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Enhancement of the Cell-Mediated Lysis of Fresh Human Leukemia Cells by Cytostatic Drugs

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A. Summary

The in vitro lysis of human leukemia cells by human natural killer cells was enhanced by pretreatment of the leukemia cells with two different cytostatic drugs, which by themselves do not cause lysis of the tumor cells within the time limits of the in vitro assay. Actinomycin D induced this higher susceptibility in four of eight leukemic samples, Cisplatin in three of four samples. Using Actinomycin D pretreatment, no enhanced lysis was seen with lymphocytes and PHA-lymphoblast targets from healthy donors nor with leukemia cell lines. Our results indicate that a larger fraction of leukemia cells than previously detectable can be recognized and destroyed by spontaneous killer cells.

B. Introduction

Attempts to increase the cell-mediated killing of leukemia cells in the past have focused on the activation of the killer cells [2, 4, 7, 8]. Various studies have shown that the killing of cell lines by cytotoxic cells or antibody plus complement can be modulated by cytostatic drug and hormone treatment of the target cells [1, 3, 5, 6]. To our knowledge, fresh human tumor material has not yet been tested with this approach. Therefore we tried to manipulate the susceptibility of the leukemic cells with cytostatic drugs, which by themselves do not kill the tumor cells under the conditions used herein.

C. Material and Methods

Peripheral blood leukemia cells (more than 80% blasts) and mononuclear cells from healthy donors were isolated by density gradient separation and used either directly or stored in liquid nitrogen in the presence of dimethyl sulfoxide. The leukemia cells were incubated with actinomycin D (ActD) or Cisplatin for 2 h, followed by labeling with ^{51}Cr in the presence of the respective drugs for 1½ h. The leukemia targets were then admixed in microtiter plates with serial dilutions of β-interferon-treated mononuclear cells obtained from healthy donors.

The killing exerted by the mononuclear cells was assessed after 6 h of incubation by counting the ^{51}Cr released into the supernatant. Representative values of specific release were taken from the linear portion of the titration curve.

D. Results and Discussion

Leukemia cells from eight patients with various types of acute leukemia were pretreated with ActD and then used as targets in a cytotoxic assay (Table 1). With this approach four of eight leukemias showed an enhanced specific release induced by ActD, including one case where even IFN-activated killers were ineffective (Exp. 11). As a second drug Cisplatin was tested on four different leukemic samples. Lysis by natural killer cells could be enhanced in three of four cases tested, indicating that other drugs besides ActD are able to increase susceptibility of fresh acute leukemia cells for cell-mediated lysis.

Table 1. Effect of actinomycin D and Cisplatin on cell-mediated lysis of leukemia cells

Target cell	Exp. No.	Donor of effector cells	E:T	^{51}Cr-release			^{51}Cr-release		
				– ActD	+ ActD	ActD (μg/ml)	– Cis-platin	+ Cis-platin	Cisplatin (μg/ml)
VG[a] (ALL)	2	ER	40:1	13.0	37.9	0.2			
RI[a] (CML-BC)	8	JO[a]	40:1	13.0	36.0	1.0			
SC[a] (AML)	10	FU[a]	37:1	11.4	23.0	0.2			
STR[a] (ALL)	11	WA	25:1	– 7.7	15.0	0.2			
SU[a] (CML-BC)	12	HB	40:1	20.3	18.5	0.5			
HU[a] (AML)	13	ZI	40:1	12.7	15.5	0.2			
MI[a] (AML)	14	ME[a]	35:1	– 0.5	2.5	0.2			
RD[a] (AML)	15	JO[a]	30:1	– 10.2	– 10.5	0.2			
KR[a] (CML-BC)	C1	ZW[a]	25:1				0.4	8.8	100
VG[a] (ALL)	C2	JO[a]	50:1				2.7	16.4	10
RI[a] (CML-BC)	C3	MA[a]	12:1				3.7	11.4	100
SC[a] (AML)	C1	ZW[a]	25:1				– 1.2	– 7.4	100

The same treatment induced no cell mediated lysis from lymphocytes and PHA-lymphoblast targets in five experiments. SDs were usually less than 5% release. Release from two leukemic cell lines (K562, Molt4) was decreased by ActD treatment (two experiments) E:T = effector to target ratio

[a] N_2 stored before use

In five experiments leukemia cells were not enhanced in their lysability. In three cases no lysis was obtained, which may be explained by a failure of the effector cells to bind to the leukemia targets. In two other instances interferon-activated killers mediated some lysis of leukemia cells, which could not be further increased by pretreatment with ActD. The level of the lysis achieved in these experiments might represent the entire fraction of leukemia cells susceptible to NK cell mediated lysis.

We found the enhancing effect to be restricted to fresh tumor cells, as leukemia cell lines were lysed to a lower degree, while in lymphocyte and lymphoblast targets no lysis was induced when treated with ActD (data not shown). More normal targets, however, will have to be tested to claim tumor specificity of the effect. Nevertheless our findings warrant similar studies employing autologous killer and leukemia cells.

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