ROLE OF SUPPRESSOR CELLS IN NEONATALLY INDUCED TOLERANCE TO TRANSPLANTABLE HEPATOMA 22a

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Increasing attention is nowadays being paid to the study of the regulatory role of suppressor cells, for they have been shown to exert their effect under pathological as well as normal conditions. Several suggestions have been put forward on the effect of suppressor cells on the formation of immunologic tolerance [4-6]. However, data in the literature on activation of suppressor cells in tolerance are contradictory, probably because the suppressive effect was tested at different times.

Accordingly in the present investigation an attempt was made to detect activation of suppressor cells at different times after injection of the antigen in the early postnatal period.

EXPERIMENTAL METHOD

Experiments were carried out on newborn C3HA mice which received three intraperitoneal injections of 0.75 mg of a saline extract of ascites hepatoma 22a on the 1st, 3rd, and 5th days after birth. Previous investigations showed stimulation of growth of a hepatoma inoculated in the adult state in such mice, and a marked fall in the level of antitumor reactions of immunity in response to immunization with extract of hepatoma cells. This was one factor confirming the presence of a state of tolerance. In control experiments an extract of liver injected into newborn mice in the same doses as the tumor cell extract did not induce tolerance to the tumor [2]. In adult mice at different times after injection of the antigen (2, 4, and 7 weeks) the spleen was removed and a cell suspension prepared, from which a T-enriched population of lymphocytes was subsequently isolated with the aid of EAC-rosette formation followed by centrifugation in a Ficoll-Verografin gradient [1]. Suppressor activity of the lymphocytes thus obtained was determined by the adoptive transfer method. For this purpose $4 \cdot 10^7$ T lymphocytes were injected intraperitoneally into adult mice which, 10 days before transfer of the living lymphoid cells, were inoculated with hepatoma in a dose of $2 \cdot 10^4$ cells. The manifestation of suppressor activity was judged by the stimulation of tumor growth and the times of death of mice with the transplanted hepatoma. Experiments were carried out on 60 mice.

EXPERIMENTAL RESULTS

The experiments showed that tumor growth in mice after transfer of T lymphocytes from animals with neonatally induced tolerance depends on the time after injection of the antigen when the lymphoid cells were removed. As Table 1 shows, transfer of T lymphocytes obtained 2 weeks after injection of antigen in the neonatal period was accompanied by significant stimulation of growth of the hepatoma. Mice in the experimental group died on average after 64.5 days, whereas the life span of the control animals was longer, namely 83.5 days. These observations suggest that stimulation of tumor growth was due to the presence of a pool of suppressor cells, depressing the protective reactions of the recipients, in the T lymphocyte population used for adoptive transfer. However, the results of experiments involving transfer of normal T cells, obtained from the spleens of 3-week-old mice, do not confirm such a conclusion. In that series of experiments, as Table 1 shows, growth of the tumor also was stimulated, possibly on account of predominance of T suppressors in sexually immature mice

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TABLE 1. Effect of Adoptive Transfer of T Lymphocytes from Mice with Induced Tolerance on Lifespan (in days) of Animals with Tumors

Type of cells transferred	Time after injection of antigen, weeks		
	2	4	7
T lymphocytes of mice with induced tolerance	64,5±5,5 $n=8$	61,0±5,0 $n=10$	100,8±9,9 $n=9$
P	<0,01	<0,002	>0,05
T lymphocytes of normal mice	63,4±5,2 $n=7$	80,0±10,0 $n=9$	91,2±13,3 $n=7$
P	<0,01	>0,05	>0,05
Control (without transfer of lymphocytes)	83,5±4,2 $n=10$		

Note: n) Number of mice.

compared with adults [7]. Stimulation of growth of the transplantable hepatoma after transfer of T lymphocytes from mice with induced tolerance 2 weeks after injection of the antigen may thus be due both to activation of suppressors, during tolerance formation, and by the larger pool of natural suppressor cells in the first 3 days of life of mice.

Significant stimulation of tumor growth (Table 1) was found also after transfer of T cells isolated from animals with induced tolerance 4 weeks after injection of antigen. Since transfer of normal T lymphocytes did not affect growth of the tumor, this suggests that the more rapid growth of the hepatoma in this group of experiments was due to activation of suppressor cells in the period of induction of tolerance. Transfer of lymphoid cells obtained 7 weeks after injection of the antigen, as Table 1 shows, in fact had no effect on the rate of tumor growth.

The suppressor activity of T lymphocytes during formation of neonatally induced tolerance is thus manifested only at certain times after injection of the antigen. It is at these same times that, as we showed previously in a study of cell-mediated immunity, that intensified migration of macrophages is observed in mice with induced tolerance [3]. Furthermore, suppressor cells were found to affect the manifestation of reactions of cellular immunity in animals with neonatally induced tolerance to the tumor and inoculated with hepatoma in the adult state [1]. It may be that T suppressors influence the effector function of T lymphocytes and prevent the production of certain mediators of cellular immunity.

The results are important in connection with the study of the intimate mechanisms lying at the basis of induction of immunologic tolerance in the early postnatal period.

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