SOURCES OF REGENERATION OF THE TRANSPLANTED SPLEEN

M. S. Gordeeva

UDC 612.6.03:612.411

The cell composition of syngeneic, semisyngeneic, and allogeneic grafts of the spleen was studied. Chromosome analysis showed that by the 15th day the lymphocytes and hematopoietic cells in the grafts were largely replaced by the recipient's cells. This replacement was complete by the 30th day. On regrafting of the semisyngeneic spleens back to the original donor's line it was found that the stroma was not replaced by the cells of the first recipient, but remained donor in origin during the 70 days that it remained in the F_1 hybrids. These results show that the reticular cells of the spleen stroma are a line of cells capable of prolonged self-support.

Several studies have been made of the sources of regeneration of the spleen. In particular, investigations on parabiotic mice using chromosome labeling have shown that after partial splenectomy on one of the partners, cells circulating in the blood stream participated in regeneration [4]. During regeneration of grafted spleens, cells of donor origin in the organ were replaced by the recipient's cells [6-8]. However, it has not been established whether cells derived from the blood stream participated in regeneration of the stroma. After syngeneic and semigeneic heterotopic transplanation of bone marrow, lymph glands, and thymus the stroma was not replaced by the recipient's cells, i.e., it remained donor's in origin, whereas the hematopoietic cells and lymphoctes of the grafts were replaced by the recipient's cells [1-3].

The object of this investigation was to determine whether cells of the spleen stroma are replaced by repopulating recipient's cells after heterotopic grafting of the organ.

EXPERIMENTAL METHOD

Mice of lines CBA and $CBAT_6T_6$, A, and also F_1 ($CBAT_6T_6 \times A$) and F_1 ($CBA \times A$) mice were used in the experiments. The spleen was cut into fragments 2-3 mm in diameter, and two such fragments were transplanted beneath the skin of the anterior abdominal wall of each mouse. At various times after their transplanation into the first recipient, some of the grafts were retransplanted into a second recipient.

For histological study the grafts were fixed with alcohol-formalin and by Zenker's method. Sections, 5 μ in thickness, were stained with methyl green-pyronine and azure-eosin.

Chromosome label T_6 was used to determine the origin of the cells in the grafts (from the donor or recipient). For chromosomal analysis cytological specimens were prepared from some of the 15-, 30- and 40-day grafts by the method of Ford et al. [5]. The specimens were fixed with a mixture of methylalcohol and acetic acid (3:1) and stained with azure-eosin.

The origin of the stroma was determined [3] by transplanting semisyngeneic grafts back into the line of the original donor. Retransplantation of syngeneic grafts within the same line was used as the control. The following series of experiments were performed: series I – syngeneic transplantation: CBA → CBA, CBACBA → $CBAT_6T_6$. Grafts were investigated 2, 20, 30, 40, and 70 days after transplanation. Series II – semisyngeneic transplanation: $CBAT_6T_6 \rightarrow F_1$ ($CBAT_6T_6 \times A$); $CBA \rightarrow F_1$($CBA \times A$). The grafts were investigated at the same time as in the experiments of series I. Series III – allogeneic trans-

Laboratories of Immunomorphology and Radiation Immunology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sceinces of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 7, pp. 92-94, July, 1972. Original article submitted December 30, 1971.

TABLE 1. Distribution of Metaphase Plates of Donor and Recipient among Dividing Cells in Grafts of the Spleen

Line of experimental mice		Day after grafting	Number of grafts	Number of metaphases of	
donor	recipient			donor	recipient
CBA	$CBAT_6T_6$	15	5	2	116
CBA	$CBAT_6T_6$	15	6	5	135
CBA	$CBAT_6T_6$	40	6	0	95
CBA	$CBAT_6T_6$	40	4	0	86
$CBAT_6T_6$	$F_1(CBAT_6T_6 \times A)$	30	8	0	120

planation: CBA→A; F_1(CBA×A)→CBA. Series IV – retransplanation within the same line: CBA→CBA→CBA. The grafts were studied 25 days after their transplanation into the second recipient, having remained in the first recipient for 70 days. Series V – retransplanation of semisyngeneic grafts into the line of the original donor: CBA→F_1 (CBA × A)→CBA. The grafts were studied 25 days after transplanation into the second recipient, having stayed for 15 or 70 days in the first recipient.

EXPERIMENTAL RESULTS

Most of the cells in all the grafts died during the first two days after transplanation. Only a layer of cells at the periphery of the grafts remained intact. These cells included: lymphocytes, cells of the myeloid and erythroid series, and stromal cells. By the 20th day regeneration was observed in the overwhelming majority (90%) of syngeneic and semisyngeneic grafts. Foci of myeloid and erythroid hematopoiesis with mitotic figures and megakaryoblasts were observed in them. These areas alternated with lymphoid follicles containing germinal centers. Lymphocytes frequently formed very extensive perivascular collections. In areas of lymphoid tissue, especially in the germinal centers, mitoses were found. Close to the follicles groups of plasma cells were usually seen. The syngeneic and semisyngeneic grafts were not significantly changed in the later periods (30, 40, and 70 days) by comparison with their appearance at 20 days, except that the content of brown pigment increased with the age of the graft and the capsule became thickened.

By the 14th day after transplantation the allogeneic grafts were rejected and replaced by scar tissue (series III).

Chromosomal analysis of the metaphase plates (Table 1) showed that after 15 days the overwhelming majority of free cells in the syngeneic and semisyngeneic grafts were derived from the recipient. Only the recipient's cells remained in the grafts aged 30 and 40 days.

After retransplantation the syngeneic and semisyngeneic grafts regenerated in fewer cases (from 55 to 70%) than after a single transplantation. The results of retransplantation of the semisyngeneic grafts back into the line of the original donor (series V) were not significantly different from the results of retransplantation within the same line (series IV).

The regrafts in which regeneration was observed were similar to the syngeneic and semisyngeneic grafts described above, from which they differed only in their thicker capsule and their somewhat smaller size.

After a period of destruction of some of the cells (during the first days after transplantation) regeneration of the lymphoid and hematopoietic tissue thus took place subsequently in the syngeneic and semisyngeneic grafts. Chromosomal analysis showed that by the 15th day the free cells of the grafts (lymphocytes, cells of the myeloid and erythroid series) were mainly replaced by the recipient's cells, while by the 30th day this replacement was complete. These results agree with those obtained by other workers [6-8] who determined the origin of the cells in grafts of the spleen by means of cytotoxic sera or chromosomal labelling.

As the results of this investigation showed, the stroma in the grafts was not replaced by the recipient's cells but remained of donor's origin. Evidence of this is given by the successful retransplantation carried out by the scheme: from CBA donors to F_1 hybrids and back to line CBA. If the stromal cells (on whose survival depends the success of the grafting) had been replaced in the first recipient (F_1) by its cells, the grafts would have been rejected as allogeneic when retransplanted back into the line of the original donor (CBA). In fact, as the results of the experiments of series III show, grafts transplanted from F_1 to CBA were rejected by the 14th day. Consequently, in heterotopic grafts of the spleen the stromal cells differ in their origin from the hematopoietic and lymphoid cells. The former belong to the donor, the latter to the recipient.

These grafts were essentially chimeric organs in which two lines of cells coexisted, at least for 70 days: stromal cells of donor's origin and hematopoetic cells and lymphocytes arising from the repopulating cells of the recipient.

The results show that the reticular cells of the spleen stroma constitutes a line of cells capable of prolonged self-support, and independent histogenetically from hematopoietic cells.

In this respect they were similar to the stromal cells of the bone marrow [3] and, according to the writer's observations, to the stromal cells of lymph glands and the thymus [1, 2].

LITERATURE CITED

1. M. S. Didukh, Interaction between Stroma and Lymphocytes in Grafts of the Lymph Glands and Thymus. Candidate's Dissertation, Moscow (1968).
2. M. S. Didukh and A. Ya. Fridenshtein, Tsitologiya, No. 7, 901 (1970).
3. A. Ya. Fridenshtein, K. V. Petrakova, A. I. Kuralesova, et al., Tsitologiya, No. 5, 557 (1968).
4. G. V. Kharlova, Byull. Éksperim. Biol. i Med., No. 12, 76 (1967).
5. C. E. Ford, J. L. Hamerton, D. W. Barnes, et al. Nature, 177, 452 (1956).
6. I. Green, J. Exp. Med., 119, 581 (1964).
7. D. Metcalf and R. Wakonig-Vaartaja, Lancet, 1, 1012 (1964).
8. M. Schlesinger and D. Hurvitz, J. Exp. Med., 127, 1127 (1968).