STIMULATION OF TRANSPLANTATION IMMUNITY AND THE PLASMA-CELL REACTION IN MICE BY INTERFERON

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Administration of interferon to allografted mice speeds up rejection of primary and secondary allografts and strengthens the cytotoxic action of the lymphocytes of these mice on target cells. Intrasplenic injection of pertussis antigen into mice together with interferon induces an intensification of the plasma-cell response in the spleens of these animals.

There is evidence in the literature which suggests a role for interferon in the development of immunologic reactivity [3-5, 8].

The writers consider that interferon is an essential factor involved in the integral immunologic response or, more precisely, interferon is an essential component for the development of immunologic reactivity. There is every reason to suppose that administration of interferon against the background of antigenic stimulation may intensify the immune response.

The object of the investigation described below was to study the effect of interferon on transplantation immunity and on some morphological indices of humoral immunity.

EXPERIMENTAL METHOD

Male and female BALB/c and (CBA × C57BL/6)F_1 mice and noninbred mice weighing 18-20 g were used. Interferon was induced by Newcastle disease virus in a culture of L-cells. The interferon titers determined in the same cell system by the degree of inhibition of the cytopathogenic action of vesicular stomatitis virus were 200-800 units/ml.

To detect the effect of interferon on transplantation immunity, the times of rejection of skin allografts and the intensity of the cytotoxic action of lymphocytes on target cells were studied. The skin grafts were transplanted by the method of Billingham and Medawar [1] using BF-6 glue for the microwounds. A skin graft was taken from the dorsal region of BALB/c mice and grafted on the same region of (CBA × C57BL/6) F_1 mice and vice versa. The size of the grafts was 1.5-2 cm^2. Altogether 123 mice were used in the experiments. Immediately after skin grafting, and then daily thereafter, the mice of the experimental groups received intraperitoneal injections of 1 ml interferon in a titer of 200-250 units/ml until rejection of the grafts was complete. The control animals received medium No. 199 in the same volume and at the same times; the other control groups of animals remained untreated after the allografting operation.

The cytotoxic test of lymphocytes in vitro was carried out in a culture of L-cells. Lymphocytes isolated from the lymph glands of the mice were added to target L-cells in the ratio of 40:1. The results were evaluated by counting the number of living target cells by staining with eosin and trypan blue after incubation for 48 h.

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TABLE 1. Lifespan of Allografts in Mice Subjected to Various Treatments

Group No.	Mice: donors	Mice: recipients	Number of animals in group	Treatment	Lifespan of allograft (in days): beginning of rejection	Lifespan of allograft (in days): complete rejection
1	(CBA× ×C57BL/6)F_1	BALB/c	31	Interferon	6,6±0,25	8,5±0,23
2	The same	The same	24	Medium No. 199	9,2±0,03 $P<0,01$	11,7±0,04 $P<0,01$
3	» »	» »	14	No treatment	9,2±0,03 $P<0,01$	11,8±0,03 $P<0,01$
4	BALB/c	(CBA× C57BL//6) F_1	33	Interferon	7,2±0,21	9,9±0,31
5	The same	The same	19	Medium No. 199	8,6±0,36 $P<0,01$	11,5±0,41 $P<0,01$
6	» »	» »	5	No treatment	7,8±0,09 $P<0,1$	11,4±0,67 $P<0,01$

TABLE 2. Lifespan of Allografts (in days) after Regrafting of Skin

Treatment after first transplantation	First regrafting: Number of animals	First regrafting: beginning of rejection	First regrafting: complete rejection	Second regrafting: Number of animals	Second regrafting: beginning of rejection	Second regrafting: complete rejection
Interferon	12	3,6±0,05	5,3±0,07	5	3,0	5,0
Medium No. 199	8	5,1±0,35 $P<0,01$	6,4±0,69 $P<0,01$	—	—	—
No treatment	8	4,8±0,43 $P<0,01$	5,8±0,43 $P<0,1$	5	5,2±0,11 $P<0,01$	6,2±0,11 $P<0,02$

TABLE 3. Number of Plasma Cells in Spleens of Noninbred Mice

Group No.	Treatment	Day of observation: 1st	3rd	4th	5th	6th	7th	8th	9th	10th	14th
1	Pertussis antigen + interferon	8,2 ±0,02	10,0 ±0,05	31,0 ±0,36	21,8 ±0,03	36,0 ±0,40	23,4 ±0,21	22,2 ±0,07	29,7 ±0,28	20,5 ±0,07	10,2 ±0,23
2	Pertussis antigen + medium No. 199	6,5 ±0,13	6,2 ±0,07	30,2 ±0,15	11,0 ±0,34	11,8 ±0,32	6,0 ±0,07	10,5 ±0,07	7,5 ±0,38	6,7 0±0,08	8,5 ±0,11
3	Pertussis antigen	5,8 ±0,08	7,2 ±0,05	26,0 ±0,21	10,7 ±0,04	17,2 ±0,19	9,0 ±0,11	12,0 ±0,10	5,0 ±0,07	6,7 ±0,07	8,7 ±0,20
4	Interferon	5,2 ±0,31	6,2 ±0,08	8,5 ±0,02	4,5 ±0,08	4,7 ±0,19	2,7 ±0,02	1,3 ±0,01	3,5 ±0,01	3,6 ±0,07	5,0 ±0,01
5	Medium No. 199	3,5 ±0,02	4,5 ±0,07	3,7 ±0,02	3,7 ±0,02	4,3 ±0,07	1,6 ±0,07	1,5 ±0,01	4,5 ±0,10	2,7 ±0,14	5,5 ±0,02

To study the effect of interferon on the plasma-cell response, interferon was injected intrasplenically into the mice in conjunction with antigen. The antigen used was a killed culture of Bordetella pertussis in a dose of 5×10^8 bacterial cells. Interferon in a titer of 800 units/ml was injected intrasplenically in a dose of 0.1 ml. The investigation was carried out on five groups of noninbred mice. Each group consisted of 50 to 60 animals. Group 1 received interferon simultaneously with antigen, group 2 received antigen with medium No. 199, group 3 antigen only, group 4 interferon only, and group 5 medium No. 199. All injections were single. To evaluate the plasma-cell response, animals were sacrificed at different times from the 1st to the 10th days and on the 14th day, imprints were taken of the spleens, and the number of cells of the plasma-cell series was counted in 1000 cells. Altogether imprints of 243 spleens were studied.

The numerical results of all the experiments were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Effect of Interferon on Lifespan of the Allografts

The lifespan of the allografts was estimated by counting the number of days from the grafting operation to the beginning and completion of rejection of the graft. The results are given in Table 1.

As Table 1 shows, if the times of the beginning of rejection and death of the allografts are compared in animals of the experimental and control groups, a statistically significant decrease in the lifespan of the allografts is observed in the mice receiving interferon.

In the next experiments the times of rejection of the allografts were studied after repeated skin grafting. Three weeks after transplantation into mice of groups 4, 5, and 6 the first regrafting operation was performed, and 4 weeks later a second regrafting was carried out, using a graft of the same size. After regrafting the mice of all groups received no further treatment. The periods of survival of the allografts are given in Table 2.

In the case of regrafting, just as of primary skin grafting, the beginning of rejection was hastened and the mean lifespan of the graft was shortened in the mice receiving interferon with the first graft by comparison with the control group.

Cytotoxic Action of Lymphocytes on Target Cells

Lymphocytes of the mice of groups 4, 5, and 6 after the first and repeated allografts were tested by the cytotoxic test in tissue culture. The results showed that lymphocytes of mice of the experimental group have a stronger destructive action on target cells than lymphocytes of the control groups of animals.

Effect of Interferon on the Plasma – Cell Response

Since the main producers of antibodies in immunogenesis are cells of the plasma-cell series [2, 6, 7], the action of interferon on the plasma-cell response in the spleen was studied in mice after injection of pertussis antigen.

The original number of plasma cells in the spleens of the intact mice was 3.7 ± 0.52. The results of these experiments are given in Table 3.

Injection of interferon and medium No. 199 had no significant effect on the number of cells of the plasma-cell series. Proliferation of plasma cells was observed in the spleens of the control groups of mice (2 and 3) between the 4th and the 6th days after injection of the antigen. Meanwhile, a study of the spleens of mice receiving pertussis antigen together with interferon revealed a marked increase in the number of plasma cells, and the plasma-cell response of this group of mice was more intensive and more prolonged. The difference between the plasma-cell response of the experimental and control groups was statistically significant ($P < 0.01$).

When the agglutination test was carried out with the mouse sera, no appreciable difference was found in the titers of antipertussis antibodies in the animals of the experimental and control groups in the period from the 4th to the 10th day. Evidently injection of a small dose of interferon (only 0.1 ml) was enough to detect only some of the morphological changes arising in the course of the humoral immunologic response.

Injection of interferon against the background of antigenic stimulation thus activates transplantation immunity and the plasma-cell response in mice. In the first case this could be recorded as acceleration of rejection of primary and secondary allografts and as intensification of the cytotoxic action of the lymphocytes of mice with allografts and receiving interferon on target cells, while in the second case it could be recorded as a marked increase in the number of plasma cells in the spleens of the mice after injection of pertussis antigen together with interferon.

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