7. M. L. Karnovsky and J. A. Badway, J. Clin. Chem. Clin. Biochem., 21, 545 (1983).
8. P. D. Lew and T. P. Stossel, J. Clin. Invest., 67, 1 (1981).
9. I. Nishigaki, T. Ozawa, and K. Yagy, Vitamin, 38, 359 (1968).
10. C. A. Rouser, W. A. Scott, O. W. Griffith, et al., J. Biol. Chem., 257, 2002 (1982).
11. R. L. Souhami and J. Bradfield, J. Reticuloend. Soc., 16, 75 (1974).
12. H. H. Thaw, J. Forslid, H. Hamberg, and J. Hed, Acta Pathol. Microbiol. Immunol. Scand., A-92, 1 (1984).
13. M. Torres and J. Hakim, in: Biochemistry and Function of Phagocytes, New York (1982), p. 429.
14. A. O. Wozencraft, H. M. Dockrell, J. Taverne, et al., Infect. Immun., 43, 664 (1984).

ACTION OF T-ACTIVIN ON ACTIVITY OF HUMAN NATURAL KILLER CELLS *IN VITRO*

S. B. Cheknev, M. Z. Saidov,
L. V. Koval'chuk, A. S. Pavlyuk,
and V. Ya. Arion

UDC 615.362.438.017:615.276.4].015.46:612.017.4

KEY WORDS: T-activin, natural killer cells

Investigations in recent years have shown that the thymus may exert its immunoregulatory function through a number of biologically active fractions [1], which can be used to correct disturbances of the T-system of immunity in the treatment of certain diseases.

This paper describes a study of the action of the Soviet preparation T-activin on activity of human natural killer cells (NKC) *in vitro*. Previous investigations showed [10] that NKC activity is depressed by the action of biologically active fractions from the thymus. However, the action of T-activin on NKC activity has not been studied. Evidence has been obtained that T-activin, under certain conditions, stimulates interferon production [2, 7], i.e., that it can stimulate NKC through the production of immune interferon.

EXPERIMENTAL METHOD

Lymphocytes were isolated from heparinized blood of 19 healthy blood donors (without any immunologic disturbances) aged between 16 and 46 years, in a Ficoll–Verografin gradient [9]. The lymphocytes were washed twice with buffered physiological saline, after which suspensions of lymphocytes were prepared in complete nutrient medium based on medium RPMI-1640 (from Serva, West Germany), containing 10% embryonic calf serum with the addition of glutamine (200 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). The initial cell concentration was 10^7 lymphocytes/ml medium. The K-562 chronic human myeloid leukemia cells, cultured *in vitro*, used as targets were labeled with ^{3}H-uridine in a dose of 3 µCi/ml culture. In accordance with the modification of the ^{3}H-uridine method of determining NKC activity, developed in the laboratory of Cellular Immunopathology and Biotechnology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR [4], after preparation of the initial concentration of target cells, namely 10^5 cells/ml, and addition of pancreatic RNase to the system in a dose of 5 µg/ml, the lymphocytes and targets were distributed in a volume of 0.1 ml into round-bottomed cells of 96-well microplates (from Nunclon, Denmark). To investigate NKC activity, the serial dilutions principle developed in the Department of Immunology, N. I. Pirogov Second Moscow Medical Institute [5] was used (NKC activity was tested with effector and target cells in the ratio of between 100:1 and 6:1). T-activin (from the Laboratory of Molecular Immunology, N. I. Pirogov Second Moscow Medical Institute), in doses of 20, 2.5, 1.5, 1, and 0.5 µg/10^6 lymphocytes, was added to the lymphocyte suspension immediately before distribution into the wells. The cells were incubated for 14 h at 37°C in an atmosphere containing 5% CO_2, after which the contents of the wells were deposited on glass fiber filters with a pore diameter of 2.5 µ and harvested by means of a multichannel biological fraction collector (from Dyna-

Department of Immunology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 101, No. 4, pp. 455-457, April, 1986. Original article submitted April 4, 1985.

0007-4888/86/0004-0487$12.50

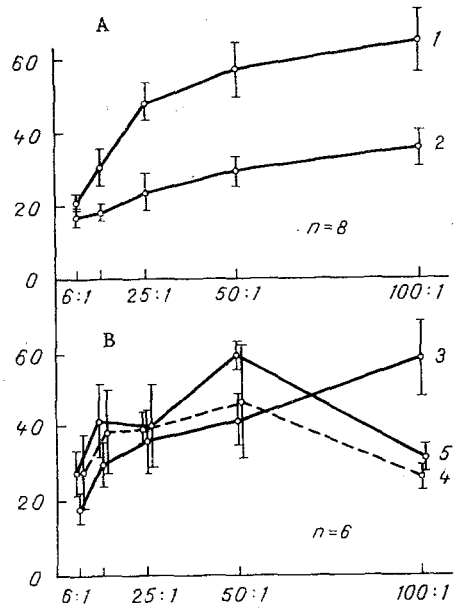

Fig. 1. Action of T-activin on NKC activity *in vitro*. Abscissa, ratio of effector to target; ordinate, CI (in %). A, B) Different series of experiments. 1, 3) Activity of NKC without T-activin; 2) activity of NKC in the presence of T-activin in a dose of 20 μg; 4) the same, in a dose of 2.5 μg; 5) the same, in a dose of 1.5 μg.

TABLE 1. Effect of T-activin in Doses of 1 and 0.5 μg/10^6 Lymphocytes on NKC Activity *in Vitro* (M ± m, n = 5)

Experimental conditions	Effector to target ratio				
	100:1	50:1	25:1	12:1	6:1
Control	59,3±10,3	41,8±7,3	36,3±8,3	30,0±6,1	18,0±4,0
T-activin					
1 μg	44,0±9,2	36,4±3,5	33,7±7,0	26,3±2,3	26,0±4,2
0,5 μg	48,2±7,3	39,0±11,0	29,0±4,0	27,5±7,5	28,0±2,0*

Legend. *P < 0.05 compared with control. Values of CI (in %) are shown.

tech, England). Residual radioactivity was counted on a scintillation counter (Intertechnique, France) and each sample was counted for 2 min. K-562 cells, incubated without lymphocytes, served as the control in the lymphocytotoxic test. To rule out any toxic effect of the preparation on NKC, the viability of the cells in the system was tested before and after incubation. The indicator of NKC activity was the cytotoxic index (CI), calculated by the equation:

$$CI = \left(1 - \frac{\text{number of counts in experimental well}}{\text{number of counts in control}}\right) \times 100\%.$$

The significance of the difference between the mean values was determined by Student's t test and also by Wilcoxon's nonparametric test for tied series.

EXPERIMENTAL RESULTS

T-activin, in a dose of 20 μg, depressed NKC activity, when the effector and target were present in all ratios (except 6:1), by 33-50% compared with the control, and it was more effective when the ratios were higher (Fig. 1A). T-activin had no toxic action on lymphocytes: the viability of the cells did not diminish during incubation.

In a dose of 2.5 μg (Fig. 1B) T-activin depressed NKC by 2.2 times when the effector and target were present in the ratio of 100:1. With other dilutions no significant effect could be detected. In a dose of 1.5 μg T-activin reduced NKC activity if the effector and target were present in the ratio of 100:1, whereas with a ratio of 50:1 CI was increased under the influence of T-activin by 1.4 times.

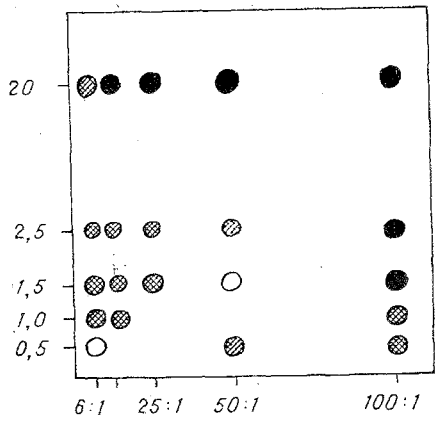

Fig. 2. Action of T-activin on NKC activity in the test system (scheme). Abscissa, effector to target ratio; ordinate, dose of T-activin (in μg/10^6 lymphocytes). Black circles — inhibition, white circles — stimulation, shaded circles — no effect discovered.

In a dose of 1 μg (Table 1) T-activin had no significant effect on NKC activity. In a dose of 0.5 μg it increased NKC activity if the effector to target ratio was 6:1.

The experimental results indicate that T-activin can depress NKC activity, but under certain conditions it can also stimulate NKC. NKC and the natural cytotoxicity system constitute a first barrier in immunologic surveillance [3, 6, 8]. Thymic hormones, under experimental conditions, can also have an antitumor action: they inhibit growth of tumor cells, prolong the life of tumor-bearing animals, and restore reactions of cellular immunity. If T-activin, under certain conditions, stimulates NKC, a mechanism is available for its antitumor action, which may be realized *in vivo*. The possibility that T-activin acts directly on target cells cannot be ruled out. This is a problem for further research.

Inhibition of NKC activity by T-activin in a dose of 20 μg is evidence in support of the previous hypothesis of the origin of NKC as precursors of T cells. T-activin facilitates expression of T-cell markers on immunocompetent cells and on differentiation of precursors of T-lymphocytes into mature forms [1]. It has recently been shown that on incubation with thymic factors, prothymocytes acquire the surface characteristics of T cells in the course of 2-6 h [11]. If NKC are early precursors of T lymphocytes, T-activin ought to realize their action in that way.

The differences in the action of T-activin, depending on the effector to target ratio, can be explained by heterogeneity of the NKC population. It may be that on dilution of the cells the suppressor influences of the regulating systems are diminished, which enables their activity, which under ordinary conditions is blocked by specific or nonspecific suppression, to be exhibited.

T-activin thus possesses immunoregulatory properties relative to NKC activity *in vitro*. Its action is determined by at least two parameters: the dose used and the effector to target ratio. Over the range of concentrations and ratios tested, a definite rule was discovered: the greater the dose of the preparation and the higher the effector to target ratio, the stronger the inhibitory action, and the smaller the dose and the lower the ratio, the more marked its stimulating effect (Fig. 2).

Further investigation of the mechanisms of action of T-activin on NKC and target cells and the search for possible ways of its clinical application are necessary.

LITERATURE CITED

1. V. Ya. Arion, in: Progress in Science and Technology. Series: Immunology [in Russian], Vol. 9, Moscow (1981), p. 10.
2. Yu. A. Grinevich, I. S. Nikol'skii, T. N. Selezneva, et al., in: Modern Methods in Immunotherapy [in Russian], Tashkent (1984), p. 25.
3. L. P. Kindzel'skii and A. K. Butenko, Eksp. Onkol., No. 3, 3 (1983).
4. M. P. Rykova, I. V. Spirande, M. S. Zedgenidze, et al., Immunologiya, No. 3, 88 (1981).

5. M. Z. Saidov, L. V. Koval'chuk, M. A. Stenina, et al., Lab. Delo, No. 9, 553 (1984).
6. R. M. Khaitov and A. V. Madzhidov, Usp. Sovrem. Biol., 97, No. 1, 3 (1984).
7. A. G. Chuchalin, V. A. Babushkina, V. Ya. Arion, et al., Byull. Eksp. Biol. Med., No. 7, 76 (1984).
8. A. S. Shevelev, Immunologiya, No. 3, . (1984).
9. A. Boyum, Scand. J. Clin. Lab. Invest., 21, Suppl. 27, 77 (1968).
10. R. Herberman, Clin. Immunol., 4, 73 (1980).
11. J. L. Touraine, M. C. Favrot, and M. El Ansary, in: Immunomodulation. New Frontiers and Advances, New York (1984), p. 1.

ISOLATION AND IDENTIFICATION OF NORMAL KILLER CELLS FROM SYRIAN HAMSTERS

V. A. Matveeva and T. E. Klyuchareva

UDC 612.112.94.017.4-086.12

KEY WORDS: normal killer cells; Percoll density gradient; granular lymphocytes; Syrian hamsters

A connection has now been proved between the presence of large granular lymphocytes (LGL) in the blood and the cytotoxic activity (CTA) of normal killer cells (NKC) in human, rat, and mouse blood [4, 5, 7, 10]. LGL can be isolated from human and rat blood in a Percoll density gradient, in the fraction containing 40-42.5% of Percoll [9, 10]. LGL possessing NKC activity can be isolated from the blood and spleen of mice in heavier fractions (55%) [4, 5]. The writers previously showed the presence of nonadherent lymphoid cells, with the properties of NKC, namely spontaneous cytotoxicity against certain target cells and ability to be activated by Newcastle disease virus, an interferon inducer, in Syrian hamsters [2, 3].

In this paper we give for the first time data on isolation of NKC from the blood and various tissues of Syrian hamsters in a Percoll density gradient and their identification on the basis of morphologic criteria and CTA.

EXPERIMENTAL METHOD

NKD were isolated from the blood, spleen, and bone marrow of Syrian hamsters aged 5-8 months. Nonadherent lymphoid cells were obtained by isolation on Ficoll followed by filtration through a column packed with nylon wadding [3]. A stepwise density gradient of Percoll [10] was used to isolate and concentrate the NKC. For this purpose, a 10% solution of 1.4 M NaCl was added to a commercial preparation of Percoll (from Serva, West Germany). All subsequent dilutions of Percoll were obtained from this solution with RPMI 1640 nutrient medium with 10% heated bovine serum and with the addition of 2 mmoles/ml of glutamine and 0.1 mg/ml of gentamycin. Later this medium also was used in the cytotoxic test (CT). The stepwise Percoll gradient was prepared in serologic tubes, starting with the highest density (from 60 to 43%), in a volume of 1 ml, and each Percoll solution was layered successively one above the other. The suspensions of the test cells, in a number of $1 \cdot 10^7$ to $5 \cdot 10^7$, were layered on the surface of the gradient in a volume of 1 ml. After centrifugation at 1500 rpm for 30 min cells from the various Percoll fractions were harvested with a Pasteur pipette into centrifuge tubes, washed twice with physiological saline, and diluted in medium. Films were prepared from each cell fraction, dried, fixed with methanol, and stained with azure and eosin by the Romanovsky–Giemsa method, and identified on the basis of morphologic criteria. In each preparation at least 200 cells were counted. For their morphologic identification the number of LGL was counted relative to the numbers of small lymphocytes and cells of the granular series. Only LGL relative to the total number of cells in the preparation were counted in spleen and bone marrow preparations isolated from the different Percoll fractions. CTA of the isolated cells was studied in the CT with target cells of a human MOLT-4 thymoma cell labeled with ^{51}Cr [2, 3].

Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 101, No. 4, pp. 457-459, April, 1986. Original article submitted February 4, 1985.

 0007-4888/86/0004-0490$12.50