# Mononuclear Leukocytes from Mice with Resected Tumor Induce Resistance to Transplantation of Tumor Cells to Animals

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Experiments on male C57Bl/6 mice with intraperitoneally transplanted Ehrlich carcinoma and DBA/2 mice with subcutaneously transplanted S-91 melanoma showed that preliminary injection of mononuclear leukocytes obtained from animals 6-8 h after tumor resection induce resistance to transplantation of malignant transformed cells. Our results suggest that not only humoral factors, but also immunocompetent cells are involved in the regulation of tumor growth. The resistance to tumor transplantation was not induced by mononuclear leukocytes isolated over the first hours and 10-12 h after removal of the primary tumor node, which excludes the direct cytotoxic effect of these cells and suggests that this phenomenon is not associated with activation of the effector mechanisms for innate and adoptive immunity.

**Key Words:** *mononuclear leukocytes; Ehrlich carcinoma; S-91 melanoma*

Removal of the primary tumor node is an experimental approach in the studies of the mechanisms of metastatic dissemination and recurrence of tumors [1]. Little is known about the mechanism underlying the effect of the primary tumors on recurrence and metastatic dissemination. Previous studies showed that the rate of cell division increases only on the first day after tumor resection. For example, the number of $G_2$/M-phase cells in the secondary inoculum increases by 10-12% 24 h after removal of the primary tumor node [4]. The authors hypothesized that this tumor produces a factor, which is accumulated in the blood of tumor animals and circulates in an inactive form. After removal of the tumor node, this factor is activated. The percentage of dividing cells increases under these conditions. However, it is impossible to confirm this hypothesis and to isolate the factor regulating tumor cell division. It should be emphasized that surgical removal of the tumor is accompanied by blood loss (*i.e.*, loss of the factor). The number of dividing cells did not exceed 140% of the control. It was not associated with the volume of blood loss and/or number of eliminated cells from the primary tumor node. Acceleration of cell division is observed only during the first 24 h after tumor removal. It can be suggested that removal of the primary tumor node is followed by a sharp increase in the concentration of a tumor-specific factor in blood plasma. Further decrease in the amount of this factor is related to activation of the negative feedback mechanism. These changes should be accompanied by the appearance of immunocompetent cells that suppress tumor growth in tumor animals over 24 h after surgery.

Here we studied the ability of mononuclear leukocytes (MNL) to induce the resistance of mice to tumor transplantation.

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## MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 and DBA/2 mice aging 2-3 months and obtained from the Stolbovaya nursery (Moscow region). Ehrlich carcinoma was transplanted intraperitoneally ($10^6$ cells in 0.2 ml RPMI 1640 medium) or intramuscularly ($10^6$ cells in 0.1 ml RPMI-1640 medium) to C57Bl/6 mice. S-91 melanoma was transplanted subcutaneously ($10^6$ cells in 0.2 ml RPMI-1640 medium) to DBA/2 mice.

Partial removal of the ascitic fluid was performed on day 7 after transplantation of Ehrlich carcinoma.

**TABLE 1.** Effect of Partial Removal of Ascitic Fluid from Mice with Intraperitoneally Transplanted Ehrlich Carcinoma on Engraftment of the Remaining Tumor Cells in Intact Animals

| Group, No. | Time after tumor removal, h | Number of mice without tumor, % |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 1 | 0 |
| 3 | 4 | 0 |
| 4 | 5 | 0 |
| 5 | 6 | 42.5±3.1 |
| 6 | 8 | 28.1±4.5 |
| 7 | 10 | 0 |
| 8 | 12 | 0 |
| 9 | 24 | 0 |
| 10 | 48 | 0 |

The remaining tumor cells ($10^6$ cells per mouse) were administered to intact animals after 0-48 h.

MNL were isolated 6-8 h after partial removal of the ascitic fluid using a Ficoll gradient (PanEco). The cell suspension was layered on Ficoll and centrifuged at 1500 rpm for 30 min. MNL were collected at the gradient density boundary and washed 2 times by centrifugation at 1500 rpm for 10 min. Peritoneal cells of mice with intramuscularly or subcutaneously transplanted tumors were obtained from peritoneal lavage in RPMI-1640 medium. The cells were injected subcutaneously ($3\times10^6$ cells per mouse) to intact animals 14 days before tumor transplantation.

Melanoma mice were operated under ketamine anesthesia on day 20-30 after transplantation (tumor size above 1 $cm^3$). MNL were isolated 0-48 h after tumor elimination. The cells were injected subcutaneously ($3\times10^6$ cells per mouse) to intact animals 14 days before tumor transplantation. The treatment was performed 1, 2, or 3 times (at 1-h intervals).

The results were analyzed by Fischer—Student test. Each group consisted of 10 mice. The data are expressed as the mean values of 3 series.

## RESULTS

Table 1 shows the effect of partial removal of the ascitic fluid from mice with intraperitoneally transplanted Ehrlich carcinoma on engraftment of the remaining

**TABLE 2.** Effect of MNL on Engraftment of Ehrlich Carcinoma

| Group, No. | Time after tumor removal, h | MNL | Number of mice without tumor, % |
|---|---|---|---|
| 1 | 4 | Splenocytes | 0 |
| 2 | 4 | Blood leukocytes | 0 |
| 3 | 4 | Peritoneal cells | 0 |
| 4 | 5 | Splenocytes | 3.7±1.8 |
| 5 | 5 | Blood leukocytes | 0 |
| 6 | 5 | Peritoneal cells | 0 |
| 7 | 6 | Splenocytes | 93.1±5.7 |
| 8 | 6 | Blood leukocytes | 66.7±5.8 |
| 9 | 6 | Peritoneal cells | 87.5±5.0 |
| 10 | 8 | Splenocytes | 98.5±3.8 |
| 11 | 8 | Blood leukocytes | 58.0±16.4 |
| 12 | 8 | Peritoneal cells | 82.5±14.9 |
| 13 | 10 | Splenocytes | 6.7±2.6 |
| 14 | 10 | Blood leukocytes | 0 |
| 15 | 10 | Peritoneal cells | 0 |
| 16 | 12 | Splenocytes | 0 |
| 17 | 12 | Blood leukocytes | 0 |
| 18 | 12 | Peritoneal cells | 0 |

**TABLE 3.** Effect of MNL on Engraftment of S-91 Melanoma

| Group, No. | Time after tumor removal, h | MNL | Number of injections | Number of mice without tumor, % |
|---|---|---|---|---|
| 1 | 6 | Splenocytes | 1 | 27.5±4.9 |
| 2 | 6 | Blood leukocytes | 1 | 12.5±5.0 |
| 3 | 6 | Peritoneal cells | 1 | 17.5±5.0 |
| 4 | 6 | Splenocytes | 1 | 40.0±8.2 |
| | 7 | Blood leukocytes | 1 | |
| 5 | 6 | Peritoneal cells | 1 | 66.7±5.8 |
| | 7 | Splenocytes | 1 | |
| 6 | 6 | Blood leukocytes | 1 | 86.7±5.7 |
| | 7 | Peritoneal cells | 1 | |
| | 8 | Splenocytes | 1 | |
| 7 | 6 | Peritoneal cells | 3 | 98.5±3.8 |
| 8 | 6 | Blood leukocytes | 3 | 82.5±5.0 |
| 9 | 6 | Splenocytes | 3 | 98.5±3.8 |

tumor cells in intact animals (Table 1). We showed that 6-8 h after partial tumor removal, transplantation of the remaining ascitic fluid to intact animals does not cause tumor growth in 28-42% specimens. Moreover, these animals acquired resistance to repeated transplantation of Ehrlich carcinoma (over all life, up to 2 years). The animals died without signs of tumor development.

We assumed that inhibition of tumor growth after removal of the primary tumor node is mediated by immunocompetent cells. Further study was conducted to evaluate the effect of MNL on Ehrlich carcinoma engraftment (Table 2). MNL were obtained from animals with Ehrlich carcinoma 6-8 h after a partial removal of the ascitic fluid. Administration of these cells was followed by resistance to tumor transplantation in 58-98% intact animals.

Similar results were obtained in experiments with subcutaneous transplantation of melanoma S-91 (Table 3). MNL were isolated from mice 6-8 h after tumor removal. Single, twofold, and threefold administration of immunocompetent cells was followed by the resistance to transplantation of S-91 melanoma in 12.5-27.5, 40.0-66.7, and 82.5-98.5% intact animals, respectively.

Our findings suggest that not only humoral factors [2,3], but also immunocompetent cells are involved in the regulation of tumor growth. Tumor removal results in a compensatory short-term increase in the rate of tumor growth, which is regulated by humoral factors. Further inhibition of their production is mediated by blood leukocytes, splenocytes, and peritoneal cells. The resistance to tumor transplantation was not induced by MNL isolated over the first hours and 10-12 h after removal of the primary tumor node. Therefore, these cells do not produce the direct cytotoxic effect. We conclude that this phenomenon is not associated with activation of the effector mechanisms for innate and adaptive immunity. This problem requires further investigations.

## REFERENCES

1. S. M. Sitdikova, B. S. Amandzholov, M. V. Kiselevskii, and F. V. Donenko, *Byull. Eksp. Biol. Med.*, **143**, No. 1, 86-88 (2007).
2. S. M. Sitdikova, B. S. Amandzholov, V. Yu. Sel'chuk, and F. V. Donenko, *Ibid.*, **135**, No. 6, 676-679 (2003).
3. F. V. Donenko, S. M. Sitdikova, A. V. Syrtsev, *et al.*, *Intern. J. Oncology*, **32**, No. 4, 885-893 (2008).
4. B. Fisher, N. Gunduz, J. Coyle, *et al.*, *Cancer Res.*, **49**, No. 8, 1996-2001 (1989).