Characterization of YB2/0 cell line by counterflow centrifugation elutriation

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With 2 figures

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Summary

The non-secreting rat myeloma cell line YB2/0 could be separated into different cell fractions by counterflow centrifugal elutriation. The obtained fractions are analyzed by morphology studies, morphometrics, clonogenic assays and flow cytometry. The methodology is extensively described.

A separation of different cell fractions according to cell cycle stages was achieved. This implies further application possibilities for clinical use like the in vitro fractionation of autologous bone marrow prior to transplantation in patients with multiple myeloma.

Introduction

Counterflow centrifugation elutriation (CCE) provides a rapid mean of separating large numbers of cells on the basis of their sedimentation properties. Since its first application for cells by LINDAHL (10) it has found a wide range of research and clinical applications (5, 16).

Blood cell neoplasms have been successfully separated by CCE into populations with different properties (3, 7, 13). Particularly the separation of cell lines at different stages of the cell cycle has been described (1, 8, 11, 12).

The non-secreting rat myeloma clone YB2/0 is a highly efficient fusion partner for the production of hybridomas. YB2/0 was initially derived from the hybrid myeloma YB2/3HL cell line after cloning in soft agar multiple times and selecting for the absence of immunoglobulin secretion. The YB2/0 cell line and its derivatives, moreover, can be propagated in (LOU X AO)F1 hybrid rats, making it a useful model for the study of neoplasms of the immune system. Although the clone has been used extensively for the production of hybridomas since its development in 1982 (9), very little is known about this rat tumor. The purpose of this study was to further characterize the YB2/0 cell line using counterflow centrifugation elutriation (CCE), flow cytometry and clonogenic assay.

Material and methods

Rat myeloma cell line: The non-secreting rat myeloma cell line YB2/0 was obtained from the American Type Tissue Collection (ATTC CTR 1662, Rockville/MD, USA) and propagated in RPMI 1640 (Flow Laboratories, McClean/VA, USA) containing 10% fetal bovine serum (FBS, Flow Laboratories). The cells were harvested in log-phase, centrifuged at 600 x g for 10 min and resuspended in 2 - 5 ml RPMI 1640 prior to CCE.

Rat splenocyte preparation: Normal rat splenocytes were obtained from LOU/C rats bred in the Johns Hopkins Oncology Center animal facility. The LOU/C rats were sacrificed by CO2 asphyxiation and their spleens removed under aseptic conditions. The spleens were gently disaggregated by passage through a wire mesh into a Petri dish containing RPMI 1640. The cell suspension was centrifuged at 600 x g for 10 min and the erythrocytes removed by 0.3% NH4Cl incubation for 5 min. The cell suspension was then washed twice in 2 - 5 ml RPMI 1640 prior to CCE.

CCE procedure: Elutriations were performed with a Beckman J-6M centrifuge with a JE-6B elutriation rotor and standard chamber (Beckman Instruments, Spinco Division, Palo Alto/CA, USA). A Masterflex peristaltic pump (Cole Palmer Instrument Co., Chicago/IL, USA) equipped with a vernier potentiometer provided precisely metered flow. Fifteen to 75 x 107 rat myeloma cells in a volume of 2 - 5 ml were loaded into the chamber at a flow rate of 16 ml/min, rotor speed 2000 RPM, and a temperature of 18°C. The rotor speed was held constant and the cells were eluted to exhaustion at flow rates of 28 ml/min and 36 ml/min. The cells remaining were collected by continuing medium flow after stopping the rotor (R/O, rotor/off fraction).
Elutriation medium: The medium was the same we used for other applications (5). It consisted of physiological saline (0.9%), D-glucose (5.55 x 10^{-3} mol/l), disodium ethylenediaminetetraacetic acid (EDTA; 3.0 x 10^{-4} mol/l), and bovine serum albumin (7.58 x 10^{-6} mol/l). The medium was sterile filtered and the pH adjusted to 7.20.

Cell counts/cytocentrifuge preparation: Cell counts were performed directly from the eluted fractions on a Coulter ZBI (Coulter Electronics Inc., Hialeah/FL, USA). The fractions were then centrifugated at 320 x g for 20 min, washed once in phosphate buffered saline and resuspended in RPMI 1640 containing 10% FBS. Cell viability was determined by trypan blue dye exclusion.

Slides for differential counts were prepared with a cytocentrifuge (Shandon Southern Instruments, Sewicky/PA, USA) using 6 x 10^4 cells/ml RPMI 1640 with 10% FBS. After staining with Wright Giemsa (Camco Quick Stain, American Scientific Products, Ocala/FL, USA), cell differentials were obtained on 100 cell counts per fraction and classified as either “lymphocytoid” or “plasmacytoid.” Lymphocytoid cells were those with slightly basophilic cytoplasm and round or slightly indented nuclei with clumped nuclear chromatin; plasmacytoid cells, however, were more basophilic with eccentric nuclei, perinuclear clear zone and secretory globules.

Morphometric studies

The mean cell surface area was determined for the cells found in the various fractions. The number of evaluated cells are 85 for the unseparated population, 93 in the fraction 28, 122 in the fraction 36, and 87 in the R/O fraction. The harvested cells had a mean area of 467.2 μm^2 (±13.1). As seen in fig. 1b, all CCE fractions had similar cell size profiles except the R/O fractions which contained significantly larger cells (p<0.001; T-test for independent samples with unequal variances). In contrast, the nuclear to cytoplasmic ratio remained fairly constant between the cells of the various fractions (fig. 1c). The mean nuclear area of the cells in the R/O fraction is 244 μm^2 as compared to 223 μm^2 for the cells of fraction 36.

Clonogenic assay

The cloning efficiency of each fraction, as compared to the unfractonated YB2/0 cells, is shown in figure 1d (3 experiments). The unseparated cells formed an average of 8.36 colonies per 50 plated cells (cloning efficiency 16.7%). The cloning efficiency of the 28 ml/min fraction was 40.7% (±9.1). The cloning capacity of the 28 ml/min cell population is significantly enhanced (p<0.05; one-sided U-test); this is an increase of 261.4% (±55.2) over the unfractonated cell population. In contrast, the cloning efficiency for the R/O fraction was 15.5% (±3.9%). The cloning efficiency of the R/O fraction is virtually identical to that of the harvested cell population (107% and 79% of the unseparated control). As demonstrated in Figure 1e, of the clonogenic cells present in the harvest, more than 85% are eluted during the cell load (at 16ml/min) and at 28 ml/min. Few clonogenic cells remained to be eluted in the large cell (R/O) fraction.
Flow microfluorometric DNA analysis

Figure 2 shows the DNA histogram of the unseparated YB2/0 cells obtained during log-phase cell growth. As compared to the diploid rat splenocytes, the unseparated YB2/0 cells are asynchronous with the majority of the cells being less than tetraploid. The DNA histograms show a progressive shift of cellular DNA content from a primar-

![DNA histogram](image)

Fig. 2. The DNA distribution of the YB2/0 cell line in comparison to normal rat splenic lymphocytes is demonstrated.

ibly G1 content in the 16 ml/min (load) and 28 ml/min fractions toward a G2 + M content in the R/O fraction. The percentage of cells in the G1, S, and G2 + M phase of each fraction, calculated from the DNA-content, is shown as a histogram in figure 1f. The 16 ml/min fraction (Load) and 28 ml/min fraction contain 86.5% of the G1 phase cells, and the last two fractions contain 41.5% of the G2 + M phase cells (28.9% and 12.6%, respectively). The majority of the G2 + M cells exit from the chamber during the load process. Of the cells present in the R/O fraction, however, 47.1% are in the G2 + M phase.

Discussion

The YB2/0 plasmacytoma cell line is a highly efficient partner for the production of hybridomas. YB2/0 are large, round cells that do not secrete any light chain immunoglobulins; both characteristics being advantageous. This same cell line, moreover, can be propagated in the (LOU × AO)F1 hybrid rat strain. This transplantable tumor offers yet another model for developing hybridisation methods and for studying the biology of both malignant myeloma as well as normal B cell regulation (4, 6, 9).

The results presented here substantiate and further define the heterogeneity of the YB2/0 plasmacytoma cell line. Like the MOPC-315 murine plasmacytoma line, the lymphocytoid and plasmacytoid cells were not separable by CCE (3). But, as with the EPSTEIN-BARR virally transformed human B lymphocyte line, LAZ-007, CCE separa-
tion of YB2/0 actually represents separation on the basis of position in the cell cycle and not on cell size (12).

The clonogenicity studies on the various fractions reveal the highest clonogenic frequency in those fractions enriched for cells in the G1 phase of the cell cycle. Although no particular fraction could be enriched for cells in G2 + M phase of the cell cycle, the 28 ml/min fraction contained the fewest and yet had the highest cloning efficiency. The small cell population appears to represent the clonogenic population with the large cells retaining only limited ability to proliferate. The proliferative capacity of YB2/0 myeloma cells with different cell size and density distributions needs further investigation both in vitro and in vivo. Perhaps with such an animal model, therapies specifically directed at the clonogenic, small cell population might be developed.

CHANG et al. (1) proved that the sensibility of hypernephroma cells towards combination treatment with interferon alpha and irradiation is dependent on their cell cycle phase. One therapeutic option in clinical hematology might include the use of CCE fractionation of autologous marrow, a method currently being used to lymphocyte-deplete allogeneic grafts for the prevention of acute graft-versus-host disease (15).

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References