ROLE OF THE THYMUS IN POSTRADIATION REGENERATION OF THE BONE MARROW IN RATS

M. I. Fedotova
and O. I. Belousova

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Quantitative methods were used to study regeneration of the bone marrow after irradiation with Co^{60} γ-rays in doses of 400 and 700 R in experiments on 470 Wistar rats undergoing true or mock thymectomy. In the thymectomized rats the cycle of regeneration of the bone marrow cells was delayed by comparison with the control. From the 10th through 15th day the delay affected mainly lymphocytes, but later it affected mainly myeloid cells. It is concluded that in the postradiation period thymocytes migrate into the bone marrow. This phenomenon is considered to be linked with the subsequent restoration of myelopoiesis.

Information confirming the presence of a two-way connection between the thymus and the bone marrow continues to accumulate. In the embryonic period migration of stem cells takes place from the bone marrow into the thymus, where they are subjected to specific differentiating influences, after which they enter the lymphoid organs, where they give rise to the population of small lymphocytes responsible for cellular immunity. This cell population is evidently not completely self-supporting and it required reinforcement in the postnatal period [9]. Repopulation of the thymus in animals irradiated with partial screening of the bone marrow takes place as the result of migration of stem cells from the protected area [8, 15], and if transfusions of bone marrow suspension are given, it takes place on account of the donor's hematopoietic precursor cells [5, 16]. On the other hand, it has now been conclusively shown that the thymus is an organ from which many cells are continuously migrating [10-12], and that one point to which migration takes place is the bone marrow [14]. Migration of the thymocytes outside the gland has been shown to increase under stress, when the total number of cells in the thymus is reduced and the number of lymphocytes in the bone marrow is increased [2, 3, 6].

In previous experiments undertaken by the writers [1] to study postradiation regeneration of the thymus in rats irradiated with doses of 150-700 R, the mitotic activity of the thymocytes was found not to correspond to the rate of increase in the number of cells in the organ, from which it was postulated that a considerable number of thymocytes migrates from the thymus after the 7th-10th day after irradiation. This fact, it is considered, must be important to postradiation regeneration in general and to regeneration of the bone marrow in particular. It was therefore decided to continue the study of the phenomenon in irradiated thymectomized animals, with special emphasis on regeneration of medullary hematopoiesis.

EXPERIMENTAL METHOD

Wistar rats (470), weighing 160-180 g, were used. Thymectomy (TE) was performed on 235 of the animals while a mock thymectomy (MTE) was performed on the rest. The animals received a single dose of whole-body Co^{60} γ-ray irradiation 5 days after the operation, in doses of 400 and 700 R. The animals were decapitated in groups of 5-10 at a time after 1, 2, 7, 10, 12, 15, 20, and 30 days and the number of myelokaryocytes in the femur was determined [13]. The myelogram was determined from impressions of the bone marrow stained by Pappenheim's method, followed by calculation of the relative percentages

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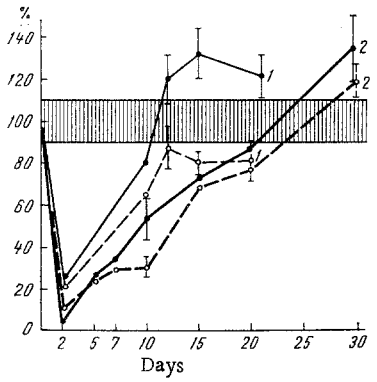

Fig. 1. Changes in total number of myelokaryocytes (in percent of control) in femur of thymectomized (broken line) and mock-thymectomized (continuous line) rats after γ-ray irradiation: 1) 400 R, 2) 700 R. Here and in Fig. 2: shaded area shows confidence interval for unirradiated rats.

of the various cell forms. The data obtained for rats subjected to TE and MTE 5 days after the operation were taken as the initial values. These animals were always taken from the same batch, and the operation and irradiation were performed at the same times.

EXPERIMENTAL RESULTS

Examination of the cell composition of the bone marrow of the intact and experimental rats showed that there were no changes in the composition of the bone marrow during the first days after thymectomy. A decrease in the number of erythroid cells, mature myeloid cells, and lymphocytes was observed only in the animals undergoing mock thymectomy.

The dynamics of the total number of myelokaryocytes in rats subjected to Te and MTE after irradiation in doses of 400 and 700 R is shown in Fig. 1. Thymectomy had no effect on the severity of the lesion in the bone marrow. Maximum destruction of the bone marrow, which developed after both doses by the 2nd day [4], was identical in the rats with TE and MTE. The beginning of regeneration and its initial rate also were identical in the two groups of animals. However, starting with the 10th day, regeneration in the rats with TE began to be slower than in the rats with MTE, and the difference was particularly marked after irradiation in a dose of 400 R.

Since bone marrow is a complex population of cells with different radiosensitivities, details are also given below of the dynamics of the three principal cell groups of bone marrow, erythroid, myeloid, and lymphoid. As Fig. 2 shows, the decrease in the number of bone marrow cells of the different types was identical in animals undergoing TE and MTE. Differences began to appear in the recovery period. The earliest and most marked differences affected the lymphocytes of the bone marrow. In the period from the 10th to the 20th day a wave of increase in the number of lymphocytes was observed in the bone marrow of the rats undergoing TE and MTE. However, the increase was much less marked in the thymectomized rats.

Restoration of the number of erythroid cells took place equally rapidly in the two groups of animals until the 10th day, at which time their initial level had been restored. Subsequently a decrease in the number of erythroid cells was observed, and this was more marked in the rats undergoing TE.

The beginning of recovery of the myeloid series of bone marrow cells was later, and whereas in the period from the 10th to the 15th days the magnitude and rate of recovery was almost identical for the rats after TE and MTE, later the degree and rate of recovery of the myeloid cell population were appreciably lower in the thymectomized animals.

It follows from these results that the most marked difference between the rats of the two groups was concerned with the number of lymphocytes in the bone marrow. To study this aspect in greater detail the number of lymphocytes in the bone marrow was studied on the 10th, 12th, 13th, 15th, 16th, and 21st days after irradiation and the mitotic index of the bone marrow lymphocytes was determined by counting 3000 lymphocytes at each of these times in impressions of the bone marrow. The results showed that between the 10th and 20th days the number of lymphocytes in the bone marrow of the thymectomized rats was 15-30 million less than in the animals undergoing the mock operation. Meanwhile the mitotic index of these cells was identical in the two groups and showed very little change during the 10 days of observation (Fig. 3).

Analysis of postradiation regeneration of the cell composition of the bone marrow in rats undergoing TE and MTE showed that it takes place more slowly in rats with TE and that differences in the period from the 10th to the 15th day were concerned mainly with the number of lymphocytes, and at later stages with the number of myeloid cells.

Many investigators are now of the opinion that the lymphoid population of the bone marrow consists mainly of cells migrating from lymphoid tissues. The nature of the exporting organs is not clear. The

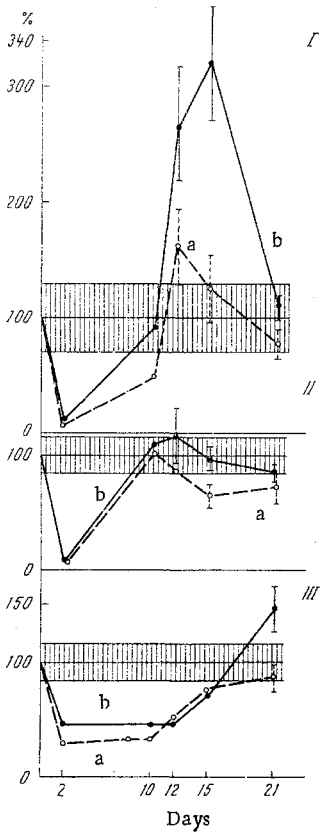

Fig. 2. Changes in number of cells of different types (in percent of control) in thymectomized (a) and mock-thymectomized (b) rats irradiated in a dose of 400 R: I) lymphoid, II) erythroid, III) myeloid cells.

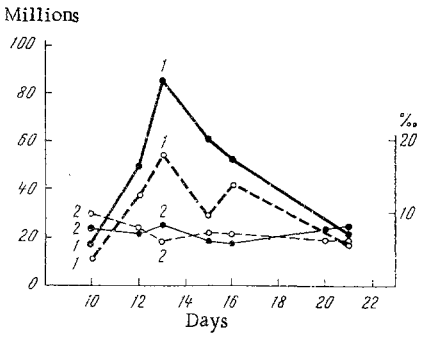

Fig. 3. Number of lymphocytes in femoral marrow and their mitotic index in thymectomized (broken line) and mock-thymectomized (continuous line) rats irradiated in dose of 400 R: 1) lymphocytes, 2) mitotic index.

results of these experiments suggest that one source of the lymphocytes for the bone marrow is the thymus gland. The wave of increase in the number of lymphocytes in the bone marrow in the period from the 10th to the 20th day after irradiation in a dose of 400 R may in fact be due to two causes: either an increase in the intensity of proliferation of the lymphocytes or their migration into the bone marrow from elsewhere. The present experiments in which the mitotic index of the bone marrow lymphocytes was determined showed that the fourfold increase in the number of lymphocytes between the 10th and 13th days and the subsequent, equally marked decrease in their number between the 13th and the 21st days were unconnected with any change in mitotic activity. This confirms the likelihood that the cells migrated into the bone marrow. The difference between the degress of migration in rats undergoing TE and MTE indicates that the thymus is one place from which this migration took place. It must be emphasized that from the 10th to the 15th day after irradiation cells capable of division reached the bone marrow, because despite considerable fluctuation in the number of lymphocytes, the mitotic index remained virtually at the same level. If cells incapable of dividing had entered the bone marrow the mitotic index at the height of the increase would have been considerably reduced because of the "dilution effect." The fate of the lymphocytes migrating from the thymus into the bone marrow is uncertain, but investigators who have observed this phenomenon attribute it to subsequent activation of myeloid hematopoiesis [7]. The results described above also suggest that these phenomena are interconnected. If regeneration of the erythroid series is unconnected with the wave of increase in the number of lymphocytes in the bone marrow, the differences in myelopoiesis detected in the rats undergoing TE and MTE after irradiation in a dose of 400 R come to light in the period of decline of the lymphoid wave. In the thymectomized rats irradiated in a dose of 700 R the number of myeloid cells was also 40% less in the period between the 20th and 30th days than in animals undergoing the mock thymectomy.

The results thus confirmed the earlier hypothesis that thymocytes migrate into the bone marrow and play a role in postradiation regeneration of the bone marrow.

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