# CYTOLYTIC ACTIVITY OF PERIPHERAL BLOOD MONONUCLEAR CELLS, T LYMPHOCYTES, AND MONOCYTES FROM LUNG CANCER PATIENTS

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Changes in the immune status of lung cancer patients remain a difficult and little studied problem. Interesting aspects of it are the extent to which the natural defenses of the body are disturbed in these patients and the role of the immune response in the course and prognosis of the disease.

The immune status of patients with lung cancer was investigated.

## EXPERIMENTAL METHOD

The group of lung cancer patients comprised 30 individuals. As the control, effector cells from 20 samples of donated blood were studied. Peripheral blood mononuclear cells (MNC) were isolated by the method in [4]. T lymphocytes were obtained by removing adherent cells from MNC on a column with nylon wadding by the method in [8]. Monocytes were isolated by adsorption of the adherent cells from an MNC suspension on plastic Petri culture dishes, previously treated with autologous plasma [7]. The proportions of T cells and monocytes in the resulting MNC suspensions, and the degree of enrichment of the populations with T lymphocytes and monocytes were determined by an immunofluorescence method, using OKT3, OKT4, OKT8, OKM1, and OKB monoclonal antibodies (orthodiagnostic system). Tumor target cells (TC) were obtained on the day of the operation by treating minced tumor tissue with a mixture of enzymes [11]. Suspensions containing not less than 80% of living tumor cells were used in the tests. To estimate the cytolytic activity of the peripheral blood lymphocytes and monocytes from lung cancer patients, autologous tumor cells, freshly isolated allogeneic tumor cells, transplantable lung cancer cell lines AKL [3], HeLa, and K-562 were used as TC. The K-562 TC and freshly isolated tumor cells were labeled with $Na_2{}^{51}CrO_4$ and introduced in a dose of $10^4$ cells of each well of 96-well micropanels with conical bottoms, and cells of monolayer HeLa and AKL cultures were labeled on the eve of the experiment and seeded overnight in a dose of $2 \times 10^4$ cells per well in 96-well panels with a flat bottom (Falcon, USA). Effector cells were added to the targets in ratios of 50:1, 25:1, and 12:1. After incubation for 18 h at 37°C with 5% $CO_2$, supernatant was withdrawn from each well for determination of its radioactivity. The percentage of lysed tumor cells was calculated by the formula:

$$\text{cytolysis} = \frac{\text{experiment} - \text{spontaneous yield}}{\text{total lysis} - \text{spontaneous yield}} \times 100\%,$$

where experiment denotes the yield of the isotope from TC on the addition of effectors; spontaneous yields denote release of the isotope by intact TC; total lysis denotes the total quantity of the isotope incorporated into TC [5].

The method of lectin-dependent cytotoxicity [1] also was used in the experiments: HeLa cells were treated for 3 h before addition of the effectors with concanavalin A (conA) in a concentration of 100 μg/ml.

## EXPERIMENTAL RESULTS

Altogether 30 patients with primary lung cancer (twenty nine men and one women), aged from 42 to 69 years (average age 54.5 years), and with spread of the disease equivalent to

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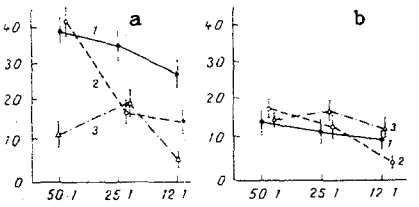


Fig. 1

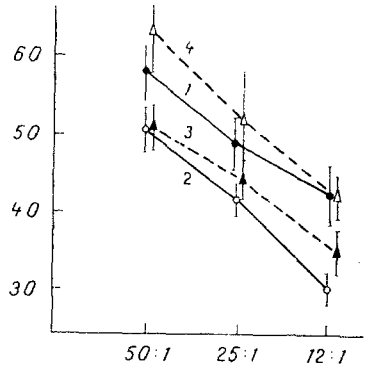


Fig. 2

Fig. 1. Cytolytic activity of peripheral blood MNC (1), T lymphocytes (2), and monocytes of patient N. against freshly isolated autologous (a) and allogeneic (b) tumor TC. Abscissa, effector—TC ratio; ordinate, number of lysed TC (in %).

Fig. 2. Cytotoxicity of peripheral blood MNC and T lymphocytes of lung cancer patients (27 patients) and normal blood donors (21) against K-562 cells. Abscissa, effector—TC ratio; ordinate, cytolysis (in %). 1) NK activity of patients' MNC, 2) the same, of donors' MNC, 3) NK activity of patients' T lymphocytes, 4) of donors' T cells.

$I^a$-III, were investigated. A squamous-cell carcinoma with keratinization was identified morphologically in 13 patients, squamous-cell carcinoma without keratinization in 10, an adenocarcinoma in 4, mixed squamous-cell-adenocarcinoma in 2, and carcinoid of the lung in 1 patient.

In 17 patients cytolytic activity of peripheral blood MNC against autologous tumor cells were studied. In 29% of cases MNC induced lysis of autologous tumor cells of between 11.5 and 40.2% (mean 23.6 ± 5.4%), in 38% of patients MNC possessed no cytolytic activity against autologous tumor cells, and in 33% of subjects studied cytolysis of tumor cells did not exceed 10% (mean 4.8 ± 1.1%), in agreement with data published previously [2, 9, 10]. To discover what type of effectors cause lysis of tumor cells, we obtained populations of peripheral blood cells enriched with T lymphocytes (91.3 ± 2.6% $OKT3^+$) and with monocytes (87.2 ± 2.9% $OKM1^+$). In 27% of patients the T lymphocytes induced lysis of autologous tumor cells on the average by 27.7 ± 5.2%. Cytolytic activity against freshly isolated allogeneic tumor cells amounted on the average to 15.7 ± 1.7% for MNC and 13.6 ± 0.9% for T lymphocytes. Peripheral blood monocytes also could cause lysis of freshly isolated tumor cells. A positive cytolytic effect was observed in two of the seven patients studied (mean 16.8 ± 1.5%). Data on the cytotoxicity of peripheral blood MNC, T lymphocytes, and monocytes against freshly isolated autologous and allogeneic tumor cells, with respect to patient N., are illustrated in Fig. 1a, b.

Monocytes had high cytotoxicity against AKL cells, and their activity, with an effector—TC ratio of 25:1, averaged 48.6 ± 4.8%. The cytotoxicity of MNC against AKL cells averaged 62.6 ± 5.4%, i.e., 1.3 times higher than T-lymphocyte activity (43.4 ± 3.9%; $p < 0.05$), which can be explained by the cytolytic action of monocytes present in the undivided MNC population (Table 1).

The level of natural killer (NK) activity of the peripheral blood of these patients, revealed on NK-sensitive K-562 TC, was rather higher than in the control. However, a significant ($p < 0.05$) decrease in NK activity of the population enriched with T lymphocytes was observed with an effector—TC ratio of 50:1. Cytolysis of K-562 TC by the patients' T lymphocytes with ratios of 50:1, 25:1, and 12:1 averaged 51.8, 44.9, and 37.0%, respectively, whereas in the control group NK activity of the T lymphocytes was 62.6, 52.6, and 41.0% (Fig. 2). Peripheral blood monocytes also possessed considerable cytolytic activity against K-562 cells, which averaged 48.3% with an effector—TC ratio of 25:1.

By the use of the lectin-dependent cytotoxicity method, which can reveal activity of killer cells circulating in patients' peripheral blood [1], we showed that the cytolytic effect of the patients' MNC increased after treatment of TC with conA on the average by 2.4 times

TABLE 1. Cytolytic Activity of MNC, T Lymphocytes, and Monocytes of Lung Cancer Patients against Tumor Cells of Transplantable AKL Line in % of Cytolysis of TC

| Patient No. | Effector cells | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MNC | | | T lymphocytes | | | monocytes | | |
| | effector–TC ratio | | | | | | | | |
| | 50:1 | 25:1 | 12:1 | 50:1 | 25:1 | 12:1 | 50:1 | 25:1 | 12:1 |
| 1 | — | 50,5 | 46,7 | — | 36,6 | 25,7 | — | 55,6 | — |
| 2 | 58,4 | 62,1 | 65,8 | 56,7 | 46,6 | 46,0 | 76,1 | 69,1 | 43,5 |
| 3 | 50,5 | 81,1 | 62,3 | 36,5 | 19,8 | 23,0 | 65,8 | 49,8 | 51,4 |
| 4 | — | 80,3 | — | — | 41,6 | — | — | 84,9 | — |
| 5 | 97,2 | 85,9 | 83,5 | 78,5 | 76,0 | 53,2 | — | 36,0 | 24,7 |
| 6 | 15,8 | 15,1 | 14,5 | 21,3 | 27,1 | 14,1 | 16,8 | 16,3 | 2,9 |
| 7 | 56,4 | 30,9 | 8,2 | 52,3 | 39,5 | 16,5 | — | 28,8 | 9,5 |
| 8 | 64,1 | 78,7 | 65,8 | 68,0 | 49,2 | 36,3 | — | 63,6 | — |
| 9 | 86,4 | 67,4 | 60,6 | 77,4 | 57,7 | 42,6 | 43,6 | 48,5 | 55,7 |
| 10 | — | 73,0 | — | — | 27,3 | — | — | 33,5 | — |

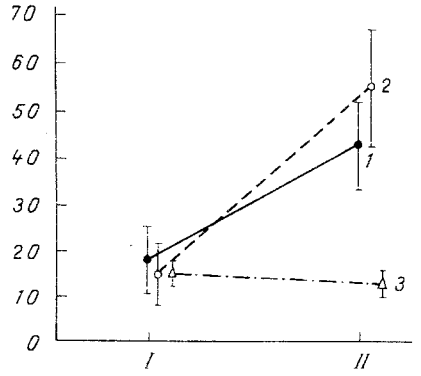


Fig. 3. Lectin-dependent cytotoxicity of peripheral blood MNC (1), T lymphocytes (2), and monocytes (3) from lung cancer patients against HeLa cells. Abscissa: I) TC not treated with conA, II) TC pretreated with conA; ordinate, number of lysed TC (in %).

($p < 0.001$), and when a population of T lymphocytes was used, activity was 3 times higher ($p < 0.001$). A study of the cytolytic activity of monocytes against HeLa TC showed that preliminary treatment of the targets with conA did not lead to any increase in cytolysis (Fig. 3).

No correlation could be found between the cytolytic activity of the patients' lymphocytes against the autologous tumor and the histological structure of the tumor. In patients with stage $I^a$–II of the disease, the cytolytic activity of the lymphocytes against the autologous tumor varied from 0 to 40.2%. whereas in stage III of the disease it did not exceed 6.1%. Consequently, this test can be used as one criterion for the prognosis of the disease.

The results show that nonspecific antitumor immunity is undisturbed in lung cancer patients. Specific immune protection of the host exists against tumors: the T lymphocytes and monocytes of the peripheral blood can recognize antigenic determinants on the surface of autologous and allogeneic tumor cells and cause their lysis [6, 9, 10]. The high cytolytic effect of MNC is due not only to the cytotoxicity of the T lymphocytes, but also to activity of monocytes. The combined cytolytic action of T lymphocytes and monocytes is higher than that of the unfractionated cells. This may be explained by competition between effectors for binding sites with the tumor cell or inhibition of some cells by others present among MNC.

Thus in patients with lung cancer peripheral blood lymphocytes and monocytes are the principal independently acting effectors in the mechanism of antitumor defense. Their activity is independent of the morphological structure of the tumor but decreases with an increase in weight of the tumor, and this must be taken into account when the course of the disease is predicted.

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# TOLERANCE TO A XENOGRAFT IN AN ADOPTIVE SYSTEM

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Induction of tolerance to xenografts is a complex task, for rejection of such grafts usually takes place very acutely. Methods of successful transplantation of allogeneic organs and tissues are in most cases unsuitable for a xenogeneic system. Positive results in this direction have been obtained with the aid of cyclosporin A, whole-body lymphocidal irradiation, and certain other procedures [3, 6-9]. In 1980, the writers developed a method of inducing tolerance to xenogeneic antigens with the aid of cyclophosphamide, including thymectomy and injection of a massive dose of xenogeneic splenocytes into the animals. Treatment of this kind resulted in prolonged (over 5 months) survival of a transplanted neonatal August rat heart in mice [4, 5].

The aim of the present investigation was to develop a method of adoptive transfer of tolerance to xenogeneic antigens in order to study the specificity of such tolerance and the mechanisms maintaining it.

## EXPERIMENTAL METHOD

Animals (male and female) of the following inbred lines were used: CBA ($H\text{-}2^k$) and (CBA × C57BL/6)$F_1$ ($H\text{-}2^{k/b}$) hybrid mice, and August ($RI^c$) rats. Erythrocytes from various species of animals — sheep, rat, and goose (SRBC, RRBC, and GRBC respectively) were used as the test antigens.

To induce tolerance, thymectomy was performed on adult mice and 3-4 weeks after the operation an intravenous injection of $1.2 \times 10^8$ rat splenocytes was given, followed 24 h later by an intraperitoneal injection of 200 mg/kg of cyclophosphamide (CP), dissolved in distilled water immediately before injection.

In the case of adoptive transfer of tolerance the recipients were irradiated from a $^{60}Co$ source in a dose of 9.5 Gy, which was followed by an intravenous injection of $2 \times 10^7$

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