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SIMPLIFIED METHOD FOR DNA AND PROTEIN STAINING OF HUMAN HEMATOPOIETIC CELL SAMPLES

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ABSTRACT

A rapid reproducible method yielding high resolution analysis of DNA and protein in human hematopoietic cell samples has been developed by modification of the propidium iodide (PI) and fluorescein isothiocyanate (FITC) procedure. Cell staining involved sequential addition of each reagent (RNase, FITC and PI) to ethanol-fixed cells and requires no centrifugation steps.

Stained cells are analysed in the reagent solutions. Analysis of bone marrow samples from multiple myeloma patients revealed mixed 2C DNA and aneuploid populations with the aneuploid cells having a significantly higher protein content.

This approach permitted differential cell cycle kinetic analysis of the 2C DNA and the aneuploid population.

INTRODUCTION

Analyses of cellular DNA by flow cytometry (FCM) provides a rapid and convenient method for studying cell cycle kinetics and population ploidy levels. However, in heterogeneous cell populations, such as found in human bone marrow, the kinetic patterns are a composite of all hematopoietic cell types and cell cycle progression profiles for subpopulations of interest are not easily derived. Furthermore, when malignant aneuploid cells become prominent in the FCM-distributions (3), cell cycle kinetic analysis by single parameter DNA measurements is not possible.

Protein content of cells reflects in a general way, the metabolic capacity of cells and, in most instances, is also a good descriptor for protoplasmic mass. Simultaneous FCM analysis of cells stained for both DNA and protein can reveal subpopulations of different protein content located throughout the cell cycle or within aneuploid cell populations. Additional descriptive information such as the nuclear to cytoplasmic ratios and the DNA to protein ratios for subpopulations can be determined by FCM (1) and this approach can further enhance the analysis of heterogeneous cell populations.

This report describes a rapid and simplified method for staining hematopoietic cells with propidium iodide (PI) and fluorescein isothiocyanate (FITC). In contrast to our previous protocol (1), all reagents are added sequentially to ethanol-fixed cells and no centrifugation steps are required. Staining time is reduced to about 10 minutes as compared to about

3 hr by our previous method.

MATERIALS AND METHODS

Fresh aspirated bone marrow obtained by sternal puncture was diluted in ACD-solution (acid citrate dextrose). Red blood cells were removed by centrifugation over Percol (density 1.085 g/ml). The supernatant cell suspension was diluted four fold with phosphate buffered saline solution containing 13mM disodium EDTA (PBS-EDTA). White blood cells were recovered by centrifugation, fixed in 70% ethanol and stored at $4^{o}C$.

For cell staining all reagents were added sequentially to ethanol-fixed cells and no centrifugation steps were required. Four ml of PBS-EDTA solution were added to 0.2 ml of ethanol fixative containing $5x10^{6}$ cells. Fluorescein isothiocyanate was initially dissolved in absolute ethanol (1 mg/ml) <u>just before use</u>, and 0.1 ml of this stock solution was added to 10 ml of PBS-EDTA solution. Two-tenths ml of the FITC in PBS-EDTA solution was added to the cell suspension and after approximately 5 min, one ml of propidium iodide (PI, 46 µg/ml) was also added to the cell suspension. After 2-3 min, 0.2 ml RNase (1mg/ml PBS-EDTA) at room temperature was added for at least 1-2 min prior to analysis. Stained cells in the reagent solutions were analyzed in a Phywe ICP 11 pulse cytophotometer modified for two-color analysis.

RESULTS

Figure I (A-D) shows the DNA-protein distributions obtained by analysis of a bone marrow sample from a multiple myeloma patient following staining with PI and FITC. The DNA profile (Fig 1A) shows a G_1/G_0 peak (major peak) representing a 2C DNA content population based on analysis of the DNA content for nucleated peripherial blood cells from a normal patient. Adjacent to this peak is the G_1/G_0 peak for an aneuploid cell population. The minor peak represents the G_2+M peak for the normal population.

The protein content distribution (Fig 1B) obtained by analysis of the green (FITC) fluorescence is bimodal, indicating at least two subpopulations of cells with respect to protein mass. The two parameter DNA-protein profiles (Fig IC and ID), particularly the contour profile (Fig ID) shows that the aneuploid cell population has a significantly higher average protein content and on this basis could be separated from the population lying between the 2C-4C DNA content range. These analyses also show that the aneuploid cell population has fewer cells in the S and G_2+M phases of the cell cycle.

DISCUSSION

Protocols for cell staining, particularly in routine clinical studies, should be simple, rapid, highly reproducible and still provide for quality analytical resolution. In this

study we have described a useful method for DNA and protein staining of human hematopoietic cell samples using PI and FITC, respectively. Cells are treated sequentially with the reagent solutions, and rinsing or centrifugation steps are not required. The approach not only increases the rapidity of the method compared to our original procedure (1)(i.e., 10 min vs 3 h, respectively), but it also minimizes cell clumping and cell loss which can be a particular problem in hematopoietic cell samples.

The two parameter profile obtained by analysis of the PI-FITC stained bone marrow sample demonstrates the high quality color resolution that is obtainable with this technique. By maintaining the stain concentrations at a low level, particularly the highly fluorescent FITC, background fluorescence remains minimal. However, the dye concentrations are still adequate to provide good resolution for detecting abnormalities such as the aneuploidy population in the DNA profile.

From a clinical standpoint, the procedure appears particularly attractive for separating the cell cycle kinetic profile of the aneuploid population from the normal 2C-4C DNA content population. In a recent autoradiographic study by Durie et al. (2), analysis of the percentage of cells in S phase (tritiated thymidine labeling index, LI) in bone marrow cells of untreated multiple myeloma patients provided helpful prognostic information. In general that study showed that pretreatment LI >3% in high cell mass patients conferred a poor prognosis compared to patients with LI<1%. A recent report by Latreille et al. (3) revealed a 65% incidence of aneuploidy in myeloma

patients and demonstrated that the frequency of myeloma plasma cells correlated with the percentage of cells with abnormal DNA content. Based on these studies it appears that simultaneous FCM DNA-protein analysis can further increase the sensitivity of the diagnostic procedure of Durie et al. (2). By analyzing the kinetic profile of the aneuploid population separate from the other cell populations it is possible to determine, in each instance, which population contributes most significantly to the elevated percentage of cells in S phase.

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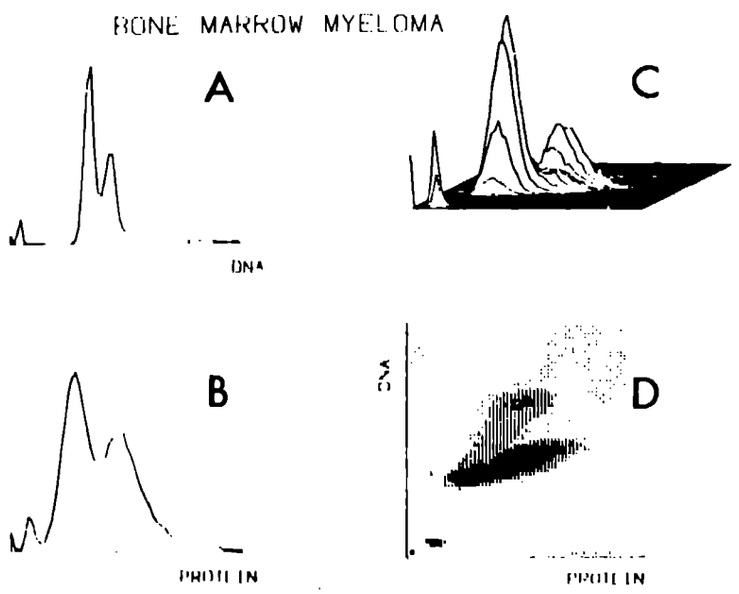

Figure 1. Single parameter DNA (A), protein (B) and two parameter DNA-protein (C, isometric and D, contour) profiles of a bone marrow sample from a multiple myeloma patient.