# MEMBRANE TOXICITY AND CYTOTOXICITY OF TUMOR CELLS TOWARD TUMOR CELLS WITH REDUCED RNA AND PROTEIN BIOSYNTHESIS OR CULTURED *IN VITRO*

A. G. Sterlina, I. V. Bogdashin, and B. B. Fuks

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KEY WORDS: tumor cell; membrane toxicity; cytotoxicity; progression of tumor; protein synthesis.

One mechanism of progression of malignant tumors is immunoselection of clones of tumor cells by lymphocytes. It was shown previously that tumor cells damage the membrane of target cells sensitive to normal killer cells [4]. We also have found that tumor cells damage the membrane of lymphocytes with reduced protein biosynthesis and that this effect is restricted with respect to the 1C-region of the H-2 complex [3]. On the basis of these data we postulated that depression of protein synthesis in tumor cells growing *in vivo* may reduce their resistance to other cells of the same tumor, i.e., make them targets for the membrane-toxic action of syngeneic tumor cells with intact protein metabolism. A connection between the level of protein synthesis and sensitivity of the cell membrane relative to cytotoxic lymphocytes was demonstrated previously [7].

The object of the present investigation was to determine whether intact tumor cells will damage the membrane of these same tumor cells with reduced protein biosynthesis.

## EXPERIMENTAL METHOD

The cytotoxicity of the tumor cell against the target cell was studied by the 4-hour test with $^{51}Cr$. The membrane toxicity of the tumor cells was determined in the test with [$^{3}$H]uridine described by the writers previously [2]. To prevent reutilization of [$^{3}$H]-RNA degradation products by effector tumor cells, they were treated with actinomycin D (1 μg/ml) for 1 h at 37°C and washed three times with large volumes of medium 199 with 10% fetal serum (Flow Laboratories, England). It was shown previously, by the present writers and others [5] that such treatment does not affect the cytotoxicity of effector cells. Tumor cells of sarcoma SA-1 (H-2$^{a}$), MCh-11 (H-2$^{b}$), thymoma (H-2$^{k}$), and leukemias EL-4 (H-2$^{b}$) and L-1210 (H-2$^{d}$) were used as effector cells. The thin tumor cells, cultured *in vitro* for 4-20 h in the presence of actinomycin D (1 μg/ml) in medium RPMI-1640 with 10% fetal serum, and also IAC-1 cells obtained by culturing IAC cells in the same medium without actinomycin D for 18 h and erythromyeloid leukemia K-562 cells were used as the target cells. The target cells were labeled beforehand with [$^{3}$H]uridine (3 μCi/ml, specific activity 24 mCi/ml) for 1 h at 37°C in medium RPMI-1640 with 10% fetal serum, and also with $^{51}Cr$ (100 μCi/ml) for 1 h in the same medium. To remove macrophages and other cells the peritoneal suspension of effector tumor cells was adsorbed on plastic Petri dishes (Flow Laboratories, No. 6120307) twice for 45 min at 37°C in an atmosphere of 5% $CO_2$ and divided on a Ficoll–Hypaque gradient (Pharmacia, Sweden, density 1.077 g/cm$^{3}$). Indices of membrane toxicity and cytotoxicity (MI and CI respectively) were calculated by the following equations:

$$MI = \left(1 - \frac{\text{target cells incubated with effector cells}}{\text{target cells incubated without effector cells}}\right) \times 100$$

$$CI = \left(\frac{\text{experiment} - \text{spontaneous yield}}{\text{maximal yield} - \text{spontaneous yield}}\right) \times 100.$$

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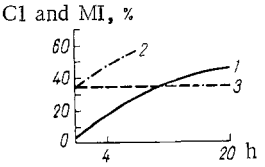


Fig. 1. Increase in cytotoxicity and membrane toxicity of sarcoma SA-1 target cells relative to erythroleukemia K-562 cells as a result of treatment of the latter with actinomycin D. 1) Test with $^{51}Cr$, 2) test with [$^{3}H$]uridine, 3) test with [$^{3}H$]uridine on K-562 cells not treated with actinomycin D (control). Abscissa, time (in h); ordinate, cytotoxicity and membrane toxicity (in %).

## EXPERIMENTAL RESULTS

The cytotoxic action of SA-1 cells on $^{51}Cr$-labeled K-562 target cells for 4 h was minimal (Fig. 1). On treatment of the target cells with actinomycin D for 2 h, cytotoxicity increased to 45%. Hence it follows that the reduction in RNA and protein synthesis in the target cell made it possible for the first time to demonstrate clearly the cytotoxic action of the tumor cell. In parallel experiments we showed an increase in the cytotoxicity of normal killer cells in the spleen of C57BL/6 mice from 20 to 54% after identical treatment of K-562 cells with actinomycin D in the test with $^{51}Cr$, and in that way we reproduced the data published by other workers [7].

In the test with [$^{3}H$]uridine, SA-1 effector cells without treatment of the K-562 target cells with actinomycin D gave a membrane toxicity index of 34% (Fig. 1). We use the term membrane toxicity to describe the increased permeability of the target cell membrane for ribonuclease molecules (mol. wt. 12 kilodaltons) during the action of the effector tumor cell. Hence, in the same experiment by means of the [$^{3}H$]uridine test an effect of injury to the membrane of the target cell (not detectable in the test with $^{51}Cr$), which did not terminate in death of the target cell, was found. This may be explained by the ability of the target cell to repair its damaged membrane [7]. A powerful argument in support of this suggestion is given by the fact that the decrease in RNA and protein synthesis in the target cell and, as a result, the decrease in repair of its membrane enabled cytotoxicity in the test with $^{51}Cr$ to be increased above the level of membrane toxicity, revealed by the [$^{3}H$]uridine test. On treatment with actinomycin D, the curves of growth of membrane toxicity and cytotoxicity were practically identical in shape (Fig. 1).

Short-term (2 days) culture of an IAC tumor transplanted into A/Sn ($H\text{-}2^{a}$) mice made it sensitive to the cytotoxic action of IAC effector cells, maintained *in vivo* in the test with $^{51}Cr$ (Fig. 2). We showed in a series of experiments that IAC-1 target cells maintained *in vitro* were sensitive to the membrane-toxic action of the following allogeneic and syngeneic tumor cells: MCh-11, SA-1 IAC, and thymoma, and also of natural killer cells (MI in the test with [$^{3}H$]uridine varied from 34.0 ± 1.1 to 48.0 ± 1.0% with effector and target cells in the ratio of 50:1). The same IAC-1 target cells were moderately sensitive to the cytotoxic action of SA-1 and IAC tumor cells (CI varied from 11.0 ± 1.2 to 19.1 ± 1.5%).

It follows from the data given above that a reduction in protein synthesis in the target cell and culture of the target cell *in vitro* leads to the same result: an increase in its sensitivity to the cytotoxic and membrane-toxic action of tumor cells growing *in vivo*. The question which requires an answer is: Is this increase in sensitivity of the target cell due entirely to reduction of its ability to repair the membrane [7] or (and) to another mechanism — a change in the chemical and physicochemical properties of the membrane, for example, an increase in the level of the glycolipid asialo-GM2 in its composition? [6].

Cytotoxicity almost reached the level of membrane toxicity after culture of IAC-1 target cells for 2 days *in vitro* (Fig. 2). This fact may be an argument in support of the suggested analogy with a change in the membrane of the target cells, leading to an increase in their

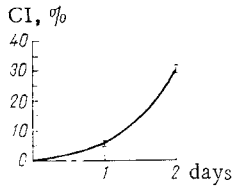


Fig. 2. Increase in cytotoxicity (in %) of IAC cells relative to IAC-1 target cells in test with $^{51}Cr$ during culture of target cells *in vitro* for 2 days. Abscissa, time (in days); ordinate, cytotoxicity (in %).

TABLE 1. Membrane Toxicity (MI) of Tumor Cells Relative to the Same Tumor Cells Treated with Actinomycin D (1 μg/ml at 37°C for 8 h)

| Effector cells | Target cells | Ratio of effectors to target cells | MI, % | Cold inhibitors | Inhibition, % |
|---|---|---|---|---|---|
| MCh-11 | MCh-11 | 50:1 | 64±1 | MCh-11 | 68 |
| | | 100:1 | 82±2 | | |
| | SA-1 | 50:1 | 14±1 | | |
| | | 100:1 | 26±1 | | |
| L-1210 | L-1210 | 50:1 | 60±2 | L-1210 | 73 |
| | | 100:1 | 80±3 | | |
| | SA-1 | 50:1 | 60±2 | | |
| | | 100:1 | 78±2 | | |
| SA-1 | SA-1 | 50:1 | 56±1 | SA-1 | 76 |
| | | 100:1 | 84±3 | | |
| | L-1210 | 50:1 | 49±2 | | |
| | | 100:1 | 76±1 | | |
| EL-4 | EL-4 | 50:1 | 58±3 | | |
| | | 100:1 | 78±2 | | |

cytotoxicity and membrane toxicity, and it suggests that membrane toxicity is the minimal reparable injury to the target cell membrane as a result of the action of effector cells (normal killer cells and tumor cells).

Previously [1] we showed the existence of mutual suppression (cytostatic effect) of a normal killer cell and tumor cell growing *in vivo*, not by the traditional method of recording a reduction of proliferation based on incorporation of [$^{3}H$]thymidine of [$^{125}I$]deoxuridine, but by recording injury to the membrane of the third party — the target cell. The results of this investigation showed that the cytostatic effect we have described cannot be revealed by the method of testing membrane toxicity. Accordingly a new technical problem arises: developing a more precise technique for recording changes in the cell membrane which lie at the basis of the phenomenon of cytostatic activity.

As Table 1 shows, membrane toxicity of tumor cells relative to syngeneic tumor cells with reduced RNA and protein synthesis in the ratio of 100:1 exceeded 78%. On the addition of "cold," i.e., unlabeled target cells subjected to the same treatment as the labeled target cells, to a mixture of effector tumor cells and labeled target cells as inhibitors, inhibition of membrane toxicity took place. Consequently, direct contact with effector tumor cells is necessary for injury to the target cell membrane.

The experiments thus showed that when protein synthesis is depressed in a certain proportion of a population of tumor cells of a given tumor, a membrane-toxic and cytotoxic effect of cells of the same tumor on them may arise. The results of the model experiments described in this paper may have a bearing on tumor progression. For instance, it can be postulated that clones appear in a tumor which have relatively higher membrane-toxic and cytotoxic potential than other cells of that tumor. As a result they may occupy the position of leaders in the given tumor, with the result that invasiveness of the tumor may be increased.

LITERATURE CITED

1. M. S. Zedgenidze, B. V. Nilonenko, I. V. Spirande, et al., Byull. Éksp. Biol. Med., No. 4, 82 (1982).
2. M. P. Rykova, I. V. Spirande, M. S. Zedgenidze, et al., Immunologiya, No. 3, 88 (1981).
3. A. G. Sterlina, B. V. Nikonenko, and B. B. Fuks, Immunologiya, No. 1, 52 (1982).
4. B. B. Fuks, M. S. Zedgenidze, I. V. Spirande, et al., Immunologiya, No. 9, 342 (1981).
5. J. Ortaldo, W. Phillips, K. Wasserman, et al., J. Immunol., 125, 1838 (1980).
6. R. Schwartz, Immunobiology, 159, 156 (1981).
7. R. Welsh and L. Kunkel, Int. J. Cancer, 27, 73 (1981).

# MORPHOLOGY OF GROWTH OF HETEROGRAFTS OF HUMAN OSTEOGENIC SARCOMA IN DIFFUSION CHAMBERS

M. V. Svyatukhin, E. M. Lysenkova, and L. A. Doronina

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Several reports of growth of human osteogenic sarcomas in tissue culture have now been published, but there is no information in the accessible literature on heterografts of such sarcomas in diffusion chambers. This paper gives the results of observations on heterografts of this kind from seven osteogenic sarcomas. The material for transplantation was obtained from the Operating Department for General Oncology, All-Union Oncologic Scientific Center, directed by Academician of the Academy of Medical Sciences of the USSR, N. N. Trapeznikov. The experimental technique was described previously [1]. Synpor filters with a mean pore diameter of 0.23-0.3 μ were used for making the chambers.

In the early stages (3-5 days after transplantation) outgrowths of elongated cells with oval nuclei, with more or less well-marked features of fibroblast-like cells (Fig. 1a), could be seen at the periphery of the graft. Some of the outgrowths were arranged in a radial direction, but some had lost this regular orientation and formed a reticular structure. In some cases, mainly in areas of more compact arrangement, groups of two or three cells lying in different planes and superposed one above the other could be seen.

A compact zone of growth, extending for a distance of up to 1.5-2 mm from the graft, was observed 1-2 weeks after transplantation around the graft or part of its circumference (Fig. 1b). Cells with oval nuclei distributed along the long axis or irregularly were oriented not quite regularly in the radial direction, and frequently were superposed one above another. Mitotic figures were found. At the periphery the compact zone gave way to a less dense reticular structure with small concentrations of flattened compact cells. Some of the cells were no longer fusiform in shape but had acquired angular outlines or had become branched.

Later the greater part of the filter or nearly the whole of its surface was covered with fields sometimes of compactly arranged cells, sometimes of less densely packed cells of different shapes: fusiform, round, triangular, irregularly polygonal, or branching (Fig. 2a). Their nuclei were round, oval, irregularly oval with invaginations of their membrane in some areas, or bean-shaped. Binuclear and, occasionally, multinuclear cells were found. In experiment 1L after 20 days, and in experiment 6D after 26 days, a distinct pattern was formed at sites of the most compact distribution of the cells grouped in different orientations (Fig. 2b). If neighboring areas of the specimen are examined consecutively a gradual transition can be seen toward regions of less compact arrangement of the cells and, finally, fields of scattered branching cells (Fig. 3a).

In experiment 2L the filter in the later stages was covered by haphazardly oriented cells with irregularly round or oval nuclei, containing course grains of chromatin, lying in different

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