

## *In vitro* assay for the quantitative measurement of apoptotic lymphocytes phagocytosis by peripheral blood monocytes

S Popovic\*, N. Arsenijevic, D. Baskic

Institute of Microbiology and Immunology, Faculty of Medicine, University of Kragujevac,  
Svetozara Markovica 69, 34000 Kragujevac, Serbia

Received: March 8, 2006

Currently used assays for the quantification of apoptotic cells uptake by phagocytes have several methodological problems. Our assay overcomes some of these problems. As a source of apoptotic cells we used peripheral blood lymphocytes obtained from the patients with chronic lymphoblast leukaemia. Apoptosis was induced by incubating cells with cycloheximide for up to 24 h. The assay was performed in suspension of peripheral blood mononuclear cells. For the visualisation of the phagocytes and phagocytosed cells and discrimination of phagocytosed from bound apoptotic cells we used Acridine orange/Ethidium bromide double staining. Here we offer a simple test which enables reliable measurement and it can show the difference of phagocytic potential between different individuals

**Keywords:** phagocytosis, apoptosis, peripheral blood mononuclear cells

Apoptosis is a physiological mechanism for the removal of unwanted or damaged cells. Apoptotic cells are phagocytosed as soon as they appear *in vivo*. Professional phagocytes, monocyte/macrophage cells, rapidly and efficiently remove these cells by phagocytosis (1, 2). The exposure of phosphatidylserine (3, 4) and modifications of membrane carbohydrate groups on apoptotic cell surface (5), soluble proteins which bind to apoptotic cells and act as “opsonins” (6, 7), and a redundant and promiscuous system of receptors on the phagocytic cell (1, 6), ensures an efficient and rapid uptake of dying cells.

Both apoptosis and clearance of apoptotic cells are essential to prevent leakage of potentially harmful intracellular contents into the microenvironment (8). Defect of phagocytosis of apoptotic cells may have deleterious consequences for neighboring healthy cells and pathogenesis of inflammatory diseases or deregulation of the immune

---

\* Corresponding author: Suzana Popovic;  
Phone: 034 364726; 063 7097063; E-mail: pop21@ptt.yu

















