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Apoptotic and necrotic cytolytic processes induced by membrane associated proteins of LAK cells

L.P. Sashchenko [a,c], O.D. Kabanova [a], T.I. Lukyanova [a,c], E.Yu. Blishchenko [b], D.K. Satpaev [b], S. Pognor [c], N.V. Gnuchev [a,*]

[a] *Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia*
[b] *Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*
[c] *International Centre for Genetic Engeneering and Biotechnology, Trieste, Italy*

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Abstract

Cytolytic processes induced by membrane-associated proteins of human lymphokine-activated killer (LAK) cells with different phenotypes ($CD3^+$, $CD16^-$, $CD8^+$ and $CD16^+$, $CD8^+$, $CD3^-$) were studied using L929 and K562 types of target cells. Independently of the phenotype of effector cells and the type of target cells, total fractions of membrane proteins induced several different cytolytic processes occuring with different rates and involving different mechanisms of genome fragmentation. The membrane fraction induced, irrespective of the phenotype of LAK cells, mostly apoptotic processes in the L929 line. At the same time, cytolytic processes induced in K562 line differed by the mechanisms of DNA fragmentation. An inhibitor of lysosome activation, NH_4Cl, and a Ca^{2+}-binding reagent, ethylene glycol bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), induced partial inhibition of short-term cytolytic processes (developing within 1–5 h) but did not affect the development of long-term cytolytic processes requiring more than 6–8 h for their development. © 1997 Elsevier Science B.V.

Keywords: LAK cells; Membrane associated proteins; Apoptosis; Necrosis

1. Introduction

As demonstrated in earlier studies, the phenotype of LAK cells changes during their stimulation by IL-2. During the first 4 days of stimulation, LAK cells express membrane antigens characteristic of the NK-phenotype ($CD16^+$ $CD8^+$ $CD3^-$) and then antigens characteristic of CTL ($CD3^+$ $CD8^-$ $CD16^-$) begin to prevail [1–3]. The alteration of the phenotype of lymphocytes correlated with changes in the composition and concentration of both secreted and membrane-associated cytotoxic proteins of LAK cells [3,4]. The death of target cells induced by LAK cells of different phenotypes involved both apoptotic and necrotic processes, the dynamic characteristics of these processes being dependent on the type of target cells and on the phenotypes of LAK cells [5–8]. In this paper we have studied the death of target cells under the action of membranes of LAK cells possessing cytotoxic activity.

2. Materials and methods

2.1. Cell culturing

L929 and K562 tumor cell lines were cultured in RPMI 1640 medium, containing 0.002 M glutamine and 10% fetal calf serum. For preparation of LAK cells, human peripheral blood leukocytes (PBL) were generated for 4 and 6 days in the presence 1000 u ml^{-1}

Abbreviations: LAK cells, lymphokine-activated killer cells; CTL, cytotoxic T lymphocytes; EGTA, ethylene glycol bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid; IL-2, interleukin II.

* Corresponding author.

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