ONCOLOGY

Interaction of Human Natural Killers with Target Cells of Line K562 and Its Multidrug-Resistant and Heat-Resistant Sublines

T. K. Davtyan, G. I. Blinova, T. N. Ignatova, Yu. T. Aleksanyan, and M. B. Meliksetyan

UDC 577.152.28:576.27

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 12, pp. 616-618, December, 1993
Original article submitted July 23, 1993

Key Words: *natural killers; lymphokine-activated killers; target cells; multiple drug resitance; heat resistance*

The resistance of tumor cells to various antitumor drugs markedly reduces the efficacy of chemotherapy. Tumor cells obtained from patients after chemotherapy and cultured multidrug-resistant (MDR) cell lines provide an appropriate model for the investigation of the molecular and genetic mechanisms responsible for cell resistance. An MDR cell which is resistant to one antitumor agent also shows resistance to a variety of cytotoxic drugs possessing no structural similarity to that particular agent [4,6,17]. The specific features of the MDR cell phenotype have been broadly characterized, a decreased accumulation of cellular toxins being a major distinguishing characteristic. An increased expression of P-glycoprotein, a plasma membrane protein which acts as an ATP-dependent pump and is responsible for drug efflux from an MDR cell, has been demonstrated [11,18].

Natural killers (NK) exercise an immunological control over the proliferation and differentiation of somatic cells. These cells are able to destroy tumor and virus-infected cells without preimmunization and are defined as non-MHC-restricted cytotoxic lymphocytes. They also help regulate hemopoiesis [13,16]. Various cytotoxicity tests and cell lines are used to evaluate the activity and to study the biology of NK. The interaction of NK with K562 cells (a human erythromyeloleukosis cell line) represents a classical test in such investigations.

The objective of this study was to evaluate the resistance of the K562 cell line and of its MDR- and heat-resistant sublines to NK, as well as to analyze the mechanism of target-cell (TC) death.

MATERIALS AND METHODS

In this study we used cell line K562 and its sublines B3 and C9 resistant to adriamycin (3 μg/ml), K562-3γ resistant to ethidium bromide (3 μg/ml), and K562-3-44 cells obtained by cloning K562 cells preincubated for 3 h at 44°C. K562, B3, and K562-3μ cells contain the amplified mdrI gene coding for P-glycoprotein [2,3]. Peripheral blood mononuclears (PBM) were isolated by gradient centrifugation [1] and employed as natural killers. For the removal of lysosome-rich cells, in some

Institute of Epidemiology, Virology, and Medical Parasitology, Ministry of Public Health, Erevan, Armenia; Institute of Cytology, Russian Academy of Medical Sciences, St.Petersburg. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0012-1520$12.50

experiments PBM were treated with Leu-Leu-methyl-ester (Leu-ME) as described previously [1]. The cells were cultured in RPMI-1640 medium containing 4 mM L-glutamine, 50 μg/ml gentamicin, 50 μM 2-mercaptoethanol and 10% fetal calf serum. Cultured PBM stimulated for 72 h with 100 U/ml recombinant human interleukin-2 (Sigma, USA) were used as lymphokine-activated killers (LAK).

The cytotoxicity assay was performed as described elsewhere [5] using the K562 cell line and its sublines labeled with ^{3}H-uridine (0.11 MBq/ml). The cytotoxicity index (CI) was calculated from the following formula: CI = [1 - (cpm in experiment/cmp in control)]×100.

The degree of DNA fragmentation in target cells (TC) which had interacted with NK was determined as follows: TC were incubated for 24 h at 37°C with ^{3}H-thymidine, washed 3 times with Eagle's medium (pH 7.4), and incubated with NK for 4 or 18 h. The TC were then washed 3 times with Eagle's medium (4°C) and lysed for 30 min on ice with a buffer containing 15 mM Tris-HCl (pH 6.8), 20 mM EDTA, and 0.5% Triton X-100. For separation of the cytoplasmic and nuclear fractions, the samples were centrifuged for 10 min at 10,000 g. The radioactivity of TCA precipitates was measured in a Beckman scintillation counter. The fragmentation index (FI) was calculated from the formula: FI = [1 - (cpm of cytoplasmic fraction/cpm of nuclear fraction)]×100.

RESULTS

To estimate the feasibility of using the proposed cytotoxicity assay in the investigation of the sensitivity of different TC to NK, we treated PBM with Leu-ME, which selectively inhibits the activity of NK [20]. When Leu-ME NK were used as effector cells, the CI decreased on average by 85%, compared with intact NK (Table 1). Consequently, this assay can be employed both for the estimation of NK activity and for the determination of TC sensitivity to NK.

It was found that the sensitivity of adriamycin-resistant B3 and C9 cells to NK is virtually the same as that of the parent K562 cells (Table 1). This holds true for K562-3γ cells resistant to ethidium bromide (Table 1). These findings agree with the literature data indicating that MDR cell lines do not differ from the parent lines in sensitivity to NK [8,19].

Heat-resistant K562-3-44 cells exhibited a very low sensitivity to NK (Table 1). In should be mentioned, therefore, that treatment of TC with γ-interferon or exposure to heat shock makes them resistant to NK and LAK, this resistance being abolished by inhibitors of protein and RNA synthesis [10]. This observation may indicate that heat shock proteins have a role in providing the resistance of TC to the effector cells of the immunological control system.

B3 and C9 cells with the MDR phenotype are more sensitive to the cytotoxic action of LAK than are K562 cells (Table 1). There are no indications in the literature of a higher sensitivity of MDR cells to LAK. On the contrary, there are several reports that MDR cells have a similar sensitivity to NK and LAK [8,14,19]. In our experiments, multidrug-resistant K562-3γ cells did not differ in LAK sensitivity from the parent cells (Table 1). The enhanced cytotoxic effect of LAK is explained by the fact that activation of the effect or stimulation of cells increase the production of cytotoxic factors (tumor necrosis factor, γ-interferon, perforins, etc.), the expression of adhesion molecules and their ligands on the cell surface, etc [9]. It should be mentioned that heat-resistant K-3-44 cells did not differ from K562 cells in their sensitivity to LAK (Table 1).

Only MDR cells were used in the studies of the interaction between NK and TC in the pres-

TABLE 1. Sensitivity of K562 Cell Line and Its Subclonal Lines to Effector Cells

Target cell	Cytotoxicity index						
	NKC		NKC + Leu-ME	KNC + interleukin-2		NKC + adriamycin	
	50:1*	100:1	100:1	50:1	1:100	50:1	100:1
K562	36.7±12.5	46.5±17.5	6.9±2.7	51.7±13.1	40.9±12.2	—	—
C9	36.2±14.9	45.9±17.5	6.3±2.9	72.8±10.7	68.5±8.9	52.7±16.4	55.6±13.9
B3	35.7±12.5	44.7±11.3	5.2±3.4	69.7±11.4	64.6±9.6	53.7±17.5	56.1±19.3
K562−3γ	32.5±11.5	42.6±15.3	—	55.3±14.7	42.4±16.8	—	—
K562−3−44	12.9±4.2	15.2±5.4	—	40.3±12.2	36.4±13.4	—	—

Note. The values are the means of 6 experiments; a dash means no experiemnt was performed. Here and in Table 2 an asterisk indicates the NKC:TC ratio.

TABLE 2. DNA Fragmentation Upon Interaction of TC with NKC

Target cell	Fragmentation index, %				Control
	after 4 h		after 18 h		
	50:1	100:1	50:1	100:1	
K562	4.9	3.9	22.1	24.8	0.03
C9	2.6	4.3	54.7	54.9	0.07
B3	6.2	8.2	52.6	55.7	0.04

ence of the anticancer agent adriamycin. This was done to eliminate a possible cytotoxic effect of adriamycin on K562 cells. C9 and B3 cells were more sensitive to NK in the presence of 3 μg/ml adriamycin that in its absence (Table 1). An enhanced cytotoxic effect was demonstrated in another test system (cloning in agar) after incubation of LAK and cisplatin with K562 cells resistant to this agent [14]. An increase in the CI for K562 cells in the presence of an antitumor agent can be explained either by activation of NK by this agent or by NK- and/or LAK-induced reversion of the MDR phenotype.

The terminal cytotoxic effect of NK may be due to their production of membrane lytic compounds (perforins, cytolysins) homologous to the complement system components or secretion of cytokines, for example tumor necrosis factor, that cause death of tumor cells [7,16]. In addition to the production of soluble cytotoxic factors, NK can activate an endogenous suicide mechanism in TC [12,21]. Activation of endonucleases leading to DNA fragmentation is a key factor in this type of cytolysis, which is known as programmed cell death, or apoptosis.

In our experiments, fragmentation of DNA was detected in TC after 4-h incubation with NK and reached a value of 25-59% after 18-h incubation (Table 2). This finding indicates that NK induced DNA fragmentation (apoptosis) both in K562 and in MDR cells. On the basis of the data given in Table 2 it can be assumed that similar mechanisms operate upon the death of these cells .

Thus, NK have a cytotoxic effect on TC sensitive to cell toxins and on MDR subclones of these TC. However, MDR cell lines are more sensitive to LAK than are the parent cell lines. Heat-resistant TC have a low sensitivity to NK and LAK. Synergism of the effects of NK and cytotoxic agents on TC resistant to these agents has been documented. Fragmentation of DNA in TC interacting with NK may be caused by activation of endonucleases.

REFERENCES

1. T. K. Davtyan, B.Kh. Nisman, T. N. Ignatova, Yu. T. Alaksanyan, *Biol. Zh. Armenii,* **44**, № 3, 235-239 (1991).
2. T. N. Ignatova, M. V. Tarunina. I. V. Kozhukharova, *et al., in: First All-Union Conference on the Human Genome* [in Russian], Moscow (1990), pp. 112-113.
3. T. N. Ignatova, V. I. Vasyukhin, L. A. Lipskaya, *et al.*, in: *Second All-Union Conference on the Human Genome* [in Russian], Moscow (1991), pp. 79-80.
4. A. A. Neifakh and A. Yu. Aleksandrova, *Dokl. Acad. Nauk SSSR,* **291**, № 4, 989-991 (1986).
5. M. P. Rykova, I. V. Spiridze, M. S. Zergenidze, *et al., Immunologya,* № 7, 148-155 (9181).
6. G. Bardley, P. E. Juranka, and V. Ling, *Biochim. Biophys. Acta,* **6**, 87-128 (1988).
7. A. Goldstein, *Nature,* **327**, 12 (1987).
8. W. G. Harker, C. Tom, and J. R. McGregor, *Cancer Res.,* **50**, 5931-5936 (1990).
9. K. Karre, N. Hansson, and R. Kiessing, *Immunol. Today,* **12**, 343-345 (1991).
10. S. H. E. Kauffman, *Ibid.,* **11**, 129-136 (1990).
11. J. Lankelma, E. C. Spoelstra, H. Dekker, and H. J. Broxterman, *Biochim. Biophys. Acta,* **1055**, 3215-3223 (1990).
12. D. J. McConkey, S. C. Show, S. Orreniuss and M. Jandal, *FASEB J.,* 4, 2661-2669 (1990).
13. D. J. Negler, S. Orenius, and H. Phillips, *J. Exp. Med.,* **168**, 120-134 (1988).
14. A. Ohtsu, Y. Sasaki, and T. Tamra, *Jap. J. Cancer. Res.,* **80**, 265-270 (1989).
15. H. F. Pross, D. Callewaert, and P. Rubin, in: *Immunology and Natural Killer Cells,* Boca Raton (1986), pp. 2-23.
16. J. N. Robinson and R. Jerome, *Blood,* **76**, 2421-2438 (1990).
17. I. B. Roninson, *Clin. Physiol. Biochem.,* **5**, 140-151 (1987).
18. A. R. Sato, R. K. Stern, and K. Chol, *Proc. Nat. Acad. Sci. USA,* **87**, 7225-7229 (1990).
19. R. J. Seheper, W. S. Dalton, and T. M. Grogan, *Int. J. Cancer,* **49**, 562-567 (1991).
20. D. L. Thiele and P. E. Lipsky, *Proc. Nat. Acad. Sci. USA,* **87**, 83-87 (1990).
21. D. S. Ueker, *Nature,* **327**, 62-69 (1987).