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The role of apoptotic and necrotic processes in cytolysis mediated by LAK cells with different phenotypes

L.P. Sashchenko[a,*], O.D. Kabanova[a], L.Yu. Blishchenko[b], T.I. Lukjianova[a], D.K. Satpaev[b], O.Yu. Chertov[b], N.V. Gnuchev[a]

[a]*Institute of Gene Biology and* [b]*Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation*

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The role of necrotic and apoptotic pathways in cytolysis mediated by LAK cells was studied. The contribution of necrotic and apoptotic processes to cytolysis depends both on the LAK cells' phenotype and the type of target cells. CD16+/CD8+/CD3− LAK cells induced necrosis of K562 and L929 target cells. The cell death induced by CD3+/CD8+/CD16− LAK cells was found to include features of apoptotic and necrotic processes.

LAK cell; Apoptosis; Necrosis

1. INTRODUCTION

Two major processes in cell death are necrosis and apoptosis. Necrosis is characterized by rapid damage of the target cell membrane with subsequent colloid osmotic lysis, while the nucleus, at least in the early stages, remains intact. Apoptosis is characterized by DNA degradation into oligonucleosomal fragments in the early stages of the process, while the kinetics of the membrane damage is significantly slower [1].

LAK cell activity was shown to be generated from phenotypically heterogeneous precursors: both T-cells [2] and NK [3] contribute to IL-2-generated cytotoxic activity. We examine here the role of the different pathways of cell death (necrosis and apoptosis) in cytolysis mediated by different subsets of LAK cells.

2. MATERIALS AND METHODS

2.1. *Cell culturing*

Human LAK cells as well as L929 and K562 target cells were cultured as described in [4].

2.2. *Cytotoxicity and DNA fragmentation assay*

Membrane permeability (P) and DNA fragmentation (F) were determined as the percentage of Trypan blue that stained cells with damaged plasma membranes [5], and the percentage of specific release of degraded [^{3}H]methylthymidine-labeled DNA [6], respectively, at a target/effector ratio of 1/20.

$$P(\%) = \frac{\text{(stained cells–spontaneously stained cells)}}{\text{(total cells–spontaneously stained cells)}} \times 100$$

$$F(\%) = \frac{\text{(experimental cpm–spontaneous cpm)}}{\text{(total cpm–spontaneous cpm)}} \times 100$$

*Corresponding author.

Abbreviations: LAK cells, lymphokine-activated killer cells; IL-2, interleukin-2; NK, natural killer cells; CTL, cytotoxic T-lymphocytes; TNF, tumor necrosis factor.

3. RESULTS

It was shown earlier [4,7] that the LAK cells' phenotype and their cytolytic activity changed during the course of IL-2 stimulation. In the first 4 days of IL-2 stimulation the surface antigens characteristic for the NK cell phenotype (CD16+/CD8+/CD3−) were expressed, then antigens characteristic for cytotoxic T-cells (CD3+/CD8+/CD16−) became predominant. We studied the mechanisms of target cell lysis induced by CD16+/CD8+ and CD3+/CD8+ LAK cells. The changes in the two parameters of cytolysis, i.e. plasma membrane permeability for Trypan blue dye (P) and DNA fragmentation (F), were compared. The contribution of necrotic and apoptotic processes to cytolysis was estimated by the P/F ratio. P/F < 1 was considered to be characteristic for apoptosis and P/F > 1 for necrotic processes [8].

Fig. 1A shows the kinetics of K562 and L929 cell lysis by CD16+ LAK cells. Membrane damage was detected after 15 min of incubation and reached a maximum within 1 h; the kinetics of DNA fragmentation was significantly slower, since DNA fragments were detected after 1 h of cytolysis. These data indicated that CD16+ LAK cells caused mainly necrotic cell death (P/F = 2.5) of both K562 and L929 target cells. As seen in Fig. 1B CD3+ LAK cells caused a complex process of cell death in K562 and L929 cells. DNA fragmentation (Fig. 1BI) was induced very rapidly (within 15 min) and reached a maximum at 3 h in K562 cells, then membrane permeability increased slowly and became maximal by 4 h. Thus the first 3 h (P/F < 1) were characteristic of apoptosis, but later, necrotic mechanisms (P/F = 1.3)

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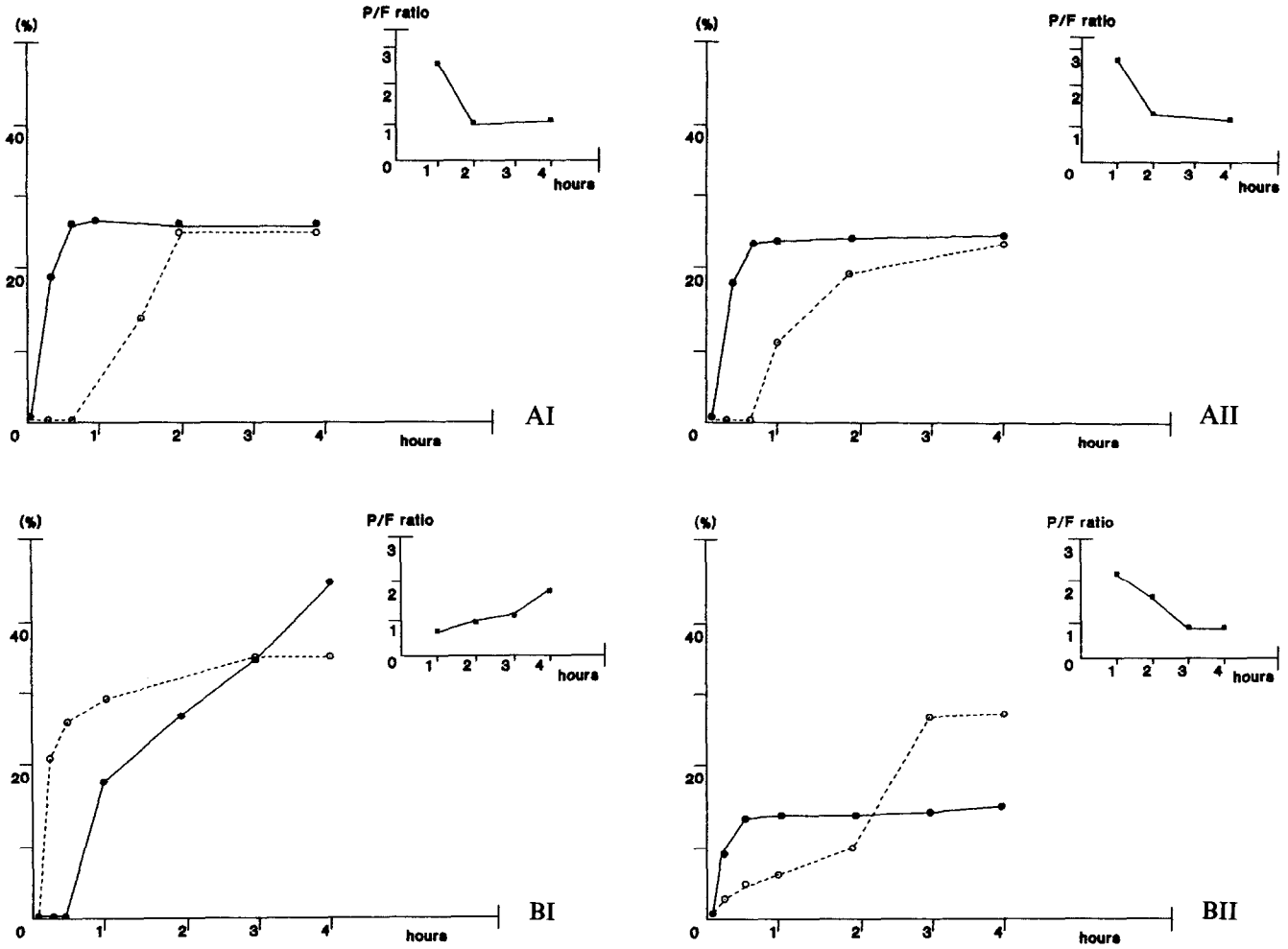

Fig. 1. Membrane permeability (filled circles) and DNA fragmentation (open circles) of K562 (I) and L929 (II) target cells induced by CD16+/CD8+/CD3− (A) and CD3+/CD8+/CD16− (B) LAK cells.

became predominant. Fig. 1BII demonstrates that CD3+ LAK cells induced necrosis in L929 cells (in contrast to K562 cells) in the early stages of cytolysis (P/F > 1), and apoptosis (P/F = 0.7) in the later stages.

4. DISCUSSION

LAK cells differ in activation mechanisms, target specificity and composition of proteins released [9,10] so the mechanisms of target cell killing may also differ in LAK cell subsets [11]. We have demonstrated that the contribution of apoptosis and necrosis to LAK cell lysis depends on the effector phenotype and the type of target cell. CD16+ LAK cells induced necrosis in K562 and L929 cells. At the same time both processes contributed to lysis mediated by CD3+ LAK cells, but the relative contribution of necrotic and apoptotic events were shown to be determined by target cell type.

The diversity of cell death pathways is the result of several factors. Heterogeneity of target cells is due to tumor cell origin, oncogene activation and subsequent homeostatic change, characteristic of tumor cells, e.g. ability to react to proliferation and differentiation signals, cell cycle stage, etc. [12,13]. These factors result in different sensitivities to lysis by potential cytolytic agents. At the same time cytolytic cells are known to express a wide spectrum of proteins which induce cytolysis [14] or participate indirectly in this process [15]. These cytolytic agents act through different receptor and second messenger systems, hence they differ in their specificity and mechanisms. Therefore cytolytic cell-mediated cytolysis must be the result of a complex mix of influences of several cytolytic and regulatory factors, subsequently resulting in the disintegration via different pathways of cell homeostasis.

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