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# ISOLATION AND IDENTIFICATION OF NORMAL KILLER CELLS FROM SYRIAN HAMSTERS

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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].

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TABLE 1. Isolation of LGL from Blood, Spleen, and Bone Marrow of Syrian Hamsters in Percoll Density Gradient (in %)

| Percoll solution | Blood | | Spleen | | Bone marrow | |
|---|---|---|---|---|---|---|
| | LGL | CTA | LGL | CTA | LGL | CTA |
| Control | 12,0 | 13,3±1,1 | 6,8 | 10,2±2,0 | 5,3 | 5,0±1,8 |
| 43 % | 4,9 | 2,3±0,3 | 6,4 | 6,7±2,1 | 3,3 | 1,0±0,5 |
| 46 % | 12,2 | 6,6±1,3 | 14,0 | 7,3±1,3 | 5,7 | 6,0±1,2 |
| 49 % | 28,8 | 16,2±1,0 | 32,2 | 20,7±2,1 | 10,7 | 9,7±1,5 |
| 52—55 % | 56,4 | 29,6±1,9 | 42,0 | 26,2±2,6 | 17,0 | 19,4±1,2 |
| 60 % | 11,1 | 7,0±1,9 | 8,8 | 8,6±0,9 | 6,3 | 8,4±1,0 |

Legend. Results of five experiments used for cells of each type. Number of LGL given as a percentage of other nucleated cells. Percentage lysis of MOLT-4 target cells determined with effector to target ratio of 50:1. Suspensions of nonadherent lymphoid cells used as the control.

## EXPERIMENTAL RESULTS

Medium-sized lymphocytes with a relatively large cytoplasm, with well-marked azurophilic granules and with a round or kidney-shaped nucleus, shifted toward the periphery of the cell, predominated among LGL. The study of lymphoid cells from the Syrian hamsters' blood, isolated in different Percoll fractions, showed that an increase in the number of LGL correlated with an increase in CTA of the cells isolated in these fractions (Table 1). The largest number of LGL (56.4%) compared with unfractionated (control) cells (12%) was isolated in the fraction containing 52-55% of Percoll. The CTA of the cells of this fraction (29.6%) was about 2.5 times higher than in the unfractionated control (13.3%).

The results obtained by isolation of lymphoid cells on the Syrian hamster spleen also confirmed the presence of relative correlation between the number of LGL and the increase in CTA. A sevenfold increase in the number of LGL in the suspension of spleen cells isolated in a 49-55% solution of Percoll was accompanied by a 2-2.5-fold increase in their CTA.

The writers showed previuusly that bone marrow cells of Syrian hamsters possess virtually no CTA but can be activated by NDV [2]. Because of the absence of data on isolation and concentration of NKC from bone marrow of Syrian hamsters and other animals, it was decided to investigate this matter, for evidence has been obtained that metastases can be prevented by injecting bone marrow cells into the blood stream of Syrian hamsters [1], and that their activity may be modified in patients with tumors [11].

Fractionation of the bone marrow lymphoid cells in a Percoll density gradient enabled a threefold increase in the concentration of LGL in the fraction containing 52-55% of Percoll, and this led to a fourfold increase in the CTA of the cells of this fraction.

The results of the experiments on Syrian hamsters thus confirm correlation between the number of LGL and the CTA of nonadherent lymphoid cells in the blood and spleen, discovered previously in a study of human, mouse, and rat NKC [6, 8, 9]. Syrian hamster NKC are closely similar in relative density to the corresponding mouse cells and they differ from human and rat NKC, which are isolated in a Percoll gradient in lighter fractions.

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# EFFECT OF TUMOR GROWTH PROMOTOR 12-O-TETRADECANOYLPHORBOL-13-ACETATE ON PROLIFERATION OF VARIOUS MOUSE TUMOR CELL CLONES IN SEMISOLID MEDIUM

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Tumors are known to be heterogeneous with respect to their cell composition, and this heterogeneity arises as a result of clonal evolution [9]. It is not clear, however, whether different clones replace one another in the course of tumor development or whether they must coexist in the tumor in order to maintain it. It has recently been shown [10] that clones of pseudonormal rat cells differ in their response to growth-stimulating factors. The question arises whether clones of tumor cells differ in their response to exogenous agents affecting cell multiplication.

The aim of this investigation was to study the ability of different clones of tumor cells to proliferate in semisolid medium in response to the action of the tumor growth promotor 12-O-tetradecanoylphorbol-13-acetate, which induces DNA synthesis in resting monolayer cultures [7], and can also induce colony formation in semisolid medium by certain types of transformed cells [6, 8].

## EXPERIMENTAL METHOD

Clone CAK-25AG$^r$, isolated from a spontaneously transformed CAK line of fibroblasts from AKR mice [1], and clones 103/11 and 3sb PS-103 [1], isolated from a culture of PS-103 sarcoma of CBA mice [4], were studied. The conditions of culture of the cells in the monolayer were described previously [1, 5]. A 1.2% solution of methyl cellulose (MC, from Sigma, USA) in the culture medium was used as the semisolid medium. The method of determination of the cloning efficiency (CE) in MC was described previously [5]. In most experiments CE of the cells on the substrate was determined parallel with CE in MC.

The growth promotor 12-O-tetradecanoylphorbol-13-acetate (TPA, from Sigma) was dissolved in acetone or dimethyl sulfoxide (DMSO) to a concentration of 1 μg/ml. The solution was kept at −70°C and the cells treated with it 72 h before seeding in MC (final TPA concentration 5 ng/ml). To test the effect of this treatment with TPA on gene amplification, colchicine (from Merck, USA) was used; colchicine was added to the culture medium in a concentration of 0.07 μg/ml.

## EXPERIMENTAL RESULTS

Clones of tumor cells used for the investigation were characterized by low CE in MC, namely about $10^{-4}$-$10^{-3}$ (Table 1). By determining the survival rate of cells of clones CAK-25AG$^r$, 103/11, and 3sb PS-103 after treatment with different doses of TPA it was possible to choose the maximal nontoxic dose for preventive treatment of the cells with the compound (5 ng/ml). The clones studied did not differ in sensitivity to the toxic action of TPA. Even so, they were found to differ in their ability to form colonies in MC in response to treatment with TPA.

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