STUDIDES ON INHIBITORY EFFECT OF HOMOLOGOUS SPLEEN CELLS ON EHRLICH ASCITES TUMOR AND YOSHIDA SARCOMA

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同種免疫脾細胞の抗腫瘍性に関する研究

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（昭和41年6月22日受付）

さきに Stuart (1963) の研究 (Landschutz ascites tumor of mice) を Ehrlich 腹水癌に就いて追試し，免疫家兎の脾細胞の抗腫瘍性はＥＡＣに対しても殆んど同程度に認められることを実験的に証明した．此の heterologous の脾細胞の免疫機構を更に検討する目的を以て，著者は homologous の脾細胞を用いＥＡＣ並に吉田肉腫に対する腫瘍発育阻止効果を比較研究した結果，両者脾細胞の阻止機構には殆んど本質的の相違を認めなかつた．唯非感受性動物と感受性動物の lymphoid cells の immunological competence には当然或程度の相違を認めざるを得なかつた．Stuart の意見と稍々異なる点は彼が胸腺細胞には阻止効果を認めず従つて抗体の形成能は細網淋巴系に平等に分布しないようだと述べたのに対し著者の実験では胸腺細胞にも阻止力が認められており従つて Stuart の見解には直ちに賛意を表し得ない．

Synopsis

In the previous paper, I have reported ona beneficial inhibitory effect of heterologous spleen cells of immunized rabbits on growth of Ehrlich ascites tumor (EAC) and discussed some aspects of the fundamental mechanism by which highly susceptible mice of DDS strain become immune against i.p. implantation of by far a larger dose of intact tumor cells. We have performed similar experiments with homologous spleen cells of mice and rats experimentally made immue against respective tumors, and recognized a similar beneficial inhibitory activity of homologous immunized spleen cells. It seemed reasonable to consider that the fundamental mechanism by which homologous immunized cells can exert their anti-tumor activity may be essentially identical with that of heterologous cells. Here I will report on experimental evidences, by which such views may be supported.

Introduction

In the previous paper, I have reported on a beneficial inhibitory effect of spleen cells of immunized rabbits on tumor growth in the recipient. The experimental results of our studies on Ehrlich ascites tumor (EAC) were almost the same as those of Stuart's work on Landschutz ascites tumor of mice [14]. Thus about 60% of mice treated with a single i.p. injection of 5×10^8 immunized spleen cells proved capable of resisting against on i.p. implantation of 10^6 intact tumor cells. They survived for more than 80 days

without clinical manifestations of EAC. The postmortim histological examination did not reveal any findings characteristic of ascites tumor.

As previously reported, mice of DDS strain can be made highly resistant against EAC or Sarcoma 180 by several means including 1) Vaccination with intact or inactivated tumor cells, 2) Vaccination with modified cell-free ascites fluid of mice suffering from ascites tumors, and 3) Therapeutic treatment of diseased mice with an anti-tumor agent named Soedomycin.

From such mice the spleens were removed to prepare spleen cell suspensions according to the Staurt's method and tested on their inhibitory effect on tumor growth in the recipient. In this test, a similar inhibitory effect was observed on tumor growth of both EAC and Sarcoma 180. Wigzell[10] did not observe any beneficial therapeutic effect on mammary tumor of mice with homologous spleen cells (1961). This striking difference between both results cannot be explained properly at the present time, however, the most probable explanation at moment seems that the difference may be due to the different properties inherent in respective tumor cells.

Stuart has suggested in his study ona cytotoxic effect of heterologous spleen cells that this beneficial action of immunized cells may be due to an antibody carried by them on their surface or interior. According to this view, immunized spleen cells adhere to tumor cells as the first step of tumor cell damage, and eventual cytolysis may occur after in vivo fixation of complement. The most obscure aspect in the mechanism of anti-tumor immunity may be the properties of coresponding antigen responsible for formation of such cellular antibody, which exerts beneficial inhibitory action against tumor growth in animals.

This experimental study was done as a step in the course of elucidation of the fundamental mechanism of anti-tumor immunity by which highly susceptible animal species can become immune against i.p. implantation of a cell dose absolutely fatal for usual animals.

Materials and Methods

Animals: Mice of DDS strain weighing about 20 gm and rats weighing about 100 gm were usesd for experiments.

Tumor cells: Ehrlich ascites tumor cells and Yoshida sarcoma cells were adopted as target cells. They have been maintained by serial transplantation from one group of mice or rats to another at appropriate intervals for more than 5 years. A dose of 10^4 to 10^7 intact tumor cells were used as a challenge dose for animals. For this purpose, ascites fluid was collected from animals with ascites tumor and after the cell count was done, it was diluted with saline solution to obtain a dilution of ascites containing 10^8 tumor cells per ml. When a challenge dose of 10^6 tumor cells was desired to use, this dilution was ten times diluted with saline solution and 0.1 ml was i.p. injected into mice.

Immunization of animals: Mice were given i.p. 10^4 tumor cells and immediately thereafter they were treated with modified cell-free ascites fluid of EAC mice (EAD, Soeda) for at least 3 days, and the spleens of mice rescued from ascites tumor were removed for preparation of spleen cell suspensions. The spleens of mice, which had developed ascites tumor or solid tumior, were also used for the same purpose. Other groups of mice were i.p. inoculated with 10^6 EAC cells and treated with combined injection of Soedomycin, an anti-tumor agent, and Marimycin, an anti-leukopenic agent. By such therapy we obtained total 13 mice which were completely rescued from ascites tumor deaths, the spleens of which were also employed to prepare spleen cell suspensions. For test with Yoshida sarcoma, rats were i.p. inoculated

with 10^6 YS cells and immediately thereafter they were treated with simultaneous injection of both Soedomycin and Marimycin. Some of rescued rats were sacrificed without further treatment and the spleens were used for experiments. The other rats were again challenged with 10^6 tumor cells, the survived rats were once more challenged with 10^7 tumor cells. By this means the mice, whichs well tolerated 3 successive challenges, were picked up to obtain spleen cell suspensions.

In some experiments, thymocytes of rats were also tested on their inhibitory effect on growth of YS in rats. Thymocyte suspensions were prepared in the same fashion.

Preparation of spleen cell suspensions: Animals were killed and the spleens were removed. All cell suspensions were prepared by grinding the spleens (or thymus glands) in a glass homogenizer with saline solution containing Streptomycin (1 mg/ml), Penicillin (1,000 units/ml) and a minute amount of heparin. They were transferred to test tubes and centrifuged at 3,000 r.p.m. for 15 minutes and after the supernatant was discarded the same saline was added to sedimented cells to obtain a proper dilution of cell suspension.

Usually 5×10^8 spleen cells were selected as a standard dose for a single injection. In some experiments, thymocyte suspensions were prepared in like manner and used for tests on their effect on tumor growth.

Experimental results

Exp. 1 Inhibitory effect of spleen cells of mice rescued by treatment with EAD

As previously reported, Soeda and Sumiyama have found a specific ultraminute agent responsible for transplantability of EAC cells in cell-free supernatant of ascites of EAC mice. Subsequently we have obtained a concentrated preparation of this agent by chemical treatment of cell-free ascites and named EAD, which means Ehrlich agent derived from ascites fluid.

It is of much interest that EAD proved capable of inducing an acquired type of immunity against EAC

Table 1. The effect of spleen cells of EAC mice cured by administration of M3 and M-2.

Group of mice	No. of mice	Dose of spleen cells	Challenge dose of EACC	Typical death(days)	2nd. challenge dose	Typical death (days)	Survival rate
Test grousp 28.7~30.0 g	1	5×10^8	10^4	39			
	2	〃	〃	29			2/ 4
	3	〃	〃	n.c.	10^5	22	
	4	〃	〃	n.c.	10^5	16	
Control group (1) 20.1~21.0 g	5		10^4	22			
	6		〃	22			
	7		〃	18			0/ 5
	8		〃	18			
	9		〃	53			
Control group (2) 15.8~22.2 g	11				10^5	17	
	12				〃	16	
	13				〃	29	0/ 5
	14				〃	33	
	15				〃	33	

in mice. Thus mice were given i.p. a single injection of 0.5 ml of EAD and 6 days later they were i.p. implanted with 10^5 intact EAC cells. By this treatment, 7 of 12 mice could escape from ascites tumor deaths and survive with no clinical signs of ascites tumor for more than 80 days. They were killed and the spleens were removed for preparation of spleen cell suspensions.

Total 4 test mice were given i.p. 5×10^8 spleen cells and 2 days later they were challenged with 10^4 intact tumor cells. Two of 4 mice did not develop ascites tumor and survived up to the 54th day without clinical appearance of ascites, when they were again attacked with 10^5 EAC cells, which killed both mice within 16 to 22 days. Total 5 control mice were inoculated with 10^4 tumor cells and nothing else. They all developed ascites tumor and died during the course of 18 to 53 days (Table 1).

Exp. 2 Inhibitory effect of spleen cells of mice with ascites or solid type of EAC

Total 18 mice with EAC were selected for preparation of spleen cell suspensions. These mice were divided into 2 groups. The first group included 7 mice which han developed subcutaneous solid tumors by subcutaneous implantations of 10^5 EAC cells. The second group included 11 mice, which were

Table 2. The effect of spleen cells of mice with typical EAC or Ehrlich solid tumors.

Group of mice	No. of mice	Dose of spleen cells	Challenge dose of EACC	Typical death days	2nd. challenge dose	Typical death (days)	survival rate
Test group 25.0～29.2 g	1	5 X10^8	10^4	n.c.	10^5	38	
	2	〃	〃	n.c.	〃	17	
	3	〃	〃	n.c.	〃	30	
	4	〃	〃	n.c.	〃	28	
	5	〃	〃	24			
	6	〃	〃	n.c.	〃	n.c.	8/10
	7	〃	〃	n.c.	〃	n.c.	
	8	〃	〃	n.c.	〃	n.c.	
	9	〃	〃	35			
	10	〃	〃	n.c.	〃	13	
Control group (1) 20.2～28.1 g	11		10^4	20			
	12		〃	20			
	13		〃	24			0/ 5
	14		〃	25			
	15		〃	23			
Control group (2) 17.7～24.0 g	16				10^5	33	
	17				〃	33	
	18				〃	17	0/ 5
	19				〃	16	
	20				〃	19	

selected among mice with ascites tumor and survived for a longer time than usual.

Total 16 test mice were treated with a single i.p. injection of 5×10^8 spleen cells and 24 hours later they were challenged with 10^4 tumor cells. Twelve of 16 mice did not develop ascites tumor. They were again attacked with 10^5 tumor cells 25 or 54 days after the first challenge and 4 mice could escape from ascites tumor deaths. In contrast to this, total 20 control mice developed ascites tumor and died within 36 days without exception (Table 2 and Table 4).

Exp. 3 Inhibitory effect of spleen cells of mice rescued from ascites tumor deaths by treatment with M-2 and M 3

In 1957, an anti-tumor agent named Marinamycin (M 2) was isolated from agitation cultures of Streptomyces mariensis (Soeda, 1957). Another antibiotic substance with an anti-leukemic activity was also obtained from cultures of the same strain and named Marimycin (M-2, Soeda, 1962). In 1965, an antitumor agent was isolated from culture broth of Streptomyces hachijoensis and named Soedomycin (M 3 Soeda, 1965).

Combined use of both Marimycin and Soedomycin proved highly active against animal tumors such as EAC or Sarcomz 180 of mice and Yoshida sarcoma of rats.

Mice were i.p. inoculated with 10^6 EAC cells and immediately thereafter they were treated with simultaneous i.p. injection of M-2 and M 3, and total 13 mice were rescued from ascites tumor deaths. From these mice the spleens were removed to prepare spleen cell suspensions.

Mice were inoculated i.p. with 5×10^8 spleen cells and 24 hours later they were implanted with 10^4 EAC cells. Three of 10 such mice remained alive without developing ascites cancer up to the 54th day, when they were again challenged with 10^5 tumor cells. They could all tolerate this test and on the 85th day they were once more attacked with 10^6 tumor cells. Two mice developed ascites tumor and died, but one mouse survived without clinical appearance of ascites and showed no characteristic findings when postmortem examination was done. In control group, 5 mice could not tolerate i.p. implantation of 10^4 tumor cells and died as a result of ascites tumor within 36 days (Table 3).

Table 3. The effect of spleen cells of cured mice with Ehrlich ascites tumors by administration of M 3 and M-2.

Group of mice	No. of mice	Dose of spleen cells	Challenge dose of EACC	Typical death (days)	2nd chal. after 54 days	Typ. death (death)	3 rdcha. after 31 days	Survival rate
Test group 25.0~30.0 g	1	5×10^8	10^4	n.c.	10^5	n.c.	10^6 n.c.	
	2	〃	〃	32				
	3	〃	〃	n.c.	10^5	n.c.	10^6 dead	
	4	〃	〃	n.c.	10^5	n.c.	10^6 dead	
	5	〃	〃	28				
	6	〃	〃	28				3/10
	7	〃	〃	27				
	8	〃	〃	30				
	9	〃	〃	30				
	10	〃	〃	26				
Control group (1) 27.9~30.7 g	11		10^4	23				
	12		〃	27				
	13		〃	27				0/ 5
	14		〃	29				
	15		〃	36				
Control group (2) 17.7~24.0 g	16				10^5	17		
	17				〃	16		
	18				〃	29		0/ 5
	19				〃	33		
	20				〃	33		

Exp. 4 Inhibitory effect of spleen cells or thymocytes of rats rescued from Yoshida sarcoma by treatment with M-2 and M 3

Rats were i.p. inoculated with 10^6 YS cells and 24 hours later they were treated for 3 days with simultaneous i.p. injection of M-2 and M 3. The rescued rats were divided into 2 groups. Three rats in the first group were killed without further treatment and the spleens and thymus glands were used to prepare cell suspensions for Test a.

Rats of the second group were again inoculated 26 days later with 10^6 tumor cells, the survived rats of which were once more inoculated with 10^7 YS cells 14 days thereafter. Two rats were selected from survived rats for preparation of cell suspensions for Test b.

Test a. Rats were given i.p. 5×10^8 spleen cells or thymocytes and 5 days later they were inoculated i.p. with 10^6 YS cells. In this test, 1 of 7 rats treated with spleen cells and 1 of 2 rats treated with thymocytes developed the typical disease and died within 14 days, but the remaining 7 rats survived without clinical signs and they well tolerated the second implantation of 10^6 tumor cells on the 33rd day. Eleven days later they were once more challenged with 10^7- YS cells. Only one rat developed ascites tumor and died 33 days thereafter, but the remaining 6 rats survived without clinical signs of the disease up to the 65th day, when they were sacrificed and postmortem examination was done. No findings characteristic of YS were observed. (Table 7).

Test b. The same experiment was done with spleen cells or thymocytes of rats of the second group. In this case, 1 of 4 rats treated with spleen cells and 1 of 2 rats treated with thymocytes developed ascites tumor and died within 14 days, but the remaining 4 rats proved insusceptible to implantation of 10^6 YS cells and further they all tolerated the second implantation of 10^6 YS cells. On the 44th day the third challenge with 10^7 YS cells, was done which killed one rat 33 days thereafter. Total 6 control rats

Table 4. The effect of spleen cells of mice with typical solid tumors which did not respond to therapy with M 3 and M-2.

Group of mice	No. of mice	Dose of spleen cells	Challenge dose of EACC	Typical death (days)	2nd. chal. after 54 days	Typical death (days)	3rd chal. after 31 days	survival rate
Test group	1	5×10^8	10^4	n.c.	10^5	27		
	2	〃	〃	34				
	3	〃	〃	n.c.	〃	27		
	4	〃	〃	n.c.	〃	21		4/ 6
	5	〃	〃	35				
	6	〃	〃	n.c.	〃	n.c.	10^6 dead	
Control group (1)	7		10^4	23				
	8		〃	27				
	9		〃	27				
	10		〃	29				0/ 5
	11		〃	36				
Control group (2)	12				10^5	17		
	13				〃	16		
	14				〃	29		0/ 5
	15				〃	33		
	16				〃	33		

Table 5. The effect of spleen and thymus cells of rats tolerated the second challenge with 10^7 YSC.

Group of rats	No. of rats	Dose of spleen cells	Challenge dose of YSC	Typical death (days)	2 nd. chal. after 32 days	Typ. death (days)	3rd.chal. after 11 days	Typ. death (days)	Survival rate
Test group 110— 160 g	1	5×10^8 (spleen*)	10^6	n.c.	10^6	n.c.	10^7	33	
	2	〃	〃	n.c.	〃	n.c.	〃	n.c.	3/ 4
	3	〃	〃	11					
	4	〃	〃	n.c.	〃	n.c.	〃	n.c.	
	5	5×10^8 (thymus*)	〃	14					1/ 2
	6	〃	〃	n.c.	10^6	n.c.	10^7	n.c.	
Control group (1) 100— 120	7		10^6	14					
	8		〃	10					0/ 3
	9		〃	12					
Control group (2)	10				10^6	11			
	11				〃	9			0/ 3
	12				〃	12			
Control group (3)	13						10^7	12	
	14						〃	12	0/ 3
	15						〃	12	

* The spleen and the thymus were taken fromNo. 32-1 rat which was made tolerable to inoculation of 10^7 YSC by combined use of M 3 and M-2.

inoculated with 10^6 tumor cells and 3-other control mice inoculated with 10^7 tumor cells developed ascites tumor and died within 14 days (Table 5).

Exp. 5. Inhibitory effect of spleen cells or thymocytes of rats which proved insusceptible to 3 successive challenges in Exp. 4.

One rat (No. 76) was selected from Test a. of Exp. 4. This rat had tolerated 3 successive challenges with respective 10^6, 10^6 and 10^7 tumor cells. It was killed 65 days after the first challenge and cell suspensions were made. Total 3 rats were treated with a single i.p. injection of 5×10^8 cells and 4 days later they received 10^6 YS cells. All rats well tolerated this test and 2 rats proved to be insusceptible to im-

Table 6. The effect of spleen and thymus cells of a rat (No. 76-2) which tolerated the second inoculation of 10^7 YSC in the former test (Table 5.)on YS.

Group of rats	No. of rats	Dose of sp. or thy. cells	Challenge dose of YSC	Typical death (days)	2nd. chal. after 20 days	Typical death (days)	Survival rate
Test group	1	5×10^8 sp.	10^5	n.c.	10^7	8	
	2	4.3×10^8 sp.	〃	n.c.	〃	n.c.	3/ 3
	3	5×10^8 thy.	〃	n.c.	〃	n.c.	
Control group (1)	4		10^5	10			
	5		〃	11			0/ 3
	6		〃	8			
Control group (2)	7				10^7	9	
	8				〃	10	0/ 4
	9				〃	7	
	10				〃	8	

Table 7. The effect of spleen and thymus cells of cured rats by administration of M3 and M-2 on YS.

Group of rats	No. of rats	Dose of sp. or thy. cells	Chal. dose of YSC	Typical death (days)	2nd. chal. after 32 days	Typ. death (days)	3rd.chal. after 11 days	Typ. death (days)	Survival rate
Test group	1	5×10^8 (sp)	10^6	n.c.	10^6	n.c.	10^7	n.c.	
	2	〃	〃	n.c.	〃	n.c.	〃	n.c.	
	3	〃	〃	n.c.	〃	n.c.	〃	n.c.	
	4	〃	〃	n.c.	〃	n.c.	〃	33	
	5	〃	〃	n.c.	〃	n.c.	〃	n.c.	6/ 7
	6	〃	〃	11					
	7	〃	〃	n.c.	〃	n.c.	〃	n.c.	
	8	5×10^8 (thy)	〃	14					
	9	〃	〃	n.c.	〃	n.c.	〃	n.c.	1/ 2
Control group (1)	10		10^6	14					
	11		〃	10					0/ 3
	12		〃	12					
Control group (2)	13				10^6	11			
	14				〃	9			0/ 3
	15				〃	12			
Control group (3)	16						10^7	12	
	17						〃	12	0/ 3
	18						〃	12	

plantation of 10^7 tumor cells done after 20 days (Table 6).

Three rats (No. 32, 38 and 41) were selected from Test b. of Exp. 4. They had tolerated 3 successive challenges with respective 10^6, 10^6 and 10^7 tumor cells. Total 7 rats were treated with a single i.p. injection of 5×10^8 cells and 5 days later they were challenged with 10^6 YS cells. Five rats did not develop ascites tumor and further they proved immune against 10^7 tumor cells. Among total 8 control rats we found a rat naturally resistant against i.p. implantation of 10^6 YS cells. This rat also tolerated the 2nd challenge with 10^7 tumor cells done after 15 days. The remaining 7 control rats developed ascites tumor and died within 14 days (Table 8).

Exp. 6. Inhibitory effect of immunized spleen cells of rats, when applied 7 days before i.p. implantation of tumor cells

In all foregoing experiments, immunized spleen cells were applied 1 to 5 days before tumor cell implantations. In this experiment, the cells were give 7 days before i.p. inoculation of 10^6 tumor cells. As shown in Table 9, rats could tolerated this challenge test, but the second attack with 10^7 tumor cells done 21 days later killed all of them as a result of ascites tumor within 8 to 22 days.

It seems likely that although enough homologous cells can survive and exert their inhibitory effect for at least 7 days in the recipient, immunological response of host animals following the first implantation of tumor cells is somewhat lower than that of groups of rats to which spleen cells were applied less than 5 days before challenge. The exact reason is not clear at the present time.

Discussion

Homologous spleen cells of mice immunized by various means were also shown capable of exerting

Table 8. The effect of spleen and thymus cells of rats which tolerated 3 successive challenges with 10^6, 10^6 and 10^7 YSC.

Group of rats	No. of rats	Dose of sp. or thy. cells	Challenge dose of YSC	Typical death (days)	2 nd. chal. after 16 days	Typical death (days)	Survival rate
Test	1	5×10^8 (sp. 38-1)	10^6	n.c.	10^7	n.c.	
	2	〃 (sp. 38-1)	〃	n.c.	〃	n.c.	
	3	〃 (sp. 38-3)	〃	n.c.	〃	n.c.	3/ 5
	4	〃 (sp. 41-2)	〃	14			
	5	3.2×10 (sp. 32-1)	〃	n.c.	〃	n.c.	
	6	10×10^8 (thymus)	〃	n.c.	〃	n.c.	
	7	5×10^8 (thymus)	〃	14			1/ 2
Control group (1)	8		〃	n.c.	〃		
	9		〃	14			
	10		〃	11			1/ 4
	11		〃	8			
Control group (2)	12				〃	9	
	13				〃	10	0/ 4
	14				〃	7	
	15				〃	8	

Table 9. The effect of mixed cells obtained from two rats, one tolerated inoculation of 10^7YS-virus (Soeda) and challenge with 10^7 YSC, and the other became resistant to 10^7 YSC-attack.

Group of rats	No. of rats	Dose of sp. or thy. cells	Challenge dose of YSC	Typical death (days)	2nd. chal. after 21 days	Typical death (days)	Survival rate
Test group	1	5×10^8 (sp)	10^6	n.c.	10^7	22	
	2	4.15×10^8 (sp)	〃	n.c.	〃	10	
	3	5×10^8 (sp)	〃	n.c.	〃	8	
	4	〃 (thy)	〃	n.c.	〃	9	6/ 6
	5	〃 (thy)	〃	n.c.	〃	10	
	6	〃 (thy)	〃	n.c.	〃	8	
Coptrol group (1)	7		10^6	22			
	8		〃	12			0/ 3
	9		〃	7			
Control group (2)	10				10^7	7	
	11				〃	7	0/ 3
	12				〃	7	

abeneficial inhibitory effect on tumor growth in the recipient. As prevoiusly mentioned, a single i.p. implantation of 10^8 EAC cells can be regarded as absolutely fatal for usual mice of DDS strain, except for naturally resistant mice very rarely found among so many mice of this strain. With a single i.p. injection of 5×10^8 immunized spleen cells of mice, 17 of 30 test mice proved to be resistant against i.p. implantation of 10^4 tumor cells (56.7%) and 7 mice of which were further shown immune against 10^5 tumor cells given 25 to 54 days thereafter, although the majority of them could not tolerate tumor cell implantation at the lewel of 10^6.

Thus with a relatively small dose such as 10^4 or 10^5 tumor cells it was clearly shown that spleen cells of mice made immune against EAC can acquire abeneficial inhibitory activity against tumor growth in

mice in almost the same manner as heterologous cells of immunized rabbits.

As reported in the previous paper, a single i.p. injection of 5×10^8 spleen cells of immunized rabbits completely rescued 17 of 30 mice from ascites tumor deaths following i.p. implantation of 10^6 EAC cells. In contrast to this, the grad of tumor inhibitory activity of immunized spleen cells of mice appears consirably lower than that of heterologous cells of immunized rabbits. Concerning their beneficial inhibitory activity, however, it seems likely that such action may be also due to an anti-tumor antibody carried by spleen cells of immunized mice. As I have discussed in the previous paper, spleen cells of insusceptible animal species may inherit by far a higher immunological competence to respond to stimulation of foreign tumor antigen, so that they may carry enough antibody for destruction of foreign tumor cells.

Wigzell did not observe any beneficial therapeutic effect on mammary tumor of mice with homologous spleen cells[10]. The difference between both results may be either due to different conditions of experiment or to variable properties inherent in respective tumor species.

As for rat tumor, homologous spleen cells of immunized rats against Yoshida sarcoma also proved capable of exerting a beneficial inhibitory effect on tumor growth in the recipient. With a single i.p. injection of 5×10^8 spleen cells of immunized rats, 21 of 25 test rats became immune against tumor cell implantation at the lewel of 10^6(complete survival rate was 80%) and further 11 of 19 such rats proved to be complately immune against tumor cell implantation of 10^7 lewel. With thymocytes of the same immunized rats, an almost similar beneficial effect was also observed, and 4 of 7 test rats inoculated with 10^8 YS cells were protected against development of ascites tumor and further they were all insusceptible to 10^7 tumor cells given i.p. as the 2nd challenge dose.

Stuart (1963)[14] did not observe any striking cytotoxic effect on Landschutz ascites tumor with heterologous thymocytes of immunized rabbits and assumed that this may be due to their failure to form antibody and that the property to form auch specific antibody may not be uniformly distributed among lymphoreticular cells. However, as far as our studies on Yoshida sarcoma were concerned, we must recognize a similar beneficial inhibitory effect of homologous thymocytes of immunized rats on Yoshida sarcoma. Whether or not thymocytes do fail to form antibody and whether or not the property of tumor cell inhibition is distributed uniformly among lymphoreticular cells may be matters of debate, however, at least we cannot neglect a possibility that thymocytes may also form antibody and exert a similar cytotoxic activity against target tumor cells, if they are intact and immunized.

At present I believe that thymocytes may also contribute to some extent to inhibition of tumor growth if they are intact and immunized, and that they may also form a specific antibody against implanted tumor cells.

The inhibitory action of immunized spleen cells of rabbits may probably be due to an anti-tumor antibody produced and carried by them. When such cells are injected into mice they can survive for a certain time within mice and exert the same inhibitory effect on implanted tumor cells. In the course of this passive cellular immunity, mice are actively immunized with some liberated tumor cell antigen as a result of cell damage by such passive antibody and acquire a certain degree of active immunity against tumor cells. Such actively acquired immunity must play a mainle role in protection of mice against subsequent implantation of tumor cells as the 2nd or 3rd challenge test. This view was supported by our studies on inhibitory effects of homologous spleen cells on tumor growth, because mice or rats could acquire a similar immunity against EAC or YS without help of passive cellular antibody carried by

immunized heterologous spleen cells.

From these experimental evidences, it seems reasonable to consider that the fundamental mechanism by which homologous spleen cells exert a inhibitory action against tumor growth might be essentially identical with that of heterologous immunized spleen cells. Quantitatively speaking, however, there seems to exist a considerable difference in anti-tumor activity between both types of cells, even if they are simil arly immunized against the same kind of tumor cells.

The most important problem which has not been fully pursued would be the properties of tumor cell antigen, toward which immunological response of lymphoid cells being directed. Now we are in study on immunization of rabbits with CAE (Soeda, 1959) in an attempt to contribute to elucidation of the properties of target agent responsible for formation of such cellular antibody, which will be reported on in near future.

Summary and Conclusion

1) Homologous spleen cells of immunized mice of DDS strain were tested on their inhibitory effect on growth of Ehrlich ascites tumor in mice of the same strain. In this test, 17 of 30 test mice pretreated with a single injection of 5×10^8 spleen cells were protected against ascites tumor when they were i.p. inoculated 24 hours later with 10^4 tumor cells, a dose absolutely fatal for untreated normal mice. The complete survival rate at the level of 10^4 EAC cells was 56.7%. Moreover, when they were again challenged with 10^5 EAC cells 25 to 54 days thereafter, 7 mice proved to be completely immune against ascites tumor. They were once more inoculated with 10^6 EAC cells, which killed 6 mice as a result of ascites tumor, but one mouse was still immune against this level of tumor cells.

Spleen cells of mice with ascites or solid type of Ehrlich tumor were also capable of exerting a beneficial inhibitory action against tumor growth in mice. This fact will indicate that immunological competence to respond to EAC cell antigen is also retained in spleen cells of mice with EAC in the same manner as in spleen cells of rabbits, and that i.p. implantation of tumor cells into susceptible animal can cause formation of immunized lymphoid cells, although their capacity to form antibody may be by far lower than that of insusceptible animals like rabbits.

2) Homologous immunized spleen cells of rats were tested on their inhibitory effect on growth of Yoshida sarcoma in rats. In this test, 21 of 25 rats pretreated with a single injection of 5×10^8 immunized spleen cells of rats did not develop ascites tumor when they were i.p. inoculated with 10^6 YS cells. The complete survival rate of test rats at the level of 10^6 YS cells was more than 80%. Moreover, about a half of such survived rats were further shown resistant against tumor cells given i.p. in a dose of 10^7.

3) Homologous immunized thymocytes also proved capable of exerting a beneficial inhibitory effect on growth of Yoshida sarcoma in rats. In this case, 4 of 7 rats pretreated with immunized thymocytes did not develop ascites tumor after i.p. implantation of 10^6 YS cells and they were further proved completely immune against tumor cells given i.p. in a dose of 10^7. Although the number of test rats was limited because of difficulty to obtain enough thymocyte suspension, it seems likely that immunized thymocytes can contribute to a certain extent to inhibition of tumor growth and that they may also form cellular antibody against implanted tumor cells.

Stuart's suggestion, that the beneficial inhibitory action of heterologous spleen cells of immunized rabbits may be due to antibody carried by them on the surface or interior, may be similarly applied to

homologous immunized spleen cells for a reasonable explantation of the experimental results of our studies. The fundamental mechanism of a beneficial inhibitory action of heterologous cells may be essentially identical with that of homologous cells.

(The main results described in this paper were published at 39th General Meeting of Japanese Bacteriology held on 6th April, 1966)

Acknowledgement

The author wishes to thank Dr. T. Moriya, President of Technical R. & D. Headquater, Dr. K. Kamiko, Director of II. Research Institute, and Dr. N. Kajii, Chief of II. Division, for their kind encouragement and help which made this publication possible.

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