LECTIN-DEPENDENT CYTOLOGICAL ACTIVITY OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES

S. N. Bykovskaya, N. G. Blokhina, E. R. Vasil'eva, V. A. Agafonov, and M. O. Raushenbakh

UDC 612.112.94.017.4.014.46:615.272:547.962.5

KEY WORDS: lectin-dependent cytotoxicity; target cells; concanavalin A; cytolytic T lymphocytes.

The cytolytic action of the killer T lymphocyte on the target cell (TC) is divided into three stages: specific binding, lethal shock, and independent lysis of TC [11]. Only immune lymphocytes, with a specific receptor for the corresponding antigenic determinants of TC, act on the first stage.

However, in the presence of phytohemagglutinin (PHA), concanavalin A (con A), and succinyl-con A, and lectins, known to be powerful mitogens of T cells, T killers can cause lysis of syngenic TC [7, 9]. It has been shown that simple nonspecific binding is not the cause of cytolysis, for the presence of various other lectins, which are not mitogens of T cells, has no effect on cytolysis [9].

The "lethal shock" stage, which according to our observations precedes the stage of activation of the secretory apparatus of T killers [1-5], is nonspecific. Not only contact with the TC membrane, but also the action of con A or PHA may be the stimulus for activation of secretion of a T killer. Hence it must be concluded that T cells possessing lytic potential can kill any target in the presence of these lectins.

This technique can be used to detect T killers in a lymphocyte population when the corresponding TC are absent or unknown. For example, an attempt can be made to estimate the number of T killers in patients with tumors or with autoimmune diseases.

The object of the present investigation was to develop a method of determining the cytolytic activity of human peripheral blood lymphocytes.

EXPERIMENT METHOD

Peripheral blood cells from 50 healthy blood donors were used. Monocytes were isolated by Boyum's method [8]. A suspension of these cells ($10 \times 10^6 - 15 \times 10^6$) was incubated in 1 ml medium No. 199 with 10% calf embryonic serum for 40 min on a nylon wadding column in an incubator with 5% CO_2 at 37°C to remove adherent cells. Nonadherent cells were eluted by 10 ml of medium 199 with 5% calf embryonic serum. The yield of lymphocytes from the column was 10-20%.

Human cytolytic T lymphocytes (CTL) were obtained in a mixed lymphocyte culture (MLC) from two healthy donors. For this purpose, peripheral blood monocytes from one donor, irradiated in a dose of 2000 rads, were mixed with an equal number of cells from the other donor in medium RPMI-1640 with 15% of inactivated human (group AB) serum, 5×10^{-3} M HEPES, 2×10^{-3} M glutamine, and antibiotics. The final concentration of lymphoid cells from each donor was one million/ml. The culture was incubated in a volume of 20 ml in Sani Glas flasks at 37°C for 6 days.

Mouse CTL were obtained from splenocytes of C3H ($H\text{-}2^k$) mice on the 11th day after intraperitoneal immunization with ascites leukemia L-1210 ($H\text{-}2^d$) cells (25×10^6 cells per mouse) by the method of Berke et al. [6].

Laboratory of Endogenous Carcinogens and Diagnostic Department, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 48-51, March, 1982. Original article submitted April 12, 1982.

 0007-4888/83/9503-0310$07.50

TABLE 1. Comparison of Cytolytic Action of T Killers Obtained from Human MLC on Stimulators and Extraneous TC

Target cells	Cytolysis, percent					
	MLC			monoculture		
	concentration of conA, μg					
	—	50	100	—	50	100
ConA blasts	25 27	— —	— —	5 3	— —	— —
HeLa	13,3 7,7 3,4	32,8 34,6 23,6	34,8 44,8 33,4	2,7 1,8 1,2	3,94 0,6 4	4,5 1,3 4,5

Legend. Duration of cytolysis 4 h. Ratio of lymphocytes: TC 20:1. Lymphocytes from a 7-day MLC were used in the experiments.

The cytotoxic test was carried out by the modified method of Cerottini et al. [10].

Human HEp-2 and HeLa fibroblasts, human conA blasts obtained by adding conA to lymphocytes of a donor whose cells were used as stimulators in MLC, and also L cells (C3H mouse fibroblasts) and ascites leukemia L-1210 cells were used as the targets.

The targets (5×10^6 cells in 0.5 ml medium 199 with 5% bovine serum) were incubated with 50-100 μCi $Na_2{}^{51}CrO_4$ for 40 min at 37°C, washed three times with medium 199, and seeded in 96-well microplates. CTL from the MLC or nonstimulated lymphocytes from healthy donors were added to the labeled blast cells in the ratio of 20 lymphocytes per TC. The microplates were centrifuged at 200 g for 5 min and incubated with 5% CO_2 at 37°C.

Lymphocytes in the ratio of 10:1 and conA in a final concentration of 10, 20, and 50 g/ml were added to the monolayer targets. The microplates were incubated with 5% CO_2 at 37°C for 4 h, if immune lymphocytes were used in the experiment, and for 18 h if peripheral blood monocytes were added.

The result was calculated by the equation:

$$\text{Percentage cytolysis} = \frac{\text{Experiment - spontaneous lysis}}{\text{Complete lysis - spontaneous lysis}} \times 100,$$

where experiment stands for liberation of ^{51}Cr by target cells interacting with lymphocytes; spontaneous lysis for liberation of the isotope by intact TC; complete lysis for liberation of the isotope by TC treated with 2% sodium dodecylsulfate solution in 0.05 M borate buffer, pH 9.0.

EXPERIMENTAL RESULTS

To demonstrate the cytolytic activity of T killers against syngeneic TC in the presence of lectins, lymphocytes from the immune spleen of C3H ($H\text{-}2^k$) mice were used and were tested on L-1210 ($H\text{-}2^d$) TC used as stimulators for immunization, on syngeneic L ($H\text{-}2^k$) TC, and also on heterologous human laryngeal carcinoma HEp-2 cells.

The experiments showed that the percentage of cytolysis of the corresponding allogeneic and syngeneic TC in the presence of PHA was practically identical (78 and 77%, respectively). The cytological activity against heterologous TC was rather lower (54% under analogous experimental conditions).

To determine the cytological activity of lymphocytes from the human MLC against TC, in response to stimulation by whose alloantigens the corresponding T killers proliferated, lymphocytes of the stimulating donor were used as TC in the MLC in the form of ^{51}Cr-labeled conA blasts (Table 1).

Lymphocytes from a 7-day MLC were lysed by 25-27% of conA blasts and 23.6-44.8% of HeLa cells in the presence of conA. The cytolytic effect of the unstimulated culture was not potentiated in the presence of lectin: 3-5% of conA blasts and 0.6-4.5% of HeLa cells were lysed in the presence of conA.

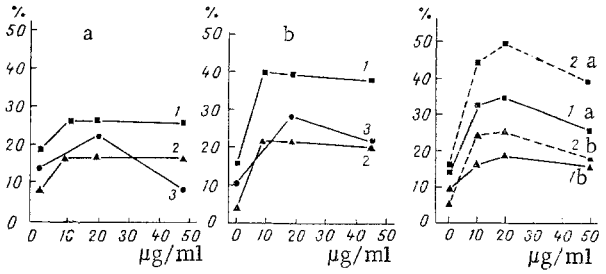

Fig. 1 Fig. 2

Fig. 1. Cytolytic activity of peripheral blood cells of five healthy blood donors on different TC. HeLa (1), HEp (2) and L (3) cells were used as targets. a) Unpurified monocytes, b) monocytes freed from adherent cells. Abscissa, conA concentration (in g/ml); ordinate, cytolysis (in percent).

Fig. 2. Cytolytic action of human peripheral blood lymphocytes in presence of conA on HeLa and HEp-2 cells. 1) Cytolysis on addition of peripheral blood monocytes to TC, 2) cytolysis on addition of monocytes purified from adherent cells. a) HeLa cells, b) HEp-2 cells. Abscissa, conA concentration (in μg/ml); ordinate, cytolysis (in percent).

To select corresponding TC, lymphocytes from five healthy donors, unpurified and purified from adherent cells, were tested simultaneously on a monolayer culture of human HeLa fibroblasts, HEp-2 cells, and L mouse cells. As will be clear from Fig. 1, the HeLa cells had the highest sensitivity in these experiments. Optimal cytolysis was obtained with small concentrations of TC ($2 \times 10^4 - 3 \times 10^4$ cells per well); although the lymphocyte:TC ratio in all versions of the experiment remained constant (10:1), the least cytolysis was observed with the highest seeding density of TC. The optimal time for determination of the cytolytic effect of the peripheral blood lymphocytes must not be less than 18 h.

Eventually, by varying the different types of TC, concentration of lectins, and duration of cytolysis it was decided to use HEp-2 and HeLa cells, to use conA as the stimulator in a concentration of 20-50 μg/ml, and to fix the duration of cytolysis at 18 h.

After these preliminary experiments, the cytolytic activity of peripheral blood lymphocytes from 50 healthy donors was investigated.

The average percentage cytolysis of HeLa cells was 11.6 without lectin, 31.1 when unpurified monocytes were used, and 50.3 after removal of the adherent population. The percentage cytolysis of HEp-2 cells increased from 19.1 during interaction with unpurified peripheral blood monocytes to 24.9 when lymphocytes purified from adherent cells were used. In the absence of lectin the cytological activity in the purified population fell from 9.4 to 5.4% (Fig. 2).

Besides T killers, natural killers and macrophages also possess cytolytic activity. Preliminary removal of adherent cells on a nylon wadding column significantly increased the cytolytic activity of the lymphocytes compared with the unpurified population. The question of participation of macrophages in this reaction is thus eliminated.

In recent years the attention of research workers has been drawn to the study of spontaneous or natural cytotoxicity against tumor cells *in vitro*. Cells with natural killer activity are not T or B lymphocytes, and their concentration in human peripheral blood is comparatively low [12]. Inevitable objections to the effect of natural killers in the system used in the present experiments can be overruled by the following arguments: activity of natural killers is exhibited mainly on myeloid leukemia K-562 cells, to potentiate their activity interferon, not lectins [12], was used, and to obtain a cytolytic effect a higher lymphocyte:TC ratio was used, namely 100:1 and 200:1, whereas in the present experiments the optimal effect was obtained with a ratio of 10:1. In addition, the cytolytic activity of the lymphocyte pop-

ulation containing T killers, interacting with allogeneic TC, corresponds to the cytolytic activity of these lymphocytes when added to a culture of syngeneic TC in the presence of lectin. It can thus be postulated that the cytolytic activity of peripheral blood lymphocytes in the presence of conA or PHA is effected by T killers. The aim of a future investigation will be to prove this hypothesis.

Theoretically, a definite number of T killers, immune to the person's own modified (virus or neoplastic) autoantibodies ought to be present in every human being.

Cytolytic activity of T killers is found, as we know, over a period of several days. Memory cells respond to repeated contact with the antigen. A cytolytic effect was observed under these circumstances as early as 24 h after re-exposure to the corresponding antigen [13]. It can be tentatively suggested that memory cells, recirculating in human peripheral blood, when adsorbed on the surface of certain "extraneous" TC in the presence of lectin for 18 h restore their lytic potential. In the course of shorter incubation time (3 or 6 h) lectin-dependent cytotoxicity is not found.

Determination of the cytolytic activity of human peripheral blood lymphocytes can thus be used as a diagnostic test in certain forms of pathology and, in particular, in tumors or autoimmune processes.

LITERATURE CITED

1. S. N. Bykovskaya, A. I. Rytenko, M. O. Raushenbakh, et al., Dokl. Akad. Nauk SSSR, 235, No. 6, 1462 (1977).
2. S. N. Bykovskaya, M. O. Raushenbakh, A. I. Rytenko, et al., Byull. Éksp. Biol. Med., No. 5, 560 (1978).
3. S. N. Bykovskaya, A. I. Rytenko, M. O. Raushenbakh, et al., Cell Immunol., 40, 164 (1978).
4. S. N. Bykovskaya, A. I. Rytenko, M. O. Raushenbakh, et al., Cell Immunol., 40, 175 (1978).
5. S. N. Bykovskaya, A. I. Rytenko, M. O. Raushenbakh, et al., Cell Immunol., 42, 197 (1979).
6. G. Berke, K. A. Sullivan, and D. B. Amos, J. Exp. Med., 135, 1334 (1972).
7. B. Bonavida and T. B. Bradley, J. Exp. Med., 138, 672 (1976).
8. A. Boyum, Scand. J. Clin. Lab. Invest., 21, Suppl. No. 92, 77 (1968).
9. R. P. Bubens and C. S. Henney, J. Immunol., 118, 180 (1977).
10. G. C. Cerottini, H. R. McDonald, H. D. Engers, et al., Prog. Immunol., 11, 153 (1974).
11. C. S. Henney, Transplant Rev., 17, 37 (1973).
12. R. B. Herberman, J. Y. Djen, H. D. Kay, et al., Immunol. Rev., 44, 43 (1979).
13. H. R. McDonald, G. C. Cerottini, and K. T. Brunner, J. Exp. Med., 140, 1511 (1974).