Acta Radiologica Oncology 22 (1983) Fasc. 5

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ABILITY OF SPLEEN CELLS FROM TUMOR BEARING MICE TO TRANSFER IMMUNOLOGIC MEMORY

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Humoral and cell mediated immune reactions are generally depressed in a tumor bearing organism (ROWLAND et coll. 1971). However, lymphocytes from tumor bearing immunosuppressed organisms react to various antigens if transplanted to syngeneic recipients. The reaction is significantly stronger than the reaction of lymphoid cells transplanted from normal syngeneic donors (JURIN & PLAVŠIĆ 1978). Further, the immune reaction to third party antigen in the tumor bearing mouse is augmented in the initial phase, i.e. shortly after tumor cell transplantation, being suppressed in advanced stages of tumor disease (PLAVŠIĆ & JURIN 1980). Immunosuppressive factors, probably released by tumor cells, prevent lymphoid cells present in sufficient numbers in lymphoid organs from reacting immunologically (PLAVŠIĆ et coll. 1982). Previous investigations indicating that lymphocytes from tumorous organisms are able to react immunologically if removed from a tumor influenced environment (PLAVŠIĆ & JURIN) have been extended to an analysis of memory cells dynamics. Spleen cells from tumorous mice were collected after rejection of allogeneic skin which had been grafted at different stages of tumor disease, and injected into lethally irradiated syngeneic recipients. These secondary hosts were grafted with the same allogeneic skin graft as their donors and the ability of transplanted cells from tumorous donors to transfer memory to allograft has been tested.

Materials and Methods

C57BL, C3Hf/Bu and A strain mice 12 to 16 weeks old and weighing 20 to 30 g were used.

Tumors. The following tumors were used: 3-methylcholanthrene induced fibrosarcoma from C57BL mice, a spontaneous lymphoma from C3Hf/Bu mice and a Lewis lung carcinoma from C57BL mice. Tumors were transplanted by a single dose of 10^6 viable tumor cells in the right hind leg. The fourth isotransplantation generation of fibrosarcoma, the fourteenth of lymphoma transplant and an unidentified of Lewis lung carcinoma were kept in liquid nitrogen at −193°C. Mice injected with frozen cells thawed just before implantation were the source of cells for the suspensions. At particular times after tumor cell injection mice were grafted with allogeneic skin (Table). Normal controls were also grafted. Three to 5 days after graft rejection the mice were killed, their spleen cells collected and injected intravenously into lethally irradiated syngeneic recipients. Each mouse was injected with 2.5×10^7 splenocytes. Eight days later they were grafted with identical skin grafts as the donors of spleen cells. Comparing the skin graft survival times made it possible to evaluate the competence of spleen cells from tumor bearing mice to transfer immunologic memory.

Accepted for publication 4 March 1983.

Table

The ability of splenocytes to transfer immunologic memory

Tumor	Days of skin allografting after tumor transplantation	Days after tumor transplantation when splenocytes were transferred in irradiated recipients	Survival of skin graft on lethally irradiated recipients ($\bar{X}\pm SE$)	Significance of difference
Fibrosarcoma	6	26	16.8±0.4	p_1 NS
	11	31	18.0±0.7	p_2 NS
	Normal mice	–	18.9±0.4	$p_3<0.01$
Lymphoma	14	32	17.2±0.5	$p_1<0.05$
	25	48	19.0±0.7	p_2 NS
	Normal mice	–	18.6±0.4	$p_3<0.01$
Lewis lung carcinoma	5	28	17.8±0.4	p_1 NS
	10	32	18.1±0.3	$p_2<0.05$
	Normal mice	–	19.4±0.5	$p_3<0.05$

$\bar{X}$ = arithmetic mean. SE = standard error of arithmetic mean. p_1=difference between early and late stages of tumor disease. p_2=differences between late stages and normal mice. p_2=difference between early stages of disease and normal mice. Fifteen mice with small and an equal number with advanced tumors were used for each tumor type. Each control group consisted of 10 mice.

Tumor cell suspensions were prepared as described previously (JURIN & PLAVŠIĆ).

Spleen cell suspension. For suspension preparation one half of the spleen was taken randomly from donor mice killed by cervical dislocation. The organs were minced in cold Hank's solution using scissors. The mash was passed through the nylon patch into a beaker and placed on ice. Five minutes later the supernatant was discarded with a syringe and the pellet was passed in the test tube and washed 3 times on 150 g for 10 min each in Hank's solution. As when preparing tumor cell suspensions the number of viable cells was determined by the Trypan blue exclusion test.

Skin grafting technique. A piece of skin approximately 1 cm×1 cm was applied to a suitably prepared graft bed and secured with a bandage. The bandage was removed 7 days later and grafts were inspected daily. A graft was scored as rejected when there was no visible remaining epithelium. Tumorous mice were grafted with allogeneic skin in early and later stages of disease. The terms of grafting in later stages were determined knowing the prolongation of graft rejection and the mean survival of animals with tumors (JURIN & PLAVŠIĆ, PLAVŠIĆ et coll. 1980).

Irradiation of mice. The mice were irradiated in a plastic box using a therapy machine at 220 kV and 5 mA using a Cu filter of 0.5 mm and Al filter of 0.1 mm. The distance from the source of radiation was 43 cm and the dose rate 0.085 Gy/min.

Statistical significance of differences between results was determined by Student's t-test.

Results

In order to find out whether splenocytes from mice sensitized with an allogeneic skin graft react with a second set immunologic reaction in lethally irradiated hosts, the allogeneic skin graft survival was compared in lethally irradiated mice reconstituted with spleen cells from normal syngeneic donors and from syngeneic donors that had already rejected an identical graft. Spleen cells from mice sensitized to the skin graft rejected the second graft in a significantly shorter period of time ($p<0.01$). This was tested on C3Hf/Bu mice and the following figures were obtained: for normal 22.4±0.7 days (arithmetic mean ± standard error) and for sensitized 18.6±0.4 days. Each group included 10 mice.

Transfer of spleen cells from mice with fibrosarcoma. The results of the experiment are presented in the Table. Six or 11 days following tumor transplantation C57BL mice received skin grafts from A mice. After the allogeneic graft was rejected, 26 or 31 days after tumor transplantation, spleen cells

were transferred into lethally irradiated recipients. Subsequently A mice skin graft rejection in lethally irradiated syngeneic recipients reconstituted with spleen cells was followed. Graft rejection was not significantly different between the mice injected with spleen cells from donors bearing the tumor for 26 or 31 days (p_1 NS), neither was it significantly different between the mice injected with spleen cells from donors bearing the tumor for 31 days and mice injected with spleen cells from normal donors sensitized to the same allogeneic skin (p_2 NS). Mice injected with spleen cells from donors bearing fibrosarcoma for 26 days rejected the skin grafts sooner than mice injected with spleen cells from normal donors that had already rejected the identical skin graft ($p_3<0.01$).

Transfer of spleen cells from mice with lymphoma. Lethally irradiated C3Hf/Bu mice injected with spleen cells from syngeneic donors bearing the lymphoma for 32 days rejected the A strain skin graft sooner than mice injected with spleen cells from donors bearing the tumor for 48 days ($p_1<0.05$) and sooner than mice injected with spleen cells from normal donors that had already rejected the identical skin graft ($p_3<0.01$). The duration of skin graft rejection was however not significantly different between recipients of spleen cells from mice bearing the tumor for 48 days and recipients of spleen cells from normal donors (p_2 NS).

Transfer of spleen cells from mice with Lewis lung carcinoma. Lethally irradiated C57BL mice injected with spleen cells from donors bearing the tumor for 28 or 32 days rejected A strain skin graft in an approximately equal period of time (p_1 NS). Lethally irradiated mice injected with spleen cells from normal donors that had already rejected CBA skin graft, rejected the same graft later than mice injected with spleen cells from donors bearing the tumor for 28 days ($p_3<0.05$) or 32 days ($p_2<0.05$).

Discussion

The tumor bearing organism is able to react to third party antigen but the intensity of reaction depends on the size of the tumor. The bigger the tumor, the weaker is the immune reaction (ROWLAND et coll., JURIN & PLAVŠIĆ, PLAVŠIĆ & JURIN). However, the transfer of spleen or lymph node cells from mice with various syngeneic tumors to lethally irradiated syngeneic recipients resulted in a significantly higher response to sheep erythrocytes than when normal donor spleen cells had been injected, as estimated by plaque forming cell assay (JURIN & PLAVŠIĆ, PLAVŠIĆ & JURIN). Further, the results of the investigation indicate that memory to third party antigens is present in tumorous animals. It is even better expressed in the splenocytes of tumor bearing organisms than in control non-tumorous hosts. Experiments were carried out using splenocytes and not lymph node cells because spleens contain enough hematopoietic elements for a lethally irradiated mouse to survive.

It should be noted that the surviving time of an allogeneic skin graft on a lethally irradiated host was shorter if the spleen cells donor had a small tumor at the time of grafting than if the donor was normal. If the donor of spleen cells which had rejected the allograft had a large tumor at the time of skin grafting, allogeneic skin graft survival on the lethally irradiated host was the same as when spleen cells were transferred from non-tumorous mice which had rejected an allogeneic skin graft, except for the Lewis lung carcinoma.

In all experiments, lethally irradiated hosts were injected with the same number of spleen cells. However, it should be kept in mind that spleens of tumor bearing mice displayed marked hematopoiesis, this being more manifest in advanced tumors (HRŠAK & MILAS 1973, JURIN & DREWINKO 1974). The hematopoiesis was most marked in fibrosarcoma bearing mice, which fits in well with other experiments (PLAVŠIĆ 1979). This means that a particular number of spleen cells from tumor bearing mice contained relatively fewer lymphoid (memory) cells than the spleen cells from non-tumorous mice. This difference should be more marked in mice with more advanced stages of tumor disease. If this is true, which could be checked by an investigation on spleen cell suspension separation, it is possible that tumor bearing organisms contain more memory cells to third part antigen than non-tumorous controls. Lymphokines released in the serum by T lymphocytes reacting to tumor antigens might be responsible for augmentation of the immune reaction (DUMONDE 1970).

SUMMARY

The ability of splenocytes from tumorous mice to transfer immunologic memory was tested. Three syngeneic experimental tumors from highly inbred strains were used; fibrosarcoma, lymphoma and Lewis lung carcinoma. Splenocytes from tumorous mice were collected after

rejection of allogeneic skin which had been grafted at different stages of the tumor disease, and injected into lethally irradiated syngeneic recipients. These secondary hosts were grafted with the same allogeneic skin graft as their donors and the ability of cells transplanted from tumorous donors to transfer memory to allograft was tested. Tumorous mice seemed to have more memory cells (T lymphocytes) in their spleens than the controls.

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