ACTION OF MOUSE ANTISERUM AGAINST ISOLOGOUS AGGREGATED IMMUNOGLOBULINS ON TRANSPLANTATION AND ANTITUMOR IMMUNITY

D. R. Kaulen, A. E. Snegireva, V. V. Khorobrykh, V. Ya. Shevlyagin, and O. S. Prigoda

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Mouse antiserum against isologous heat-aggregated immunoglobulins (MAAS), on repeated injection into animals, prolonged the survival of skin allografts, facilitated the development of Moloney sarcoma, reduced the latent period of appearance of tumors, sharply increased the yield of tumors which in most cases led to death of the mice, disturbed the normal age barrier for induction of fibrosarcomas, and sharply reduced the intensity of development of Rauscher leukemia. This effect of MAAS on transplantation and antitumor immunity is immunological in nature.

KEY WORDS: allograft; Moloney sarcoma virus; Rauscher leukemia; transplantation and antitumor immunity.

Previous experiments showed that during the primary immune response to sheep's red blood cells changes take place in the structural organization of the immunoglobulin receptors of B lymphocytes. Antigen-binding receptors of these cells can be blocked by means of rabbit or mouse antiserum against heat-aggregated mouse immunoglobulins [2, 3]. Inhibition of antigen-binding receptors of rosette-forming cells suggested that antiserum against isologous heat-aggregated immunoglobulins may participate in the reactions of transplantation and antitumor immunity.

In the investigation described below the effect of antiserum against isologous aggregated mouse immunoglobulins on the survival of skin grafts and of the development of Moloney sarcoma and Rauscher leukemia was studied.

EXPERIMENTAL METHOD

To assess the action of mouse antiserum against isologous heat-aggregated immunoglobulin (MAAS) on transplantation immunity a skin grafting method was used [8]. CBA ($H\text{-}2^k$) mice served as recipients and C57BL/6 ($H\text{-}2^b$) mice as donors. Daily for 5 days the recipients were given an intraperitoneal injection of 0.1 ml MAAS obtained in CBA mice as described previously [4], before and from the 7th to the 13th days after transplantation of the skin graft inclusive. Recipients receiving normal mouse serum (NMS) of CBA mice in accordance with the same scheme served as the control.

The action of MAAS on the development of Moloney sarcoma and Rauscher leukemia was studied in BALB/c mice of different ages. Activity of the viruses of Moloney sarcoma and Rauscher leukemia was determined by titration in BALB/c mice, calculated by Kärber's method, and expressed in infectious units (IU). Moloney virus was injected as a single dose of 0.035-3.5 IU subcutaneously (in the interscapular region) or intramuscularly (into the thigh), whereas Rauscher virus was injected intraperitoneally in a dose of 10 IU. MAAS obtained in BALB/c mice was injected into the animals simultaneously with the virus in a dose of 0.1 ml, and subsequently on alternate days, between five and 15 times altogether depending on the age of the animals and the development of the tumor. Mice receiving injections of virus and either NMS or physiological saline in accordance with the same scheme served as the control.

EXPERIMENTAL RESULTS

Analysis of the results showed primary taking of the skin allografts in all the recipi-

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TABLE 1. Effect of Isologous Serum Against Heat-Aggregated Mouse Immunoglobulins on Development of Moloney Sarcoma Induced by Different Doses of Virus

Dose of virus, IU	Reagent	No. of injections	No. of mice	Time of appearance of tumors, days, M ± m	% of tumors	Mortality of animals with tumors		Survival period of mice with tumors, days
						time of death, days	% dying	
3,5	MAAS	10	21	10,0±0,3	95,2	10—27	95,2	17,4
	NMS	10	19	13,4±0,4	100	—	0	>35
	Physiological saline	10	18	13,8±0,7	94,5	22	5,5	>35
	—	—	10	12,9±1,1	100	—	0	>35
0.35	MAAS	12	22	14,6±0,7	75,2	17—29	57,1	21,7
	NMS	12	21	19,0±0,4	9,5	—	0	>35
	Physiological saline	12	12	17,0	8,3	—	0	>35
0,035	MAAS	12	24	25,7±0,5	12,5	30—35	100	32,3
	NMS	12	20	—	0	—	0	—
	Physiological saline	12	6	—	0	—	0	—

TABLE 2. Development of Sarcoma Induced by Moloney Virus in a Dose of 3.5 IU in BALB/c Mice of Different Ages After Injection of MAAS

Number of animals	Age of mice, days	Reagent	No. of injections	Time of appearance of tumors, days, M ± m	% of tumors	Mortality of animals with tumors		Survival period of mice with tumors, days
						time of death, days	% dying	
14	0—4	MAAS	5	4,1±0,2	100	6—9	100	8,4
11		NMS	5	6,3±0,4	100	9—15	100	12,4
10		Physiological saline	5	6,8±0,3	100	9—16	100	13,0
21	17—21	MAAS	10	10,0±0,3	95,2	10—27	95,2	17,4
19		NMS	10	13,4±0,4	100	—	0	>35
18		Physiological saline	10	13,8±0,7	94,5	22	5,5	>35
6	45—55	MAAS	15	14,5±2,6	66,7	—	0	>45
4		NMS	15	—	0	—	—	—

ents. In the control animals rejection of the graft took place 10.9 ± 0.4 days after the operation in the case of animals receiving NMS, and 11.1 ± 0.3 days after the operation in animals receiving physiological saline. In mice receiving MAAS the survival period of the skin grafts was 15.8 ± 0.7 days. The difference in the survival period of the skin grafts in the experimental and control animals was statistically significant ($P < 0.01$). Consequently, MAAS prolonged the survival of the allografts by 5-6 days, possible evidence of its immunosuppressive action.

Considering the inhibitory effect of MAAS on receptors of lymphocytes [2, 3] and transplantation immunity, as well as the data in the literature on the effect of immunosuppressants during the development of tumors induced by oncogenic viruses [1, 5], an attempt was made to evaluate the action of MAAS on the development of Moloney sarcoma in BALB/c mice aged 2.5-3 weeks (Table 1). It was found that after repeated intraperitoneal injection both of NMS and of physiological saline, a sarcoma induced by Moloney virus developed in 8-100% of animals (depending on the dose virus — 0.35-3.5 IU). However, the tumors did not cause death of the animals and regressed completely. In animals of the experimental groups which received MAAS, tumors appeared earlier ($P < 0.05$), developed more rapidly, ulcerated, and caused death of the mice in 57-95% of cases. Virus in a dose of 0.35 IU caused the appearance of sarcomas in animals receiving MAAS eight times more frequently and the survival period of the mice with tumors was 21.7 ± 1.0 days.

In the group of animals receiving Moloney virus in a dose of 0.035 IU tumors appeared only in the mice receiving MAAS (in 12.5% of cases).

It can be concluded from these results that MAAS facilitates the development of Moloney sarcoma, shortens the latent period of appearance of tumors, sharply increases the intensity of their development, and causes death of the animals. This was clearly shown by the results of experiments in which the virus was injected in threshold or subthreshold doses (0.35-0.035 IU). In most cases when MAAS was given the animals with tumors died after ulceration of the sarcoma, whereas in the control groups the tumors regressed.

This conclusion was confirmed by experiments to study the effect of MAAS on the development of Moloney sarcoma in animals of different ages into which the virus was injected in a dose of 3.5 IU (Table 2). In mice aged up to 4 days and 2.5-3 weeks, tumors appeared in 100% of cases both in the experimental and in the control groups. However, after injection of MAAS the latent period of onset of the sarcoma and the survival period of the animals with tumors were both reduced ($P < 0.05$). Furthermore, Moloney virus in a dose of 3.5 IU did not cause tumors to arise in adult animals, whereas when MAAS was given sarcomas developed on the 13th-17th day and were recorded until the 35th day. Morphological analysis confirmed the development of fibrosarcomas in the animals with tumors.

In the next experiments the development of Rauscher leukemia was investigated in BALB/c mice after 25 injections of NMS or MAAS into the animals. After intraperitoneal injection of Rauscher virus in a dose of 10 IU leukemia developed in the animals of the control and experimental groups in 100% of cases. However, when NMS was given, the survival period of the mice was only half as long (48.1 ± 5.4 days) as in the animals receiving MAAS (97.6 ± 8.3 days). The intensity of development of Rauscher leukemia in the mice receiving MAAS was considerably less than in the control. For instance, 60% of the animals receiving NMS died from leukemia before the 40th day and the remaining 40% died between the 40th and 60th days. After injection of MAAS death of the animals was not observed before the 40th day, and only 30% of the mice died between the 40th and 60th days.

The results of these experiments are evidence that MAAS prolongs the life of skin allografts, facilitates the development of Moloney sarcoma, disturbs the normal age barrier for induction of sarcomas, and sharply reduces the intensity of development of Rauscher leukemia. The experimental results suggest that MAAS acts mainly on cellular immunity, as has been shown for induction of polyoma in thymectomized animals or animals treated with antilymphocytic serum [5-7]. Inhibition of development of Rauscher leukemia in mice treated with MAAS probably may be connected with the synthesis of immunoglobulins blocking the development of leukemia. Indirect evidence in support of this view is given by experiments in which MAAS, after five injections into animals immunized with sheep's red cells, caused an increase in the titer of 7S hemagglutinins in their blood serum and an increase in the number of antibody-forming cells in the spleen after 2 weeks. The mechanism of action of MAAS on transplantation and antitumor immunity is evidently not yet fully clear and requires further experimental investigation.

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