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Analysis of cycloheximide-induced apoptosis in human leukocytes: Fluorescence microscopy using annexin V/propidium iodide versus acridin orange/ethidium bromide

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Abstract

Apoptosis is a highly regulated and programmed cell breakdown process characterized by numerous changes. Since it is implicated in many pathological as well as physiological processes, it is vital to have reliable methods for detecting cell death.

In this study, we compared several methods for detecting apoptosis and necrosis in human leukocytes. Apoptosis was induced either by incubating the cells with various doses of cycloheximide (CHX) or by 312 nm UVB irradiation. The methods used for detecting apoptosis were light microscopy (May Grunwald–Giemsa and trypan blue staining), fluorescence microscopy (acridin orange/ethidium bromide and annexin V/propidium iodide staining) and agarose gel electrophoresis of fragmented genomic DNA.

Our study showed that CHX-induced apoptosis in cultured peripheral blood mononuclear cells but had no effect on apoptosis in polymorphonuclear cells, so its effect depends on cell type. Evaluation and comparison of the methods for detecting apoptosis showed the following. A Giemsa-stained cytospin allows the main morphological characteristics of necrotic and apoptotic death to be recognized. Trypan blue staining, widely used for estimating cell viability, is valueless for detecting apoptosis. Both fluorescence methods provided reliable and reproducible results and distinguished clearly between subpopulations of apoptotic cells, and were closely intercorrelated. Although applicable to a wide spectrum of cell types, agar electrophoresis of extracted DNA cannot be applied to all cell types and apoptotic conditions. Generally, microscopic examination of acridin orange/ethidium bromide stained cells can be recommended as the most reliable of the methods tested.

Keywords: Apoptosis; Method; Microscopy; Cycloheximide; Leukocytes

1. Introduction

Apoptosis is a well-controlled, tightly-regulated physiological process, in which the cells participate in self-destruction. A large body of evidence suggests that apoptosis is a central mechanism in embryogenesis and morphogenesis, immune system regulation, hematopoesis and control of normal tissue turnover (Vaux and Korsmeyer, 1999), but it has been also implicated in a variety of diseases (Arends and Wyllie, 1991). Failure of cells to undergo normal apoptotic cell death, or increased cell loss by apoptosis, may be involved in the pathogenesis of cancer, autoimmune disorders, neurodegenerative disorders, AIDS and myelodisplastic syndromes.

Apoptosis, or programmed cell death, is essential for the normal development, homeostasis and function of the immune system (JJ Cohen et al., 1992; Golstein et al., 1991). Leukocyte apoptosis must be tightly controlled to allow normal lymphocyte differentiation and neutrophil function in inflammation, and to prevent malignancy and autoimmunity

Abbreviations: CHX, cycloheximide; PBMC, peripheral blood mononuclear cells; PMNC, polymorphonuclear cells; CLL, chronic lymphocytic leukemia; AO/EB, acridine orange/ethidium bromide; AnnV/PI, annexin V/propidium iodide.

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