wilke, *Biotechnol. Bioeng.,* **32,** 947 (1988).