OBSERVATIONS CONSISTENT WITH AUTOCRINE STIMULATION OF HYBRIDOMA CELL GROWTH AND IMPLICATIONS FOR LARGE-SCALE ANTIBODY PRODUCTION

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Abstract

Existence of autocrine growth factors (aGFs) may influence the serum requirement for growth of hybridoma cells and thus significantly influence process economics. For the murine hybridoma cell line S3H5/γ2bA2, critical inoculum density (cID) and serum requirement for growth were inversely related for cultivation in both T flasks and spinner flasks. In spinner flasks, an inoculum density of $10^6$ cells/ml was necessary for the cells to grow in RPMI 1640 medium without serum supplement, and an inoculum density of $10^5$ cells/ml was necessary in RPMI 1640 medium with 10% serum. In T flasks, where the local cell density is higher than in spinner flasks, an inoculum density of $10^6$ cells/ml was necessary for the cells to grow in RPMI 1640 medium without serum supplement, and an inoculum density of 1 cell/ml was also necessary in RPMI 1640 medium with 10% serum. Further, immobilized cells at high local cell density could grow under conditions where cells in T flasks at corresponding overall cell density could not grow. The cells at high inoculum density were less sensitive to shear induced by mechanical agitation than the cells at low inoculum density. Taken together these observations support the existence of secreted aGF(s) by the hybridoma cell line used. Since the specific MAb production rate was independent of cultivation method and inoculum density, the existence of autocrine growth factors would suggest that the use of immobilized cells should improve the economics of MAb production.

INTRODUCTION

Autocrine stimulation of cell growth has been described as the molecular and cellular basis for several malignancies (Sporn and Roberts, 1985; Cooper, 1990). The well known critical inoculum density (cID) requirement for growth in the culture (Tharakan and Chau, 1986; Velez et al., 1986; Lee et al., 1989) has led to the hypothesis that hybridoma cells produce autocrine growth factors (aGFs) needed for their own growth. Some supporting evidence for this phenomenon has been reported; spent serum-free medium was found to accelerate growth rate of hybridoma cell line 167.4G5.3 (Ozturk and Palsson, 1990).

The possible existence of aGFs has significant implications for large-scale cultivation of hybridoma cells. The most expensive item for large-scale cultivation is serum. If the aGFs are also contained in the serum, elimination of serum requirement (by adaptation, genetic manipulation, aGF supplementation, etc) might be desirable. Detailed mechanistic understanding and identification of a particular aGF may become necessary but the first step would be to establish aGF requirement phenomenologically.

We therefore set out to address three hypotheses. First, if the aGFs are serum contained and are produced by the cells at low levels, then there should exist an inverse relationship between the cID and the serum level. If the serum level is low then high inoculum densities would be required to make enough aGF to enable growth, whereas high serum levels should satisfy the aGF requirement and allow growth at low inoculum densities. Second, if an aGF is secreted then the mode of cultivation would influence the cID. Cultivation methods that have high local cell densities (such as T flask where the cells sit in close proximity on the bottom of the flask, or cells immobilized in a matrix) should exhibit lower cID as compared to cultivation methods in which
Third, cells grown at high cell density would be exposed to high levels of aGFs which are known to stimulate cell growth. Cells that are actively growing are less susceptible to shear than cells in lag (Petersen et al., 1988) and stationary phases (Lee et al., 1988a; Petersen et al., 1988). Therefore, cells at high cell density may be less sensitive to shear induced by mechanical agitation than cells grown at low cell density. Further, an important issue from an antibody production point of view is whether the specific monoclonal antibody (MAb) productivity is altered by aGFs. Interleukin 6 (IL-6), which may be an aGF in hybridoma cells, is known to stimulate immunoglobulin synthesis in lymphoid cells (Hirano et al., 1986; Balkwill, 1989).

An affirmative answer to these hypotheses is suggestive of the presence of aGF but a conclusive answer can only be obtained by identifying the particular aGF responsible for the growth stimulation. However, phenomenological results are important for MAb production. Here, we show that the answer to the three hypotheses addressed is affirmative, thus justifying a search for the particular aGF responsible. Further, we show that for the cell line we used growth rate and the specific MAb production rate are decoupled.

MATERIALS AND METHODS

Cell line, medium and culture maintenance The murine hybridoma used was S3H5/γ2bA2. The antibody produced by this cell line is an IgG2b, directed against the idiotype of murine lymphoma 38C13 (Bergman and Haimovich, 1977). The cell culture medium was RPMI 1640 (Sigma, St.Louis, MO) supplemented with 10% fetal bovine serum (FBS, Gibco Laboratories, Grand Island, NY), 100 units/ml of penicillin, and 100 μg/ml of streptomycin (Sigma). The cells were maintained in T 25 cm² plastic cell culture flasks (Bellco Glass, Inc., Vineland, NJ) at 37°C in a humidified CO₂ incubator. The cells were diluted 1:5 with fresh medium every other day.

Cell cultures Exponentially growing cells were inoculated into 25 cm² T flasks and spinner flasks (Bellco) at the inoculum density in the range of 10² to 10⁶ cells/ml. The total working volumes of T flasks and spinner flasks were 10 ml and 50 ml, respectively. RPMI medium with various FBS concentrations was used. For static culture, thirty T flasks were inoculated at each initial cell density and three flasks were taken each time for assays. Since a significant amount of S3H5/γ2bA2 cells attach at the bottom of T flasks during the culture, it was necessary to sacrifice T flasks to obtain accurate cell counts (Lee et al., 1988). Exponentially growing cells were also inoculated into 6 well plates at the inoculum density in the range of 1 to 10 cells/ml to determine a critical inoculum density required for growth in media with various serum concentrations. For agitated cultures, cell cultures in spinner flasks were carried out at two agitation speeds of 100 and 200 rpm. All the experiments were performed in a humidified CO₂ incubator adjusted to 37°C.

Immobilization The hybridoma cells were entrapped in a gel matrix of calcium alginate as follows: (1) Exponentially growing cells were centrifuged at 1000 rpm for 10 min. After discarding the supernatant, cells were resuspended in buffered saline (0.9% NaCl buffered with 2.2 mM HEPES at pH 7.4 with 150mg/l glucose) and the stock sodium alginate solution (2% in 0.9% NaCl) to form a final concentration of approximately 10⁵ cells/ml in 1.0% sodium alginate. One ml of a mixture of viable cells and alginate solution was packed in a syringe. (2) The mixture was added dropwise to 1.3 % CaCl₂ buffered with 13 mM HEPES. Eighty one uniform-sized gel particles (ca. 2 mm in diameter) were obtained in this manner. (3) Gel-entrapped hybridoma cells were allowed to stand for 10 minutes in order to achieve proper gelation. The supernatant solution was removed by decantation, and the immobilized cells were washed 3 times with buffered saline. (4) Gel particles were resuspended in 0.05%(w/v) poly-L-lysine (PLL, molecular weight 22,000, Sigma) for 6 minutes to form the membrane. This PLL treatment is necessary when RPMI 1640 is used, because high concentrations of phosphate in RPMI medium will dissolved the calcium alginate gels (Lee and Palsson, 1990). The supernatant solution was removed by decantation, and the immobilized cells were washed 3 times with fresh RPMI 1640 medium with 1% FBS. Twenty gel particles were transferred to 75cm² T flask containing 30 ml of RPMI 1640 medium with 1% FBS. The initial cell concentration was ca. 800 cells/ml of medium.

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. The specific growth rates were determined from the slope of the logarithmic viable cell concentration versus time during the exponential growth period. The cell growth in the