Heterotransplantation of Ehrlich Ascites Tumor Cells in Rats

Pre-Treated with Cytostatics and Antimetabolites

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Pre-treatment of rats with various doses of cytostatics and antimetabolites (cyclophosphamide, mitomycin-C, 6-mercaptopurine, 6-azauridine and 5-azaorotic acid) permits successful heterotransplantation of Ehrlich ascites tumor, as indicated by the progressive growth of the tumor cell population. Experiments with cyclophosphamide suggest a direct dose dependence of the rate of tumor growth in heterologous recipients.

A number of authors have reported the successful transplantation of Ehrlich ascites tumor (EAT) cells to species other than mice, as well as the transplantation of human tumor to animals after treatment of the heterologous recipients with X-rays. Cortisone, or both deal with the gradual rejection and regression of EAT, transplants in untreated rats. The protective effect of cyclophosphamide on the transplantation of Yoshida sarcoma in rats has also been described.

The present experimental study was carried out to investigate whether progressive growth of EAT transplants can be achieved in rats pre-treated with cytostatics (cyclophosphamide, mitomycin-C) and antimetabolites (6-mercaptopurine, 6-azauridine and 5-azaorotic acid) in order to suppress their immune response.

Materials and methods

Experimental animals: Male albino Wistar rats, weighing 150–160 g each, were divided into groups 7 days before the experiment. The EAT was maintained in 25–30 g mice of the Agnes-Bluhm strain.

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**Tumor:** The experiments were carried out with the hyperdiploid variant of EAT, known in the literature as the Lettré strain, Landschütz sarcoma I, or Ehrlich-Lettre hyperdiploid strain. On the eighth day after transplantation, the ascites contained 95—97% EAT cells and 3—5% leucocytes and lymphocytes. The mitotic index was 3—4 per cent. The number of EAT cells per ml totalled 160·10⁶ on the average.

**Transplantation techniques:** The experimental animals were injected intraperitoneally with 0.4—0.6 ml ascitic fluid under sterile conditions. Subcutaneous transplants were placed between the shoulder blades.

**Cell counting techniques:** The cell concentration in the ascitic fluid was determined by counting the cell in a Bürker chamber. The percentage of tumor cells as well as the mitotic indices were estimated from smears of the ascites fluid and from squash preparations stained with acetoorcein. The concentration of true blood cells in the ascitic fluid was determined from smears stained after Pappenheim.

**Solid tumors, as well as various internal organs, were fixed in 3:1 ethanol-acetic acid, embedded in paraffin, sectioned and stained with methylgreen-pyronin and haematoxylin-eosin.**

**Cytostatics and antimetabolites:** Cyclophosphamide, mitomycin-C, 6-mercaptopurine, 6-azauridine were commercial preparations. 5-azaorotic acid was synthesized and purified p.a. by Mr. Georgi Karamanov of this laboratory. The substances were dissolved in distilled water, or suspended by homogenization in a 0.5% solution of carboxymethylcellulose (Schuchardt tylose). The substances were injected intraperitoneally under sterile conditions.

**Results**

In the majority of our experiments, we used cyclophosphamide. Five successive experiments in a total of 140 rats were carried out with different doses of this compound. The animals were treated for two days, and after a 24 hr interval the EAT was transplanted.

In all cyclophosphamide-treated animals an effect on the heterotransplantability of EAT cells was observed. To follow the process of tumor growth in groups of animals treated with different doses of cyclophosphamide, two animals were killed daily, beginning with the second day after transplantation. In the control animals, tumor growth was insignificant. During the first week, surviving tumor cells without mitosis were observed, revealing signs of progressive degeneration. No signs of an inflammatory process in the abdominal cavity were detected, nor where there any haemorrhagic exudates or solid tumor nodes at the site of injection in the peritoneal cavity.

In the experiments with cyclophosphamide the fate of the heterotransplants varied according to the doses applied. At a dose of 7 mg/kg body weight, there was no transplantability, whereas at a dose level of 35—70 mg/kg regression of the transplants only began after a period of 8 days. At doses of 100 mg/kg and 140 mg/kg, no effect on the transplants was observed before the 15th day, and at a dose of 200 mg/kg the treatment resulted in the death of the animals due to the rapid development of the tumor.

In none of the groups animals were killed by an overtreatment with cyclophosphamide; only a loss of weight was noticed during the first 4—5 days, accompanied by diarrhoea and bristling of hair.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total dose [mg/kg] for 2 days</th>
<th>Number of animals</th>
<th>Number of animals with successfully transplanted tumors</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ascitic form + solid tumors</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>7</td>
<td>10</td>
<td>No significant heterotransplantability</td>
<td>Up to 7th—8th day — small increase in the number of tumor cells, followed by tumor regression</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>10</td>
<td>10</td>
<td></td>
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<tr>
<td></td>
<td>70</td>
<td>10</td>
<td>10</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>40</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>6-mercaptopurine</td>
<td>130</td>
<td>10</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Mitomycin-C</td>
<td>2.5</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>6-azauridine</td>
<td>150</td>
<td>14</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5-azaorotic acid</td>
<td>1500</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. Heterotransplantation of EAT cells after treatment with different doses of cytostatics and antimetabolites.
Table 1 gives the data on heterotransplantation of EAT cells after treatment with different doses of cyclophosphamide. Fig. 1 shows the results of the application of various doses of cyclophosphamide, and Fig. 2 shows the effect on i.p. heterotransplants of EAT cells in rats, pretreated with 0 and 140 mg/kg cyclophosphamide, respectively, on the 9th day after heterotransplantation.

The mitotic index was at its maximum towards the fifth day after transplantation, and decreased towards the seventh day. Similar values are obtained in EAT developing in homologous mice. The percentage of degenerated tumor cells increased progressively as a function of time. The number of blood elements in the ascitic fluid (mainly lymphocytes and leucocytes) also increased with time. The colour of the ascitic fluid changed from milk-white to yellow-brown; the viscosity of the ascitic fluid decreased progressively.

In parallel with the increase in the amount of ascitic fluid in some animals, there was a marked development of solid tumor inside the abdominal cavity, as well as at the site of the puncture of the abdominal muscles and the peritoneum. Tumors in the abdominal cavity appeared during the first 5 – 6 days in the form of white oval kidney-shaped cell clusters ranging from 0.5 – 1 cm in diameter, and floating freely in the ascitic fluid. Towards the 8th – 9th day, the solid nodes caught a firm hold of bowel tangles, omentum, spleen and liver, becoming up to several cm in diameter and 20 g in weight. Histologically, the solid tumors were characterized as conglomerates of EAT cells with a central necrosis and a periphery of viable tumor cells (Fig. 3).

As a criterion for cell viability under the conditions of heterotransplantation, the test of Scherk was used. A direct proof of tumor cell viability was provided by the successful back-transplantation to mice. For this purpose, ascites from rats was taken on the eighth day after transplantation. Mice were either injected intraperitoneally with 0.2 ml of ascitic fluid (about 30–10⁶ cells), or received transplantats of pieces of solid tumor. In all cases and in all animals typical growth of EAT was observed after back-transplantation.

The scheme devised for treating recipient rats with cyclophosphamide was also adopted for the other compounds. The results obtained with these substances are summarized in Table 1, which shows the applied doses, the number of experimental animals, and the cases of successful heterotransplantation. With mitomycin-C, maximum tumor growth

Fig. 1. Development of tumor-heterotransplant in rats pretreated with various doses of cyclophosphamide: o—o 35 mg/kg; •—• 70 mg/kg; ▲ ▲ ▲ 100 mg/kg; △ △ △ 140 mg/kg.

Fig. 2. Effect of i.p. heterotransplantation of EAT in rats pretreated with 140 mg/kg cyclophosphamide. 9th day after heterotransplantation. a — control animal; b — pretreated animal.
was observed on the fifth and sixth day after transplantation, when the quantity of EAT cells was six times higher than in the initial inoculum. A quick regression of the transplantation followed thereafter, resulting in a complete rejection of the transplants on the eighth day after transplantation. Mice were uridine and 5-ataorotic acid the picture was similar. The overall quantity of tumor cells increased nearly fivefold by the sixth day after transplantation and was then followed by rapid regression. As indicated by the data in Table 1, the number of animals with successful heterotransplantation varied in our experiments.

**Discussion**

The results obtained reveal that in rats pretreated with varying doses of cytostatics and antimetabolites, successful heterotransplantation of EAT cells is observed in a number of cases. The clearest results were obtained with cyclophosphamide, which is known as a powerful immunodepressor. This finding is in agreement with results of several studies on homotransplantation 9-11. The rate of tumor growth (see Table 1 and Fig. 1) and the immunosuppressive effect after pretreatment of the recipients with this drug appear to be dose dependent. Whereas after doses of 35 mg/kg and 70 mg/kg, tumor growth is at its maximum between the fifth and sixth day (whereafter it gradually slows down and ends in the complete rejection of the heterotransplant), the survival of the tumor cells is considerably increased (up to 15 days) after application of higher doses (100 mg/kg and 140 mg/kg). After doses of 200 mg/kg, the treatment results in the death of the animals due to excessive tumor growth.

![Fig. 3. Histological section of a solid EAT from the abdominal cavity of a rat pretreated with 140 mg/kg cyclophosphamide.](image)

The present experiments show that under the above mentioned experimental conditions, the effect of 6-mercaptopurine is less pronounced. It might, however, be assumed that with an appropriate change of experimental conditions, the compounds which exerted smaller immunosuppressive effects in the present study, may prove to be more active. Whereas back-transplantation of EAT cells from rats to mice was consistently successful after cyclophosphamide, this was not the case after pretreatment with mitomycin-C ad 6-mercaptopurine. This implies that in the first case the retransplants contained a large number of viable cells (up to 97%), whereas after pretreatment with the other compounds the percentage of viable tumor cells in the ascitic fluid was smaller. In the latter case, a considerable number of true blood elements and phagocytizing cells was observed in the ascites fluid. With regard to the untreated control animals, our results do not differ qualitatively from data reported by other authors.

As a source of possible errors in the quantitative evaluation of tumor growth after heterotransplantation of EAT to rats could be the frequent presence in the abdominal cavity not only of single EAT cells but also of cell conglomerates especially in necrotic sites, and of blood elements. Irrespective of this shortcoming, we believe that the heterotransplantation of EAT to pretreated rats could be used as a quick semi-quantitative method for the estimation of immunodepressive effects of cytostatics, antimetabolites and other compounds.

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