A STUDY OF THE POSSIBILITY OF INDUCTION OF HISTOIMMUNE SHOCK IN AN ISOLOGOUS GENETIC SYSTEM

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A single injection of homologous [2, 3, 4] or of heterologous spleen cells into mice has been found to cause severe dysfunction of the immune system, manifested by depression of the reaction to the primary [2, 3, 5] and, in particular, to the secondary [2, 3, 4] injection of antigen. We have called this state "histoimmune shock." The induction of histoimmune shock has been found to require the injection of foreign or isologous (for F_1 hybrids) spleen cells. During transplantation of isologous cells into F_1 hybrids, histoimmune shock developed as a result of the reaction of the living transplanted cells against the host, whereas in the case of injection of strictly homologous and heterologous cells depression of the immunological reactivity developed after injection of both living and nonviable cells. It is not clear, however, whether heterogeneity of the transplanted cells or their ability to react against the host is essential for the induction of histoimmune shock.

In the present study an attempt was made to discover whether histoimmune shock can develop in an isolinear system, excluding all possibility of a reaction of the graft against the host (the transfer of isolinear spleen cells to recipients of the same sex as the donor) and also in conditions favoring this reaction (the transfer of cells from females to irradiated males).

In some strains of mice, grafts of male skin are known to be rejected by females of the same line [5]. It has been suggested that this phenomenon may be attributed to the presence of a specific sex antigen in the males, linked with the Y-chromosome and absent in females. These differences are seen most clearly in mice of line C57BL. Theoretically, in this genetic system, induction of a reaction of the graft against the host may develop if immunologically active lymphoid cells of adult females are injected into newborn or adult irradiated males of the same line, whose immunological reactivity is sharply depressed. In these conditions the cells of the graft may react against the host's sex antigen, which is foreign to them, although the host cannot reject the transplanted cells.

Attempts to find such a possibility in animals irradiated with a lethal dose and treated with isologous bone marrow have yielded conflicting results. In some experiments it was found [6] that the late mortality during the secondary disease whose main cause was evidently the reaction of the immunologically active bone marrow cells against the host was highest in the case when cells were injected from females into males; other investigators [7], however, failed to draw this conclusion.

METHOD

Mice of lines C57BL/6 and hybrids (C57BL/6 X CBA)F_1 aged 12-16 weeks were used as recipients and donors. Spleen cells were obtained by mincing the organ in a hand-operated glass homogenizer, suspending the cells in ice-cold Hanks's solution, and filtering the suspension through a silk mesh. The viability of the cells was determined by staining with 0.2% eosin solution.

The recipients were irradiated in the RUM-11 apparatus (voltage 180 kV, current 15 mA, field 20 × 20 cm, focal length 40 cm, filters 1 mm Al and 0.5 mm Cu; dose rate 44-48 R/min.

Effect of Transplantation of Isologous Spleen Cells into Sublethally Irradiated Mice of Line C57BL/6 on Mortality Rate and Antibody Production

Character of procedure	Dose of radiation (in R)	No. of cells, $\times 10^6$	No. of animals: total	No. of animals: dying during 30 days	Titer of antibodies: primary reaction Me	primary reaction X	primary reaction P	secondary reaction Me	secondary reaction X	secondary reaction P
Expt. No. 1 (single immunization of donors):										
Injection of cells from females into males	500	180	8	6	32	—1,43	>0,05	—	—	—
Injection of cells from females into females	500	180	8	3	32	+0,16	>0,05	—	—	—
Control	500	—	7	0	32	—1,39*	>0,05	—	—	—
Expt. No. 2 (triple immunization of donors)										
Injection of cells from females into males	350	70	10	0/7	256	—1,50	>0,05	2 048	—5,44	<0,01
Injection of cells from females into females	350	70	10	0/1	512	—1,55	>0,05	5 120	—3,90	<0,05
Control	350	—	10	0/3	512	+2,28	>0,05	16 384	—3,44	>0,05
Control	—	—	10	0/2	256	—	—	16 384	—	—

Note. The donor females were immunized with spleen cells of males. Numerator—number of mice dying before transplantation of sarcoma; denominator—number of mice dying during 60 days after transplantation of sarcoma. Me—medians of antibody titers (reciprocals); X—value of X in relation to irradiated control animals.

* The value of X is given in relation to the unirradiated control animals.

Spleen cells were injected intraperitoneally 2-4 h after irradiation. Immediately after irradiation the experimental and control animals were injected intraperitoneally with Salmonella typhi cells (strain ty-2), killed with formalin. The second injection of antigen was given 18-23 days after the first. At different periods after irradiation and transplantation, blood was taken from the caudal veins of the recipients, and the titer of agglutins was determined individually for each mouse. The statistical analysis of the results was conducted by means of the rank criterion X [1].

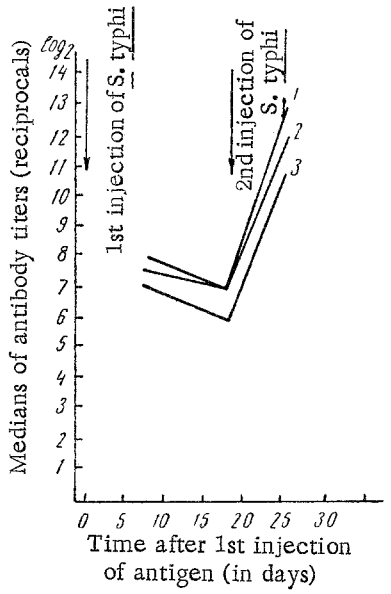
RESULTS

Transplantation of Spleen Cells of Female Hybrids (C57BL/6 × CBA)F_1 into Females (C57BL/6 × CBA)F_1

Transplantation of 20 × 10^6 living spleen cells from females (of line C57BL/6 × CBA)F_1 into females C57BL/6 × CBA)F_1 irradiated in a dose of 500 R did not produce signs of homologous sickness. No statistically significant depression of antibody production was observed in the experimental mice in response to the primary or secondary injection of antigen ($P > 0.05$; see figure) by comparison with the control irradiated mice not receiving spleen cells. In this experiment irradiation itself likewise had no effect on antibody production.

The Study of the Possibility of Induction of Histoimmune Shock on the Basis of Differences in the Sex Antigen

It was assumed that, since the sex antigen is a weak antigen, in this case the induction of a reaction of the graft against the host in sublethally irradiated recipients, whose immunological reactivity had been depressed to a lesser degree than after lethal irradiation, requires the following essential conditions: injection of a large number of immunologically competent cells (especially spleen cells) and artificial potentiation of the specific immunological reactivity of the donor females as a result of preliminary immunization with spleen cells from males.



Effect of transplantation of isologous spleen cells into sublethally irradiated (500 R) hybrids (line C57BL/6 × CBA)F_1 on antibody formation. Titers of antibodies in control nonirradiated mice (1), in control irradiated mice (2), and in hybrids receiving isologous spleen cells (3). Each point on the curve corresponds to the median of 10 observations of the antibody titer.

The results of the corresponding experiments are given in the table.

It is clear from the table that the injection of massive doses of spleen cells obtained from females did not cause death of males irradiated in a sublethal dose (experiment No. 2), or the mortality in this case was only slightly different from that observed when the cells of specifically sensitized females were injected into females (experiment No. 1). In experiment No. 2, 30 days after irradiation in a dose of 350 R and injection of spleen cells into the experimental and control mice, a sarcoma 298, previously subjected to passage into mice of line C57BL, was transplanted. In conditions favoring the reaction of the graft against the host, the more rapid death of the recipients was observed by comparison with the mortality among the other groups of animals.

It also follows from the table that the injection of cells of immunized females into males and females had no effect on the primary reaction of antibody formation in the recipients. However, the secondary reaction of antibody formation was much more histosensitive in these experiments: the injection of spleen cells taken from females into males caused a highly significant depression of the secondary reaction ($P < 0.01$). Injection of the same cells into females produced a less marked, yet still significant ($P<0.05$) depression of the secondary immunological response.

Hence, on the basis of differences in the sex antigen, it is evidently possible to induce a reaction of the graft against the host in sublethally irradiated isologous recipients, but in the conditions of the present experiments this reaction was manifested mainly by depression of the immunological memory and by some degree of transplantation immunity. Depression of the secondary reaction of antibody formation in experiment No. 2 and the presence of lethal issues in experiment No. 1 after injection of the cells of sensitized females into irradiated females may be partly explained by the presence of a sex antigen, foreign to the recipients, in the tissues of donors immunized with spleen cells from males.

The results described above demonstrate the extremely high histosensitivity of the secondary reaction of antibody formation, for it was depressed as a result of the action of such a weak stimulus as the reaction of the graft against the host on the basis of differences in the sex antigen. At the same time, during isolinear transplantations between individuals of the same sex (see figure), doses of spleen cells which, as a rule, caused well marked histoimmune shock in response to the injection of isologous cells of F_1 hybrids had no effect on antibody production.

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