INDUCTION OF HISTOIMMUNE SHOCK DURING TRANSPLANTATION OF HETEROLOGOUS SPLEEN AND BONE MARROW CELLS

(UDC 612.017.1-06:[612.411+612.419]:612.6.02)

A. S. Shevelev, V. F. Semenkov, E. A. Fedosov, E. E. Stysina, and A. A. Fomina

Department of Microbiology (Head, Professor V. A. Yudenich), Smolensk Medical Institute
(Presented by Active Member AMN SSSR, N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 2, pp. 68-71, February, 1966
Original article submitted June 11, 1964

It has been shown previously [2,3,5] that the injection of homologous spleen and bone marrow cells depresses the process of antibody production in the recipients, and this is most clearly seen in animals irradiated with sublethal and mean-lethal doses. These findings demonstrate the need for a detailed study of the histosensitivity of immunological reactions, by which is meant the sensitivity of the immune system to injection of foreign or related (for F_1 hybrids) cells [5].

The study of the histosensitivity of immunological reactions in a homologous genetic system has shown that the injection of homologous spleen and bone marrow cells causes a profound dysfunction of the immune system of the host, which the authors have called histoimmune shock. This takes the form of nonspecific and tolerant-like phases: the first is shown by a non-specific depression of antibody formation following injection of various corpuscular antigens, while the second develop after a primary injection of antigen immediately after transplantation and is characterized by a sharp depression of the ability to give a secondary immunological response.

The object of the present investigation was to discover whether histoimmune shock develops as a result of the transplantation of heterologous spleen cells.

EXPERIMENTAL METHOD

In most cases, the donors were adult inbred "August" rats and the recipients CBA mice, but in a few experiments the recipients were mice of the C57BR line and hybrids of the (C57BL/6xCBA)F_1 line or mice of no particular line. Most of the recipients had been irradiated in a sublethal dose. Conditions of irradiation: apparatus RUM-11, voltage 180 kV, current 15 mA, filters 1 mm Al and 0.5 mm Cu, field 20 × 20 cm, focal distance 40 cm, dose rate 44-48 R/min.

Bone marrow was obtained by washing out the femur, the tibia, and the humerus three times with Hank's solution, and the suspension of spleen cells by mincing the tissue in a manual glass homogenizer with the addition of Hank's solution. The cell suspensions were kept on ice and injected intraperitoneally (spleen cells) or intravenously (bone marrow) 2-4 h after irradiation and 1.0-1.5 h after preparation.

Immediately after transplantation, the experimental and control mice received intraperitoneal injections of $100 \cdot 10^6$ formalin-killed typhoid bacteria (strain ty = 2) per animal. Between 15 and 46 days after the first injection of antigen, the second dose was injected, equal to the first. In some experiments, three injections of antigen were given. At various periods after the first and second injections, blood was taken from the tail vein of the mouse and the agglutinin titer determined in each mouse. The results were analyzed by statistical methods using the ranking criterion X [1].

In some experiments, a sarcoma 298 was transplanted into the experimental and control animals.

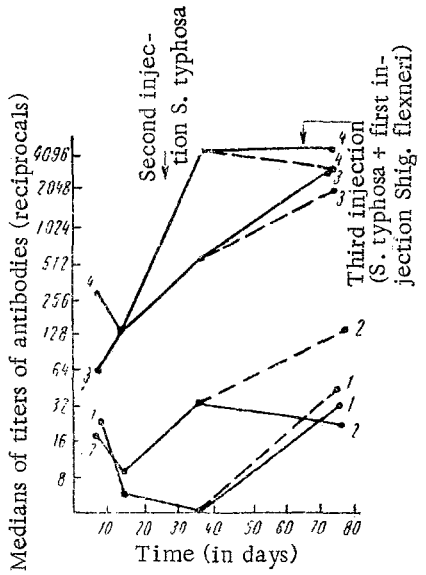


Fig. 1. Effect of transplantation of $135 \cdot 10^6$ spleen cells of "August" rats on antibody synthesis in sublethally irradiated CBA mice: 1) injection of living spleen cells of "August" rats and irradiation (500 R); 2) injection of spleen cells of lethally irradiated (1000 R) "August" rats and irradiation (500 R); 3) control of irradiation, 500 R; 4) control of immunization (immunization without irradiation and without injection of cells). Continuous lines – medians of antibody titers against S. typhi; broken lines – medians of antibody titers against Shig. dysenteriae flexneri.

EXPERIMENTAL RESULTS

Transplantation of Bone Marrow. The intravenous injection of $100 \cdot 10^6$ bone marrow cells from young "August" rats (weight 35-70 into 12-week CBA mice irradiated in a dose of 750 R produced a severe disease in the mice and 50% of the recipients died on the 13th-16th day after transplantation, while 100% of the control irradiated mice or the irradiated mice receiving intravenous injections of $6 \cdot 10^6$ cells of isologous bone marrow survived. The recipients receiving rat bone marrow developed total nonspecific immunological reactivity 7 and 14 days (titer less than $1/5$) and 37 days (titer less than $1/10$) after the primary injection of antigen, and partial reactivity after the second injection of antigen (46 days after the first injection).

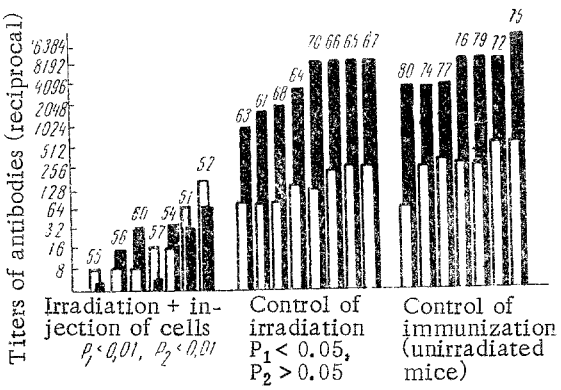
Transplantation of Spleen Cells. The results of transplantation of spleen cells of "August" rats into CBA mice and $(C57BL/6xCBA)F_1$ hybrids were studied in five experiments (200 mice).

Induction of Heterologous Sickness. In 8-12-week-old sublethally irradiated (400-500 R) CBA mice, injection of $40\text{-}135 \cdot 10^6$ spleen cells of adult "August" rats produced a severe sickness ending in death of the greater part of the recipients. The body weight of the animals fell sharply, the hair became shaggy, and the animals drooped and became emaciated. Blood analysis showed a hemolytic anemia and the serum contained bile pigments. Marked splenomegaly was found at autopsy. Lethal irradiation of the donors (1000 R) as a rule prevented death of the recipients. Injection of $75 \cdot 10^6$ spleen cells of "August" rats into CBA mice aged 9 months did not produce the sickness. Preliminary immunization of the donors with spleen cells of mice of the CBA line increased the aggressiveness of their spleen cells in this particular genetic system.

Antibody Formation. In all the experiments, injection of spleen cells of "August" rats caused a statistically significant depression of the primary, and especially of the secondary, reaction of antibody formation in sublethally irradiated CBA mice by comparison with control irradiated animals not receiving spleen cells. Histoimmune shock also developed in those experiments in which the animals did not develop homologous sickness, but it was intensified with a reduction in the age of the recipients and an increase in the number of transplanted cells. This type of shock was also observed in unirradiated recipients (by comparison with unirradiated, immunized animals not receiving spleen cells).

The results of an experiment in which the effect of transplantation of $135 \cdot 10^6$ spleen cells of "August" rats nine months old, immunized with spleen cells of CBA mice, was studied on the synthesis of antibodies in 10-week-old CBA mice are given in Fig. 1. In this experiment, a highly significant depression of the primary and secondary reaction of antibody formation was observed following injection of spleen cells, not only of normal donors, but also of lethally irradiated (100 R) donors. On the 68th day after the primary injection (43 days after the secondary injection) of antigen, a third injection of S. typhi was given, mixed with an equal number of formalin-killed cells of Shigella flexneri (total dose $100 \cdot 10^6$). It is clear from Fig. 1 that the formation of antibodies against S. typhi in the experimental mice after the third injection of antigen was still less marked than after the second, and much weaker than the formation of antibodies after the primary injection of Shig. flexneri, demonstrating the specificity and the duration of depression of the secondary immunological response in the experimental animals.

The individual reactions of the experimental CBA mice receiving spleen cells of lethally irradiated "August" rats and of the control animals following primary (first injection of S. typhi) and secondary (third injection of S. typhi) immunological response are shown in Fig. 2. It is clear from Fig. 2 that, in the sublethally irradiated animals and the unirradiated control animals, a normal secondary reaction was observed (a sharp increase in the titers of antibodies by comparison with the primary immunological response). Irradiation itself (500 R) produced only a



Individual titers of antibodies in experimental and control mice.

Fig. 2. Effect of transplantation of $135 \cdot 10^6$ spleen cells of "August" rats on antibody formation in sublethally irradiated (500 R) CBA mice. Shaded columns – antibody titer 8 days after first injection of S. typhi. Black columns – antibody titer 9 days after 3rd injection of S. typhi; P_1 – significance of depression of primary reaction; P_2 – significance of depression of secondary reaction; the numbers above the columns represent the serial numbers of the mice. The second injection of S. typhi was given 25 days after the first injection, and the third injection 43 days after the second injection.

moderate depression of the primary reaction and had no effect on the secondary immunological response. In contrast to these findings, the injection of spleen cells of irradiated donors not only produced a highly significant depression of the primary and secondary immunological response in the CBS mice by comparison with the control irradiated animals, but also gave rise to a sharply distorted reaction in 50% of the experimental mice, i.e., the secondary immunological response either was completely absent or was much weaker than the primary response.

Homotransplantation Immunity. Transplantation of sarcoma 298, following passage in mice of C57BL line, into 9-month-old CBA mice 34 days after sublethal irradiation and injection of $75 \cdot 10^6$ spleen cells gave negative results (absence of survival of tumors in the experimental and control animals). Injection of spleen cells ($135 \cdot 10^6$) from 9-month-old "August" rats into sublethally irradiated (500 R) CBA mice produced moderate depression of the transplantation immunity in the case when the tumor was transplanted 26 days after the beginning of the experiment; in all three mice surviving after transplantation of the spleen cells of unirradiated donors, and in 3 of the 9 mice receiving spleen cells from irradiated rats, temporary survival of the tumors was observed (reaching the size of a cherry), followed by their absorption.

The results show that, following transplantation of adequately large doses of spleen and bone marrow cells of "August" rats into sublethally irradiated CBS mice, the recipients may develop a disease due evidently to the immunological reaction of the transplanted cells against the host. This suggestion was confirmed by the fact that preliminary immunization of the donors with spleen cells of future recipients strengthened the pathogenic action of the spleen cells of the donors, and irradiation of the donors considerably weakened the pathogenic properties of their spleen cells.

The results also demonstrate that a single injection of heterologous cells may give rise to severe histoimmune shock, during which the immunological memory (the second immunological response) was particularly sharply depressed and distorted. It is important to note that histoimmune shock also developed after injection of the cells of lethally irradiated donors, i.e., in conditions preventing the development of heterologous sickness. The depression and distortion of the secondary immunological response may be regarded as a state to some extent reminiscent of the complete (absence of secondary immunological response) or partial (depression of secondary reaction) acquired immunological tolerance in adult animals. However, in contrast to the classical immunological tolerance, reproducible in newborn animals, in which the induction of tolerance to bacterial antigens can be obtained with great difficulty or not at all [4], in the present experiments an analogous state developed comparatively easily.

The results of these experiments showed that the transplantation of heterologous spleen cells may also give rise to moderate depression of the transplantation immunity in relation to homotransplanted tumors 26 days after the beginning of the experiment.

LITERATURE CITED

1. I. P. Ashmarin and A. A. Vorob'ev, Statistical Methods in Microbiological Investigations [in Russian], Leningrad (1962).
2. G. O. Bain, Canad. J. Biochem, 38, 621 (1960).
3. G. O. Bain, Canad. J. Biochem, 41, 573 (1963).
4. M. Hasek, A. Lengerova, and T. Hraba, in the book: Advances in Immunology, Vol. 1, New York (1961), p. 1.
5. A. S. Shevelev, Folia biologica, 11,177 (1965).