Cell Separation

METHODS AND SELECTED APPLICATIONS

EDITED BY

THOMAS G. PRETLOW II AND THERESA P. PRETLOW

VOLUME 3

1984

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Institute of Pathology
Case Western Reserve University
Cleveland, Ohio

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Preface

In 1975, we published a general review of methods of cell separation. Because of the interest in this review, we planned a sabbatical year to write a book with the same scope. Between the writing of the first review (1973–1974) and the attempt to write a book (1977–1978), the references to be cited increased from somewhat more than five hundred to somewhat more than seven thousand. Our bibliography pertinent to this methodology was expanding at a rate of two to four dozen articles weekly, and we were compelled to face the fact that it was no longer feasible for one or two authors to address this area adequately. The rapid growth in this area led us to plan this multivolume, multiauthored treatise.

In approaching this work, it was our goal to select critical authors with considerable personal familiarity with the design and/or application of methods for the separation of cells. Rather than attempt comprehensive reviews, they were asked to address relatively finite subjects and to include sufficient references to direct those readers who want more information to the appropriate sources. We have attempted to address this work to a heterogeneous audience of experimental oncologists, hematologists, immunologists, cell biologists, endocrinologists, and others who are not already expert in the use of methods for cell separation. We are grateful that most of those invited to contribute to this work found the time to do so, and we hope that their critical, quantitative approaches to problems in cell separation will stimulate new investigators to examine critically many of the "accepted" methods for cell separation.

THOMAS G. PRETLOW II
THERESA P. PRETLOW

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Chapter 1

Separation of Malignant Lymphoid Cells by Countercurrent Distribution

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I. Introduction

To determine tumor cell characteristics important in tumor metastasis cell subpopulations with altered survival and malignant properties have been isolated from heterogeneous populations of parental tumor cells. In general, two strategies have been used: (1) selection sequentially *in vivo* or *in vitro* to obtain variant cell lines differing in their metastatic properties and (2) cloning *in vitro* to obtain cell clones with discrete metastatic potentials. These approaches have proved invaluable in examining particular cell-surface properties and their role in the metastatic process (see reviews by Nicolson, 1982; Nicolson and Poste, 1983).

Recent evidence indicates that the metastatic properties of highly selected tumor subpopulations or cell clones may be unstable during growth *in vivo* or in tissue culture (Chow and Greenberg, 1980; Fidler and Nicolson, 1981; Poste *et al.*, 1981; Miner *et al.*, 1982). Therefore, tumor cell subpopulations should be obtained as quickly as possible so that random cellular changes which can occur during cell growth after selection or cloning are not su-

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perimposed on cellular differences due to metastatic or other properties. For this reason rapid separation techniques based on cell density have been used to isolate metastatic cell subpopulations. That such techniques can be utilized for the separation of cells with differing malignant properties was shown by Grdina *et al.* (1977), who separated cells from a methylcholanthrene-induced fibrosarcoma on linear density gradients of methyl-glycamine 3,5-bis(acetylamino)-2,4,6-triliodobenzoate. When analyzed for metastatic potential, low-density cells were more efficient in lung colonization assays than high-density cells from the same tumor. Using the B16 melanoma of spontaneous origin Baniyash *et al.* (1981) showed that cells from the low lung-colonizing B16-F1 subline had a higher mean density profile in collodial silica isopycnic density gradients than cells from the high lung-colonizing, *in vivo*-selected B16-F10 subline.

Viable cell subpopulations can be quickly and easily obtained by subfractionation using cell partitioning in two-polymer aqueous phases (reviewed in Walter, 1977). This cell separation technique is based on subtle differences in cell surface properties that occur when cells are undergoing changes in differentiation, maturation, aging, and transformation. Albertsson and Baird (1962) used aqueous, isotonic solutions of two different water-soluble polymers, such as dextran and poly(ethylene glycol), which can be mixed at specific concentrations to yield two-phase systems suitable for cell separation and subfractionation via partitioning procedures. By careful selection of polymer type, ionic composition and concentration in each phase, cells can be separated based on subtle differences in their surface charge properties, lipid-related membrane characteristics or polymer affinity of cell surface constituents (Walter, 1977). The sensitivity of this technique stems from the relationship between partitioning coefficients and the cellular properties that determine them; for all practical purposes this is an exponential rather than a linear relationship (Walter, 1977).

If subfractionation by partitioning of cells is to be based predominantly on surface charge characteristics, salts are added to the aqueous phases. Since salts, such as phosphates and sulfates, have different affinities to polymers like dextran and poly(ethylene glycol) (Johansson, 1970), they partition unequally between the phases, and electrostatic potential differences can be established between a more electronegative dextran-rich bottom phase and a more electropositive, poly(ethylene glycol)-rich top phase (Reitherman *et al.*, 1973). Such two-phase systems can be used to separate cells with minor differences in charge-associated surface properties (Walter, 1977).

We have used sequential aqueous partitioning (countercurrent distribution or CCD) in dextran-poly(ethylene glycol) phases that possess electrostatic potential differences to separate malignant lymphoma cells (Miner *et*

al., 1981). These CCD cell separations have shown that highly malignant cell variants exist in and can be obtained from the low-malignant parental tumor cell population by fractionations based on differences in their cell surface properties.

II. Experimental Methods

A. Tumor Cell Lines

Parental large cell lymphoma line RAW117-P was obtained from spleen cultures of BALB/c mice infected by Abelson leukemia virus (Raschke *et al.*, 1975). This cell population has been used to sequentially select *in vivo* for highly malignant liver-colonizing variant sublines (Brunson and Nicolson, 1978). Cell growth and passage conditions of these cells are described elsewhere (Brunson and Nicolson, 1978; Reading *et al.*, 1980a,b).

B. Preparation of Dextran-Poly(ethylene glycol) Aqueous Phase Systems

Two different phase systems were used for CCD which take into account the differences in charge-associated membrane surface properties between low- and high-malignant RAW117 cells (Miner *et al.*, 1981). They were prepared as described by Walter (1977). Phase system 1 consisted of 5% (w/w) dextran T500, 4% (w/w) poly(ethylene glycol) 6000, 160 mos*M* sodium phosphate buffer, pH 7.4, 120 mos*M* NaCl, and 5% fetal bovine serum (FBS) (heat inactivated); phase system 2 contained the same polymer concentrations but with 135 mos*M* sodium phosphate buffer, pH 7.4, 144 mos*M* NaCl, and 5% FBS. The electrostatic potential difference between the phases is higher in phase system 1 than in phase system 2. The phase systems were filtered through a 0.45-μm filter (Nalge), equilibrated at 4–5°C in a separatory funnel, and the top and bottom phases were separated.

C. CCD Separation of RAW117 Lymphoma Cells

RAW117 lymphomas cells at a density of 2–3 $\times$ 10^6 cells/ml were suspended in 4 ml of the top phase of the CCD system and were placed into the first cavity of a thin-layer CCD apparatus (Albertsson, 1970) consisting of two circular Plexiglas plates with 120 concentric cavities and a bottom phase capacity of 0.7 ml. In this apparatus the bottom plate was a stator plate, and the top plate was a rotor plate. We were able to run simultaneous CCD separations on two cell preparations at 4–5°C in the identical phase

systems beginning at opposite ends of the plate without overlap (Miner *et al.*, 1981). The automatic cycle consisted of shaking for 25 seconds and settling for 6 minutes followed by a transfer. After the transfers (50 or 59) were completed, tumor cells were collected directly into sterile plastic centrifuge tubes. Adjacent tubes were pooled into groups of four. Tumor cell viability ranged from 60 to 96% as determined by the trypan blue dye exclusion test. The tumor cells were washed with growth medium plus 10% FBS and gentamicin sulfate (50 μg/ml), and were incubated for 12 hours at 37°C prior to conducting the *in vivo* assays for metastatic potential.

In one of the experiments, cavities 8–19 and cavities 28–43 were pooled separately and cultured overnight. They were then subjected separately but simultaneously to a second CCD separation in a phase system having the same composition as that used in the original fractionation. This is a standard method to test whether cells from the left and right ends of a distribution are truly different (i.e., have different partition coefficients) or are merely distributed on a random basis (Walter *et al.*, 1981). Aliquots of the cell suspensions obtained from different portions of the CCD extraction train were electronically counted with an Electrozone Celloscope.

D. *In Vivo* Assays

RAW117 cells were assayed for organ colonization (experimental metastasis) after intravenous injection of 5000 viable cells into at least 10 animals per group (Brunson and Nicolson, 1978). After 14–23 days, organs were removed, and the numbers of tumor colonies in each organ were determined visually. Organ colonization was confirmed histologically by staining thin sections of paraffin-embedded tissues with hematoxylin (Reading *et al.*, 1980a).

E. Analysis of Cell-Surface Proteins

Cellular glycoproteins were identified by autoradiography after sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis (SDS-PAGE) using ^{125}I-labeled lectins (Maizel, 1971; Irimura and Nicolson, 1983).

III. Fractionation of RAW117 Lymphoma Cells

A. CCD Patterns of RAW117 Lymphoma Cells

The CCD distribution curves of low-malignant potential RAW117-P and high-malignant potential, liver-selected RAW117-H10 cells indicated that there were cell-surface differences between these cell populations (Fig. 1).