Growth of Human Lymphoid Cells (Raji Strain) in a Five-Liter Fermentor

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Received for publication 10 March 1971

Procedures used to produce an established line of mammalian cells (L cell) in a New Brunswick fermentor were adapted to propagate human lymphoid cells in suspended culture. Control of pH within defined limits was more effective for regulation of cell metabolism than control of oxidation-reduction potential. An unusually high rate of agitation was required.

Human lymphoid cells have become an important tool for research in the field of transplantation immunity, yet the methodology of their production has received little attention other than that of small-scale laboratory culture. In this report, we show that basic procedures used to produce mammalian cells (L cell) in fermentors (New Brunswick Scientific Co., New Brunswick, N.J.; 2) may be used to grow human lymphoid cells; however, regulation of cellular metabolism is best accomplished by control of pH rather than oxidation-reduction potential (ORP).

Human lymphoid cells of the Raji strain, established in vitro in Nigeria (3) from Burkitt’s lymphoma, were grown in chemically defined medium (1) supplemented with heat-inactivated fetal calf serum (Flow Laboratories, Rockville, Md.), 0.1 unit of insulin per ml, crystalline (Sigma Chemical Co., St. Louis, Mo.), 50 units of nystatin per ml (E. R. Squibb & Sons, Inc., New York, N.Y.), 100 units of penicillin per ml, and 100 μg of streptomycin per ml. All suspension cultures were grown in the presence of 0.10% methylcellulose of 15-centipoise viscosity. Stock cultures and inoculum for initiating a new cycle of growth were maintained at a cell concentration of about 7.5 x 10⁶ cells/ml in liquid nitrogen vapor (−150 C) and upon reculturing were fast-thawed (37 C) and inoculated directly into suspension culture.

Growth vessels consisted of 125-ml metric Pyrex bottles (Corning Glass Co., Corning, N.Y.); different-sized Erlenmeyer flasks; 1-liter, water-jacketed, cylindrical, Pyrex-jacketed spinners (Bellco Glass, Inc., Vineland, N.J.); or a 5-liter Pyrex New Brunswick fermentor. The latter two vessels were operated under conditions where ORP control was present or absent as described in earlier work (2). Viable cell counts were determined by using the erythrosin B dye exclusion test (2).

Lymphoid cell cultures were readily initiated from inoculum cultures stored at −150 C by holding the frozen cultures at 37 C in a water bath until thawed; then transferring 2 ml into 23 ml of fresh medium in a 125-ml, metric Pyrex bottle; and growing on a New Brunswick gyratory shaker at 128 rev/min at 37 C. The culture bottles were stoppered for the first 24 hr and then vented with a 13-gauge needle. Growth occurred readily and at a rate equivalent to that observed for L cells (1). No problems were observed during routine build-up to a 200-ml volume in Erlenmeyer flasks by 1:1 splits after a cell density of 10⁶ cells/ml was obtained [Fig. 1 (1–2) and (1–3), curves B, C, and D]. Each 24 hr, pH was adjusted to 7.0 with 0.5 M NaHCO₃.

When growth of lymphoid cells was attempted in the Bellco spinner under the conditions earlier established for L cells (i.e., ORP, 75 ± 15 mv, pH 7.0, agitation 200 rev/min), poor growth or a decrease in viable cells occurred [Fig. 1 (1)]. Growth did not occur until the agitation was increased to 600 rev/min. Moreover, because the pH dropped to 6.4 ± 0.1 in 24 hr, pH was adjusted each 24 hr to 7.0 and continuous control of ORP was eliminated. Under these conditions, growth rates equal to those obtained in Erlenmeyer flasks occurred [Fig. 1 (1–2), (1–3), curve H]. This high rate of agitation did not affect viability adversely, even when the cells were in stationary growth.

If pH was not adjusted to 7.0 [Fig. 1 (1–2) and (1–3), curve E–F], no growth occurred, although good viability was maintained. However, when pH was adjusted to 7.0, normal growth occurred [Fig. 1 (1–2) and (1–3), curves F–G and H–I]. Growth in a New Brunswick fermentor also
required the same conditions as a Belco spinner. Cell growth was stationary or declining at 200 and 400 rev/min of the agitator [Fig. 1 (1-3), curves I-J], but growth equivalent to that in Erlenmeyer flasks occurred when agitation was increased to 600 rev/min.

We were unable to extend and complete this work but wish to communicate that it appears to be quite feasible to grow human lymphoid (Raji) cells in large culture systems by using methods similar to those used for growth of L cells (2). However, growth of the lymphoid cells differs from L cells in requiring an unexpectedly high rate of agitation and not responding to the more sophisticated methods of continuously controlling pH, namely ORP or gas exchange (2).

We acknowledge the assistance of William I. Jones, Jr., in the preparation of biological media.

LITERATURE CITED

1. Higuchi, K. 1970. An improved chemically defined culture medium for strain L mouse cells based on growth responses to graded levels of nutrients including ion and zinc ions. J. Cell. Physiol. 75:65-72.
2. Klein, F., W. I. Jones, Jr., B. G. Mahlandt, and R. E. Lincoln. 1971. Growth of pathogenic virus in large-scale tissue culture system. Appl. Microbiol. 21:265-271.
3. Pulvertaft, R. J., V. 1965. A study of malignant tumors in Nigeria by short-term tissue culture. J. Clin. Pathol. 18:261-273.