GLOBULIN FORMATION BY RAT SPLEEN CELLS TRANSPLANTED INTO MICE SOON AFTER BIRTH

D. N. Mayanskii

UDC 612.65'411.017.1

Rat spleen cells were transplanted into mice during the first 12 h after birth. Immunoglobulins of types IgG and IgM were found in their blood 2.5 weeks later. Type IgG immunoglobulins could still be detected in some cases 5 weeks after transplantation of the cells. A correlation is observed between the appearance of donor's immunoglobulins in the blood of the chimeras, the accumulation of cells containing alkaline phosphatase in the bone marrow, and the intensity of the graft versus host reaction.

To identify the donor's hematopoietic cells in heterologous rat–mouse chimeras, various methods are used [3, 10, 11]. In some cases, however, it is important to obtain some idea not only of the survival, but also of the level of function of the donor's lymphocytes in the recipient's body. Attempts have therefore been made, not always successfully, to detect rat immunoglobulins, the products of lymphocyte function, in rat–mouse radiochimeras [7, 8, 12].

It is also interesting to discover how long heterologous lymphocytes can function actively when injected not into an irradiated recipient, but into an immunologically immature recipient, in the adaptive period. This would help to evaluate the participation of these lymphocytes in the mechanisms of development of the immunologic conflict which goes under the name of the "graft versus host" (GVH) reaction [5, 12].

The object of this investigation was to study the formation of immunoglobulins by rat spleen cells when transplanted into mice in the early postnatal period.

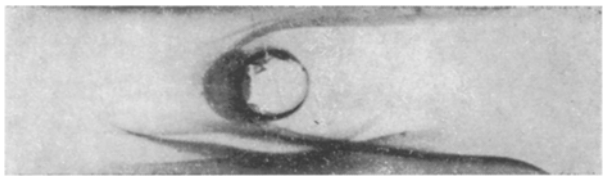

Fig. 1. Immunoelectrophoresis (+ on left). Central well contains serum of 2.5-week mouse which received $2.2 \cdot 10^7$ rat spleen cells in first 12 h after birth. Bottom gutter contains rabbit antiserum against rat proteins; top gutter contains same serum after exhaustion with mixture of serum antigens from 5 normal mice. Top precipitation arcs were formed by rat immunoglobulins of types IgG and IgM.

EXPERIMENTAL METHOD

During the first 12 h after birth, mice of different groups received an intraperitoneal injection of spleen cells from Wistar rats in doses of $1 \cdot 10^6$–$1.2 \cdot 10^6$, $6.9 \cdot 10^6$, $1.1 \cdot 10^7$–$1.22 \cdot 10^7$, $1.9 \cdot 10^7$–$2.12 \cdot 10^7$, and $4.2 \cdot 10^7$–$4.7 \cdot 10^7$. A cell suspension was prepared in a glass homogenizer [2] and contained 80–85% of viable nucleated cells. The mice were sacrificed at the ages of 2.5, 5, and 10 weeks. To identify the donor's hematopoietic cells, smears were taken from the spleen, lymph glands, thymus, and bone marrow, and cells containing alkaline phosphatase were determined in them by the method of simultaneous azo-coupling (using α-naphthyl phosphate as substrate and fast blue B as dye) [4]. Rat proteins in the blood of the rat–mouse chimeras

Department of Pathological Physiology, Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 1, pp. 61-64, January, 1970. Original article submitted August 23, 1968.

TABLE 1. General Indices after Transplantation of Different Doses of Donors' Cells

Group of mice	Number of spleen cells injected during first 12 h after birth	No. of mice	Age of mice (in weeks)	Weight of mice (in g)		Splenic index		Number of phosphatase-positive cells in bone marrow (in %)*	Precipitation arcs against globulins
				variations	P	variations	P		
Control	—	28	2,5	7,2—9,3	—	0,3—0,9	—	—	—
		15	5	17,2—18,8	—	0,2—0,8	—	—	—
		11	10	21,8—23,5	—	0,5—1,1	—	—	—
1	$1 \cdot 10^6$—$1.2 \cdot 10^6$	25	2,5	6,8—8,8	$< 0,05$	0,6—1,1	$< 0,05$	—	—
2	$5.9 \cdot 10^6$	22	2,5	7,3—8,2	$< 0,05$	0,7—1,2	$< 0,05$	—	—
3	$1.1 \cdot 10^7$—$1.2 \cdot 10^7$	18	2,5	4,8—7,6	$< 0,05$	0,8—3,1	$< 0,01$	0,03—0,05	—
4	$1.9 \cdot 10^7$—$2.2 \cdot 10^7$	16	2,5	3,7—5,8	$< 0,001$	1,3—3,2	$< 0,001$	0,2—0,5	IgG and IgM
	$1.9 \cdot 10^7$—$2.2 \cdot 10^7$	8	5	13,7—15,2	$< 0,02$	0,7—2,1	$< 0,05$	0,03—0,08	KIgG
5	$1.9 \cdot 10^7$—$2.2 \cdot 10^7$	10	10	21,0—25,5	$< 0,05$	0,5—0,8	$< 0,05$	—	—
	$4.2 \cdot 10^7$—$4.7 \cdot 10^7$	9	2,5	3,8—5,1	$< 0,001$	1,5—3,8	$< 0,001$	0,6—0,8	IgG and IgM

*Cells containing alkaline phosphatase counted in 100 fields of vision, each of which contained about 100 cells.

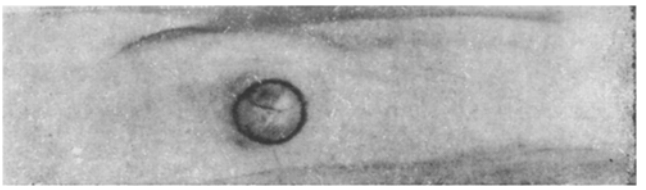

Fig. 2. Immunoelectrophoresis (+ on left). The same as in Fig. 1, but central well contains serum of mouse of same group but aged 5 weeks. Bottom arc formed by rat immunoglobulins of type IgG.

Fig. 3. Immunoelectrophoresis (+ on left). The same as in Fig. 1, but central well contains serum of 2.5-week mouse which received $4.7 \cdot 10^7$ rat spleen cells in first 12 h after birth. Top arcs were formed by rat immunoglobulins of types IgG and IgM.

were estimated by immunoelectrophoresis. A rabbit antiserum against rat serum proteins, preliminarily exhausted with a mixture of sera from 5-6 intact mice, was used for this purpose. Completeness of exhaustion was judged by disappearance of precipitation arcs to serum antigens of normal mice during immunoelectrophoresis. To identify the muscle proteins, a rabbit antiserum was prepared against mouse serum antigens, preliminarily exhausted by a mixture of sera from 5-6 intact rats. The degree of activity of the GVH reaction was assessed from the clinical manifestations of runting and the splenic index. In the control, newborn mice were injected with rat spleen cells in a dose of $5 \cdot 10^7$–$5.5 \cdot 10^7$, the cells first being broken up by freezing and thawing three times.

EXPERIMENTAL RESULTS

Mice receiving $1 \cdot 10^6$–$1.2 \cdot 10^6$ rat spleen cells in the first 12 h after birth had no identifiable donor's cells (Table 1). After transplantation of $1.1 \cdot 10^7$–$1.2 \cdot 10^7$ cells marked retardation of general development and a regular increase in the splenic index compared with the control ($P < 0.01$) were observed. Isolated phosphatase-positive (PP) cells appeared in the bone marrow of some of the mice. No rat proteins were found by electrophoresis in the blood of these mice.

Rat immunoglobulins of types IgG and IgM appeared in the blood of 11 of the 16 mice 2.5 weeks after transplantation of $1.9 \cdot 10^7$–$2.2 \cdot 10^7$ rat spleen cells (Fig. 1). The bone marrow of the mice of this group contained 0.2–0.5% of phosphatase-positive cells, and many of them died with severe manifestations of runting (Table 1). No alkaline phosphatase was found in other lymphoid organs or in the blood, because of their inadequate content of cells of the myeloid series [1, 3].

After 5 weeks rat immunoglobulins of type IgG continued to be found in mice of this same group, in some cases, but the precipitation arcs were less distinct (Fig. 2). By this time many of the PP cells had disappeared from the bone marrow, and the manifestations of runting were much less pronounced.

Rapid development of runting coincided with the accumulation of large numbers of PP cells in the bone marrow of the mice receiving $4.2 \cdot 10^7$–$4.7 \cdot 10^7$ rat spleen cells. Clear precipitation lines were formed with their sera because of the presence of rat immunoglobulins of types IgG and IgM (Fig. 3).

Besides rat immunoglobulins, all the experimental mice contained their own immunoglobulins in their blood.

Lymphoid cells of rats, injected into mice during the first 12 h after birth, thus survive, proliferate, and function actively for 2-3 weeks from the moment of transplantation. This is confirmed by the appearance of products of their function in the blood of rat–mouse chimeras. Meanwhile, in chimeras which survive the GVH reaction, a gradual elimination of the donor's cells takes place, in agreement with other published data [6, 8, 9].

LITERATURE CITED

1. Yu. M. Zaretskaya, Radiobiologiya, No. 1, 892 (1961).
2. D. R. Kaulen, Byull. Éksperim. Biol. i Med., No. 1, 61 (1963).
3. R. V. Petrov and Yu. M. Zaretskaya, Transplantation Immunity and Radiation Chimeras [in Russian], Moscow (1965).
4. M. Burstone, Enzyme Histochemistry [Russian translation], Moscow (1965), p. 214.
5. R. Billingham et al., Ann. New York Acad. Sci., 87, 457 (1960).
6. M. J. Fox, Immunology, 5, 489 (1962).
7. N. J. Gengozian, J. Immunol., 83, 173 (1959).
8. P. Grabar et al., Immunology, 5, 673 (1962).
9. J. Howard et al., Brit. J. Exp. Path., 42, 478 (1961).
10. T. Makinodan, Proc. Soc. Exp. Biol. (New York), 92, 174 (1956).
11. T. Makinodan and N. Andersen, Blood, 12, 984 (1957).
12. M. E. Phillips and G. J. Thorbecke, Internat. Arch. Allergy, 29, 553 (1966).