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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 meyeloma 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 × 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, centrifugated at 600 × g for 10 min asnd 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 CO_2 asphyxiation and their spleens removed under aseptic conditions. The spleens were gently disaggregated by passage through a wire mesh into a Petri dish containing RPM1 1640. The cell suspension was centrifugated at 600 × g for 10 min and the erythrocytes removed by 0.3% NH_4Cl incubation for 5 min. The cell suspension was then washed twice in RPMI 1640.

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 75×10^7 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 (5.55×10^{-3} mol/l), disodium ethylenediaminetetraacetic acid (EDTA; 3.0×10^{-4} mol/l), and bovine serum albumin (7.58×10^{-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 $320 \times g$ 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 6×10^4 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.

Morphometrics: Morphometric analyses were performed with computer assisted image analysis similar to that described by Diamond et al. (2). A Zeiss-3 microscope with a 40× objective and an Olympus OM-2 camera were used to photograph the stained slides. A GTCO digitizer pad attached to an IBM Personal Computer (IBM, Boca Raton/FL, USA) with modified GAP 1 software was used to trace the cell perimeter and nuclear perimeter from projection slides after calibration with a projection slide showing the grid of a Neubauer counting chamber.

Clonogenic assay. Fifty cells per fraction were plated in triplicate in 2 ml semi solid agar preparation in 12×75 mm sterile capped tubes (Falcon, Oxnard/CA, USA). A 1 : 10 dilution of Agar Noble (Difco Laboratories, Detroit/MI, USA) in Dulbecco's Modified Eagles's medium (Gibco, Grand Island/NY, USA) was prepared with 20% FBS, 10% NCTC-109, 2% L-glutamine, 1% oxaloacetate-pyruvate-insulin. The cell cultures were incubated for 14 days in a 37°C humid chamber with 5% CO_2 atmosphere. Visible colonies of more than 50 cells were counted on day 14.

Flow microfluorometric DNA analysis: The fractionated rat myeloma cells (1×10^6 cells/fraction) and normal rat spleen lymphocytes were resuspended in 1 ml propidium iodide cocktail as described by VINDELOV (14). The cocktail consisted of 7.5×10^{-5} mol/l propidium iodide (Cal Biochem, San Diego/CA, USA), 0.1% Nonidet P-40 (Bethesda Research Lab., Bethesda/MD, USA), 1.0×10^{-2} mol/l NaCl, 3.4×10^{-3} mol/l sodium citrate and 700 units/l ribonuclease. After resuspension, the cells were stored overnight at 4°C in the dark.

Flow cytometry was performed with FACS II Instrumentation (Becton Dickenson, Sunnyvale/CA, USA) using a 5 watt Argon laser at 488 nm wavelength. The propidium iodide fluorescence was measured using a 580 LP filter.

Results

Cell separation characteristics and recovery

The viability of the cultured YB2/0 cells ranged from 83 to 92% (mean 85%) for the 5 separation experiments. As expected, the vast majority of the non-viable cells were eluted at 15 ml/min, during the loading process. The remaining fractions had more than 95% cell viability consistently as determined by dye exclusion.

Figure 1a shows the cell recovery profile. Of the viable cells recovered, 45.1% (±8.8) were recovered in the load fraction, 31.4% (±5.6) in fraction 28, 14.7% (±3.0) in fraction 36, and 8.9% (±2.9) in the R/O fraction (standard mean errors in brackets). On examining the cytocentrifuge preparations obtained from each fraction, no separation between lymphocytoid and plasmacytoid cells could be delineated. Eighty percent of the unseparated cells had lymphocytoid morphology with other fractions having between 73% and 88% lymphoid-like cells.

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 provfiles 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 unfractionated 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 clonogenic capacity of the 28 ml/min cell population is significantly enhanced ($p \leq 0.05$; one-sided U-test); this is an increase of 261.4% (±55.2) over the unfractioned cell population. In contrast, the cloning efficiency for the R/O fraction was 15.5% (±3.9%). The clonogenicity 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.

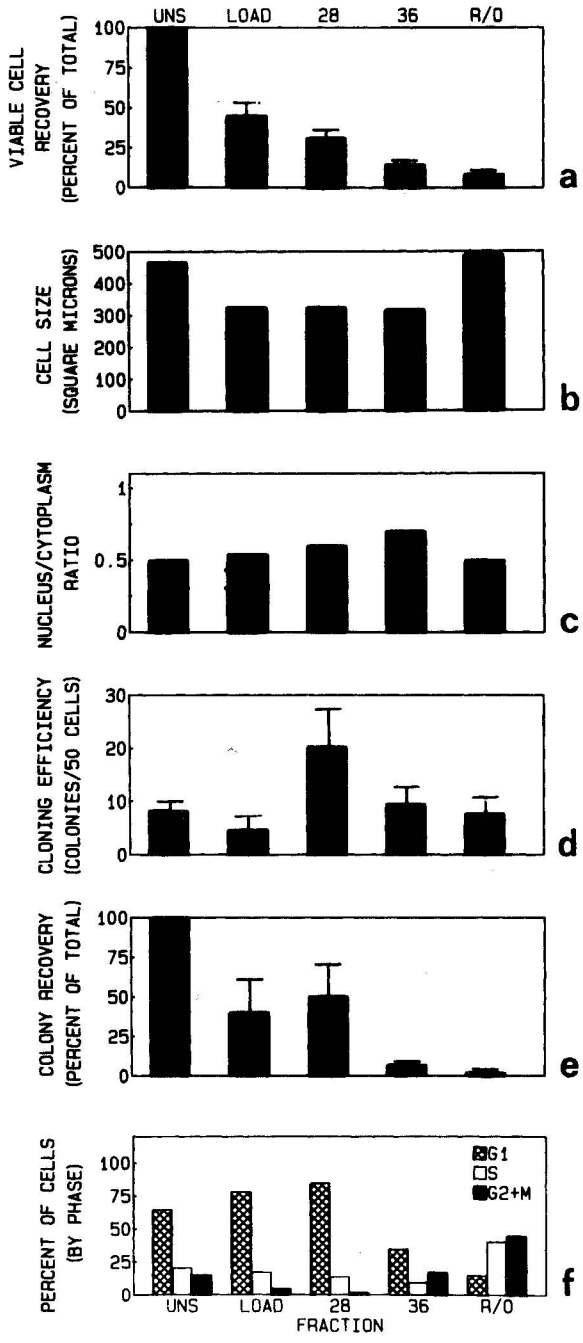

Fig. 1a–f. Properties of the YB2/0 cell line before separation (UNS) and of the obtained fractions: The fractions are named according to their pump flow rate during the elutriaton process (while the rotor speed was held constant). LOAD: loading pump flow rate 16 ml/min, 28: pump flow rate 28 ml/min, 36: pump flow rate 36 ml/min, R/O (rotor off): the cell fraction which was eluted after the rotor was stopped. Cell distribution of the recovered cells (**a**), cell size (**b**), nucleus/cytoplasm ratio (**c**), cloning efficiency (**d**), colony recovery (**e**) and number of cells by cell cycle phase (**f**) are shown in histograms. Bar extension indicate standard mean errors.

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 primarily 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.

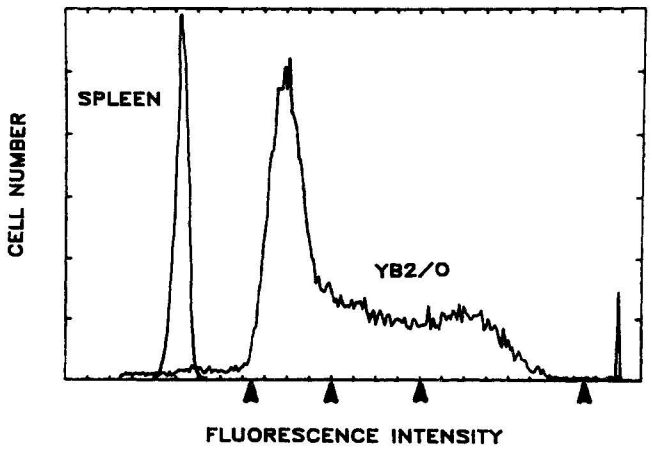

Fig. 2. The DNA distribution of the YB2/0 cell line in comparison to normal rat splenic lymphocytes is demonstrated.

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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