ELSEVIER

Immunology Letters 53 (1996) 25–29

immunology letters

Different pathways of the release of cytotoxic proteins in LAK cells[1]

L.P. Sashchenko[a,c], T.I. Lukyanova[a,c], O.D. Kabanova[a], I. Mirkina[b], O.N. Yatskin[b], S. Pongor[c], N.V. Gnuchev[a,c,*]

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

Received 15 May 1996; accepted 23 July 1996

Abstract

Human LAK cells were shown to release cytotoxic proteins by both Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. $CD3^{+}CD8^{+}CD16^{-}$ and $CD16^{+}CD8^{+}CD3^{-}$ LAK cells were co-incubated with target cells in the presence of 4 mM EGTA. Although EGTA inhibited the exocytosis of cytolytic granules, supernatants obtained were cytotoxic for target cells. Cytotoxicity of $CD3^{+}$ LAK cells and $CD16^{+}$ LAK cells was due to cytotoxic proteins with MW 75 (p75), 35 (p35) and 22 (p22) kDa. LAK cells were also shown to release cytotoxic proteins by way of continuous secretion. After co-incubation in the absence of target cells LAK cells can secrete cytotoxic proteins with MW 75 (p75), 55 (p55), 38 (p38), 35 (p35), 25 (p25), 22 (p22) and 17 (p17) kDa.

Keywords: Lymphokine-activated killer cells: secretion; Calcium ions; Cytotoxic proteins

1. Introduction

The cytolytic activity of effector lymphocytes is known to be expressed both in the presence and in the absence of exogenous calcium [1–4]. Ca^{2+}-dependent cytotoxicity is associated mainly with secretion of soluble cytotoxic proteins and Ca^{2+}-independent cytotoxicity with the activity of membrane-associated proteins [5,6]. However, the possibility of protein secretion in the absence of Ca^{2+} ions was not ruled out also, but no experimental evidence has been obtained supporting this possibility [7].

We have studied the cytolytic action of LAK cells and demonstrated that their activity is determined by secreted and membrane-associated cytotoxic proteins. During 8-day incubation of lymphocytes with IL-2, the phenotype and cytotoxocity of LAK cells changed. During 4 days of incubation, antigens characteristic of the phenotype of NK cells ($CD16^{+}CD8^{+}CD3^{-}$) were expressed on the membrane of LAK cells, then antigens characteristic of CTL ($CD3^{+}CD8^{+}CD16^{-}$) prevailed. The change of the phenotype correlated with the change in the composition of secreted and membrane-associated cytotoxic proteins [8,9].

The aim of the present work is to study the possibility of secretion of cytotoxic proteins by LAK cells in the absence of Ca^{2+} ions and the characteristics of proteins secreted by the Ca^{2+}-independent mechanism

---

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

* Corresponding author. Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia.

[1] The research was supported by Russian state scientific programms Russian state Foundation for Fundamental Researches' and INTAS-93-2411.

Published by Elsevier Science B.V.
PII S0165-2478(96)02609-0