NONSPECIFIC SUPPRESSIVE ACTION OF ASCITES FLUID OF EHRLICH'S TUMOR ON TRANSPLANTATION IMMUNITY

A. S. Belokhvostov and N. K. Zelenkova

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The effect of syngeneic ascites fluid from an Ehrlich's tumor on the survival of allogeneic skin grafts was investigated in CC57BR mice. Some delay of rejection of the grafts (by 3-4 days) was observed after injection of this fluid. Preliminary transplantation of such a graft into mice with an Ehrlich's tumor caused no increase in the immunodepressive action of the ascites fluid taken from it. The results indicate that antigen-dependent immunodepressive factors of ascites fluid of tumors play a dominant role in the suppression of transplantation immunity *in vivo*.

KEY WORDS: immunodepression; tumor; transplantation; blocking factor.

The development of a tumor leads to the appearance of immunodepressive substances in the biological fluids of the host, including a blocking factor [1, 4, 5, 7, 8]. The blocking factor depresses the cytotoxicity of immune lymphocytes against the corresponding target cells *in vitro* [8], but it is not clear how actively it protects target cells *in vivo*. Antitumor immunity has much in common with transplantation immunity. Consequently, the study of the effect of a tumor and of the biological fluids of an animal affected with a tumor on rejection of grafts *in vivo* could be a very valuable source of information on the mechanism of immunodepression in cancer.

The object of this investigation was to study the antigen-independent effect of ascites fluid from an Ehrlich's tumor on transplantation immunity and also to study antigen-specific blocking activity.

EXPERIMENTAL METHOD

Male CC57BR and C3HA mice, weighing 18-20 g, from the Rappolovo Nursery, Academy of Medical Sciences of the USSR, were used in all the experiments. Skin grafting was carried out by the method of Billingham and Medawar in Ogurtsov's modification [6]. C3HA mice were used as donors of the skin graft, except in a few cases when CC57BR mice were used. The recipients were CC57BR mice. The hair was removed from the skin of the back of all the mice 24 h before grafting. The operation was performed under hexobarbital anesthesia (0.3 ml of a 1% solution of hexobarbital per mouse). The graft for transplantation measured 0.8-1.0 cm^2. Skin from one donor was grafted onto two recipients. The subcutaneous cellular tissue was carefully removed from the graft. The subcutaneous cellular tissue of the recipient, on the other hand, was preserved as the graft was laid upon it. The edges of the graft were trimmed to the same size as the graft bed and compressed firmly against it by means of a cotton swab. BF-6 bactericidal medical glue was then applied around the edges of the graft and surrounding skin.

An Ehrlich's ascites tumor was transplanted into CC57BR mice by intraperitoneal injection of a dose of 0.2 ml (30-40 million tumor cells). After 6-8 days, when the volume of ascites fluid was 5-7 ml, it was centrifuged for 5 min at 3,000 rpm in the TsLR-1 centrifuge to remove cells. The resulting cell-free ascites fluid was centrifuged for a further 10 min at 3,000 rpm and used immediately thereafter for injection into the animals, on the grounds that keeping and freezing might influence its immunodepressive activity.

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TABLE 1. Effect of Ascites Fluid of Ehrlich's Tumor on Survival of Allogeneic Graft

Group and number of animals in it	Period of survival of allograft, days
Control 1 (n = 11): first grafting	8.5 ± 0.5
Experiment 1 (n = 5): first grafting (ascites fluid from tumor injected into recipient)	12.0 ± 0.4
Experiment 2 (n = 11): first grafting (ascites fluid injected into recipient from mice having previously rejected similar graft)	11.7 ± 0.4
Control 2 (n = 4): repetition of allografting	6.8 ± 0.2
Experiment 3 (n = 5): repeated allografting on mice after experiment 2	12.6 ± 0.5

The cell-free ascites fluid was injected into CC57BR mice in a dose of 1.5 ml per mouse daily, starting four days before skin grafting. The injections were continued until the day of rejection of the graft. These experiments were intended to show nonspecific immunodepressive activity of the ascites fluid of the Ehrlich's tumor in relation to transplantation immunity (experiment 1).

Meanwhile an attempt was made to demonstrate the antigen-dependent suppression of transplantation immunity under analogous conditions. For this purpose, to obtain ascites fluid, CC57BR mice were first grafted with skin from C3HA mice and, 7-10 days after rejection of the graft, an Ehrlich's tumor was transplanted into the recipients. This ascites fluid, because of the presence of the tumor, could contain modified antibodies against the allogeneic graft, i.e., blocking factor. Ascites fluid of mice which had previously rejected an allogeneic graft was also injected in a dose of 1.5 ml per mouse daily, starting four days before skin grafting (experiment 2). Skin grafting was repeated 20 days later on these same mice (experiment 3).

EXPERIMENTAL RESULTS

Intralinear skin grafting (CC57BR → CC57BR) was carried out as the positive control of graft survival. On the 4th-7th day the syngeneic grafts had united with the surrounding skin and the boundary between them was imperceptible. On average on the 13th-15th day hair began to grow on the graft, and by the 19th-21st day it was of the normal length. During subsequent observation for 4-5 months no signs of rejection of the graft were observe in any animal. A control of the normal allogeneic graft rejection reaction (C3HA → CC57BR) also was set up. In this case grafts were indistinguishable from syngeneic until the 5th-6th day, but then signs of rejection began to appear; the boundary between the graft and the surrounding skin became more sharply defined and small subcutaneous hemorrhages appeared. Complete rejection of the graft took place on the 7th-10th day after transplantation (Table 1).

On injection of freshly obtained ascites fluid from an Ehrlich's tumor into the mice the survival of allogeneic grafts was prolonged on average by 3-4 days to 12.0 ± 0.4 days (Table 1). The beginning of rejection also was observed 2-3 days later than in the control, and rejection took place less intensively. Considering that the ascites fluid was taken from CC57BR mice, i.e., mice of the same line as the recipients, the delay of rejection of the graft could be explained by nonspecific suppression of transplantation immunity on account of products of the tumor and substances secreted by the host in the presence of the tumor.

Injection of tumor ascites fluid obtained from animals which had rejected an allogeneic graft into mice also induced delay of rejection of the graft. No significant difference compared with the previous group was found (Table 1).

On repetition of the grafting of CC57BR mice which, however, received no other treatment, the second graft was rejected more rapidly (control 2). For instance, by the 3rd-4th day the first subcutaneous hemorrhages appeared, and on the 5th-6th day rapid death of the graft took place, followed by the formation of a dry scab (Table 1).

On regrafting skin from C3HA mice into mice treated with ascites fluid during the first graft, an interesting fact was discovered. Death of the graft was delayed. Rejection under

these circumstances was of the primary type, or it even took place more slowly than in the control mice after the first skin grafting. For instance, a more sharply defined boundary appeared between the graft and the surrounding skin on the 7th-8th day, and the first changes in the graft itself appeared about the same time. Complete rejection of the second graft occurred on the 11th-14th day.

Suppression of transplantation immunity by tumor ascites fluid is a known phenomenon. At the same time it has been shown [2] that injection of any antigen into an animal followed by induction of ascites leads to the accumulation of corresponding antibodies in the ascites fluid. In the present experiments, if antibodies did accumulate, this had no effect on the survival period of the allograft. It was also observed that injection of ascites fluid prevented the formation of an immunologic memory. This was shown by the fact that a second graft, transplanted 15 days after the first and 19 days after the beginning of injection of ascites fluid, was rejected more slowly than in the control. Suppression of transplantation immunity by tumor ascites fluid does not rule out the possibility of an immunodepressive effect of leukemia viruses present in these fluids [6].

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