Reaction of Different Functional Zones in Mouse Thymus and Spleen Lymphoid Tissue to γ-Irradiation

M. P. Sapin, L. M. Erofeeva, D. E. Grigorenko, and B. S. Fedorenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 4, pp. 381-384, April, 1998
Original article submitted January 16, 1997

It is shown that immune organs respond to single γ-radiation of 6.9 Gy in a cyclic manner. Acute reaction characterized by spontaneous lymphocyte lysis in the thymus and spleen develops on day 1 postirradiation and takes 3 and 7 days, respectively. This is followed by enhancement of thymocyte mitotic activity and migration of young cells to the thymic cortex and splenic lymphoid tissue. Twenty-one day postirradiation lymphoid cell populations in the thymus and spleen recover to 70-90 and 55-70%, respectively.

Key Words: *thymus; spleen; lymphocyte; γ-irradiation*

Organs of the immune system are characterized by high sensitivity to irradiation. Disturbances in the thymus and other immune systems organs induced by various types of radiation and their postirradiation recovery have been previously described in detail [1,5, 6,12]. However, peculiarities of the reaction of different functional zones in these organs remained usually beyond the scope of these investigations. The dynamics of postirradiation reparative processes is little studied. We have previously demonstrated a cyclic pattern of reparative processes in the immune system after exposure to some factors (toxicants) [4,7,8]. In the present study we investigated changes in cell populations of different thymic and splenic functional zones in mice exposed to γ-radiation in a single dose of 6.9 Gy.

MATERIALS AND METHODS

Experiments were carried out on 48 adult male BALB/c mice (8 mice per group). Experimental animals were exposed to γ-radiation (^{137}Cs, 6.9 Gy) in a Svet apparatus. Intact mice served as the control. The animals were decapitated 1,3,7,15, and 21 day postirradiation. Standard histological techniques and methods of morphometry analysis were used.

Laboratory of Functional Anatomy, Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow

RESULTS

Single irradiation in a dose of 6.9 Gy induced marked morphological alterations in lymphoid organs (thymus and spleen) as soon as 1 day postirradiation. The thymus in experimental mice was 2-3-fold smaller than in the control. We observed inversion of layers within thymic lobules, which disappeared only by the 15th days postirradiation. Cortical substance was represented by naked stroma with groups of destructively changed cells and numerous macrophages (10-fold more abundant than in the control).

In the spleen, lymphoid nodules with germinal centers disappeared on day 1 and appeared again only on day 15 postirradiation. On days 1-3, the white pulp shrank 2-3-fold in comparison with the control, but than it returned to the initial volume. The marginal zone surrounding lymph nodes and periarteriolar lymphoid sheath (PALS) was minor or absent throughout the observation period. The red pulp (on days 7-21 postirradiation) was markedly shifted to the periphery, sinuses were enlarged and

0007-4888/98/0004-0416$20.00

contained little or no lymphoid cells. Signs of karyorhexis and karyopyknosis were seen in the spleen and thymus throughout the experiment. Numerous cells underwent apoptosis [10].

Morphometrical analysis showed activation of destructive processes and complete inhibition of cell mitotic activity (MA) in the thymus 1 day postirradiation. The most pronounced changes are seen in the cortical zone 1 day postirradiation. The content of destructively altered cells in the subcapsular and central zones increased 29- and 10-fold, respectively (Fig. 1, *a*). Dividing and blast cells disappeared (Fig. 1, *b, c*). Marked reduction in the number of thymic lymphocytes (Fig. 1, *d*) was due not only to cell death and disturbances of cell proliferation and differentiation, but also to lymphocyte release into circulation. One day postirradiation we observed considerable influx of mast cells to the thymus increasing vascular permeability and facilitating migration processes (1.72±0.86%).

The content of defective cells in deep cortical layers increased, while the content of mature lymphocytes decreased until postirradiation day 3. The 3rd day was the peak of acute reaction to irradiation followed by gradual recovery of the spleen, characterized by appearance of blasts and dividing cells in the deep cortical layer. On day 7, we found only few dividing cells in functional zones of the thymus. In the subcapsular zone, MA did not returned to the control even 15 days after irradiation. It should be noted that the total number of immature cells (blasts and large lymphocytes) in the subcapsular zone increased on day 7 and to the 15th day attained 61 and 93% of the control, respectively, while the number of small lymphocytes in the subcapsular and deep layers attained 82 and 70% of the control, respec-

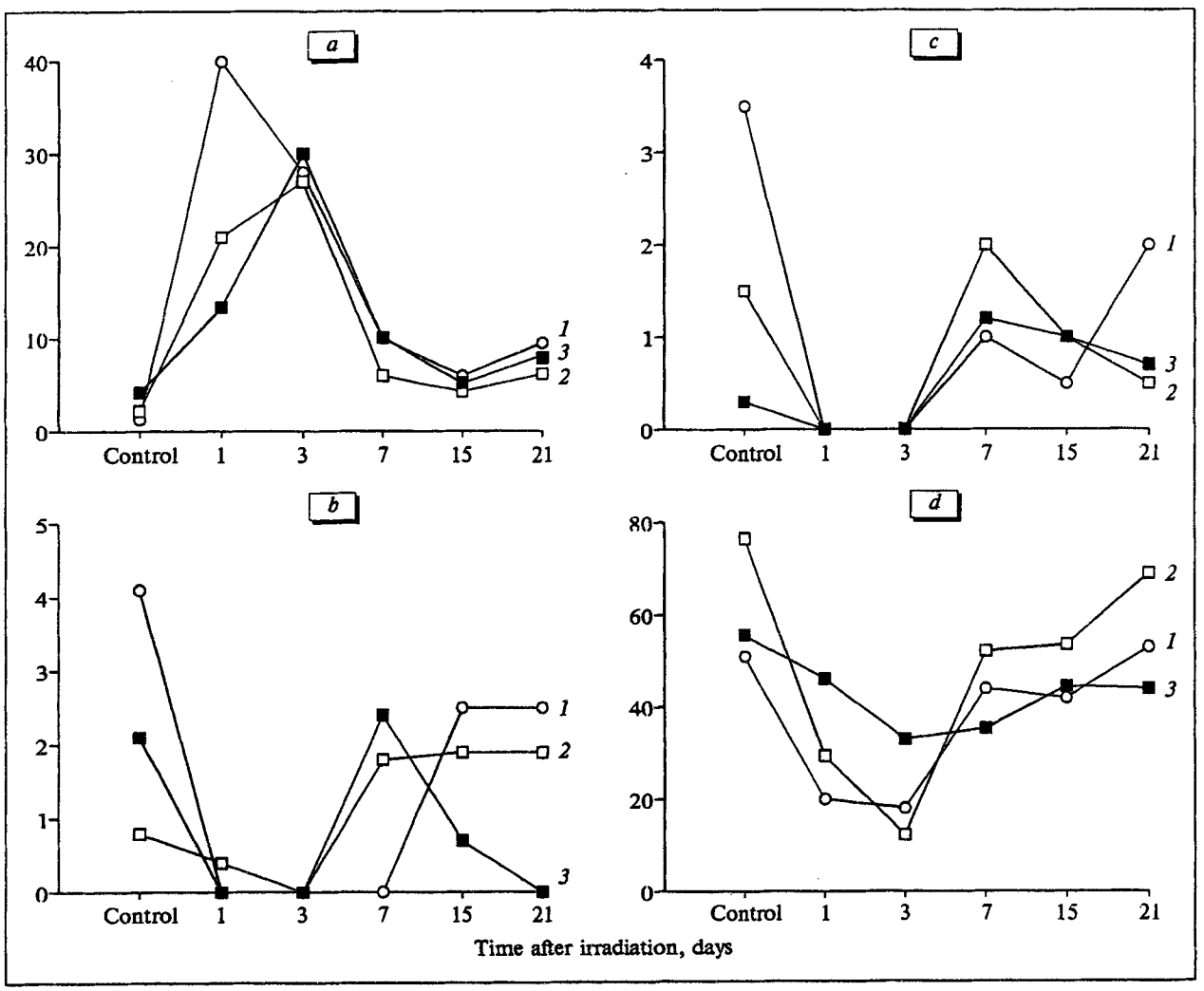

Fig. 1. Content of destructively altered cells (*a*), blasts (*b*) mitotic figures (*b*), and small lymphocytes (*d*) in different functional zones of the thymus of BALB/c mice exposed to single γ-irradiation. *1*) subcapsular zone, *2*) cortical zone; *3*) medullary zone. Here and in Fig. 2: ordinate is content of cells, %.

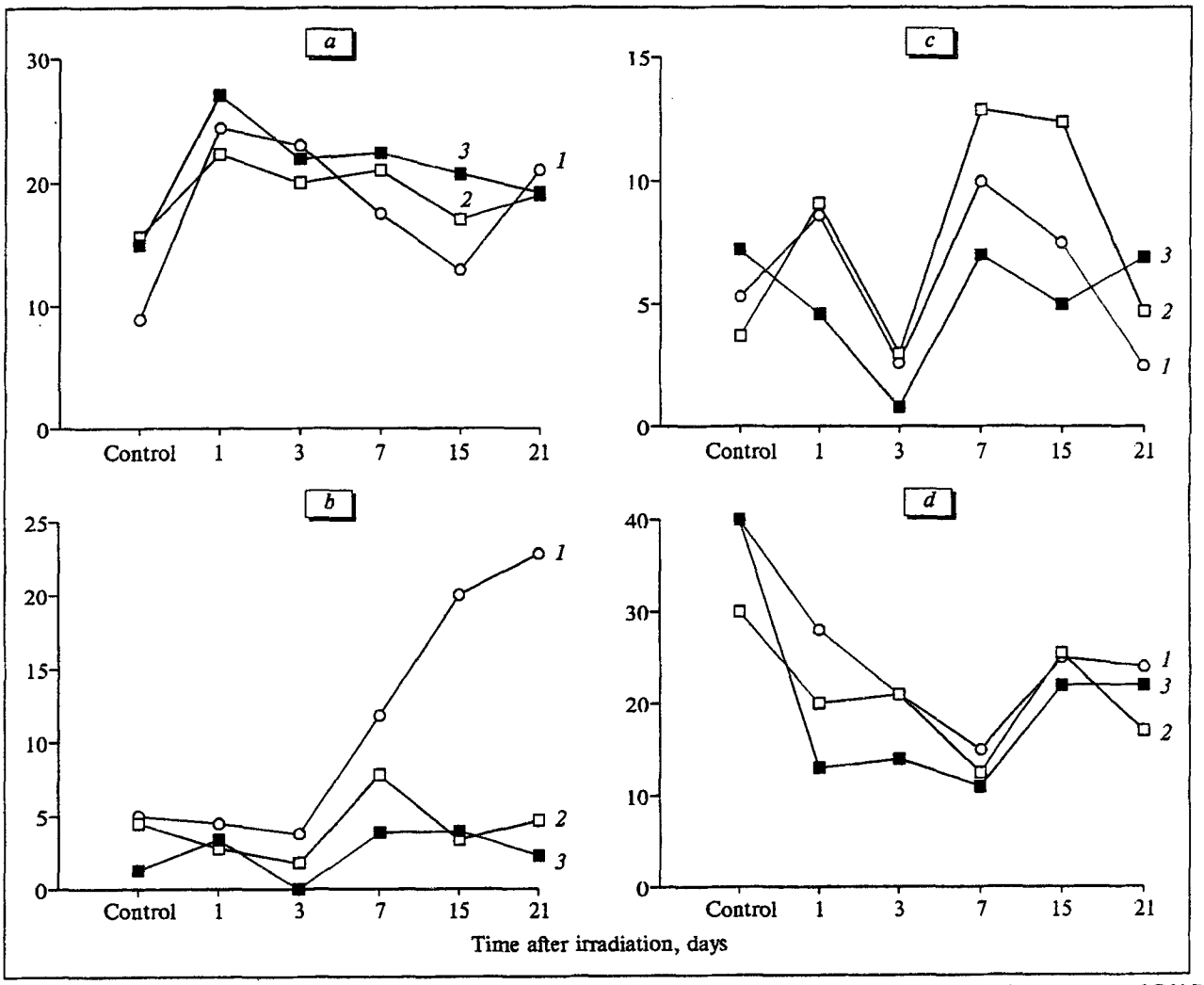

Fig. 2. Content of destructively altered cells (*a*), blasts (*b*) mitotic figures (*b*), and small lymphocytes (*d*) in splenic structures of BALB/c mice exposed to single γ-irradiation. *1*) lymphoid nodule without germinal center; *2*) periarteriolar lymphoid sheath; *3*) lymphoid elements of the red pulp.

tively. After 21 day, the content of small lymphocytes in the cortex was recovered by 90%, while there were still numerous defective cells, MA being 2-fold lower than in the control (Fig. 1, *a*, *b*).

Apart from reaction of the lymphoid component, irradiation induced the appearance of eosinophils and neutrophils in the thymic cortex. These cells usually appeared in the thymus under pathological conditions [11] associated with allergic reactions [14]

In the medullary zone of the thymus changes in the number of lymphoid cells were less pronounced; the number of destructively changed cells increased 3- and 7-fold on days 1 and 7, respectively, in comparison with the control (Fig. 1, *a*). By contrast, reparative processes were more intense in this zone. On postirradiation day 7 we observed a compensatory burst of MA: the number of mitotic figures 4-fold surpassed the control values and the content of blasts increased. However, on day 15 the number of blasts sharply decreased and on day 21 these cells were completely absent. The content of small lymphocytes in the medullary zone attained 80% of the initial level and remained at this level to postirradiation day 21.

There are several stages in the reaction of splenic lymphoid tissue in the postirradiation period (from day 1 through 21, Fig. 2). The first stage (postirradiation day 1) is characterized by intense destructive processes in all structures (Fig. 2, *a*), which are most pronounced in lymphoid nodules without germinal centers (2.4-fold compared with the control) and less active in the red pulp and PALS (1.8- and 1.4-fold, respectively).

The second stage is characterized by a decrease in the number of immature cells (primarily, large lymphocytes) and plasma cells on day 3 postirradiation (Fig. 2, *b*, *c*). The number of plasma cells in

the red pulp decreases 9-fold in comparison with the control, while large lymphocytes completely disappear. In lymphoid nodules and PLAS these changes are less pronounced: the number of large lymphocytes and plasma cells decreases 1.3- and 2.0-fold in the lymphoid nodules and 2.5- and 1.2-fold in PALS.

The third stage (postirradiation day 7) is the most dramatic period: previous cytological changes lead to a sharp depletion of all splenic structures with small lymphocytes (Fig. 2, *d*), which is most pronounced in the red pulp (3.6-folg compared with the control) and less intense in lymphoid nodules without germinal centers and PALS (2.6- and 2.4-fold, respectively).

In lymphoid nodules without germinal centers and PALS mitotic figures were noted only on day 1 and 7 postirradiation (0.9 and 0.2%, respectively), while in the red pulp and PALS they were absent throughout the observation period. In contrast to previously reported enhancement of MA in other organs of the immune system on day 7 postirradiation [3,9], reproductive processes and lymphocytopoiesis in the spleen are markedly suppressed.

The rate of recovery of splenic lymphoid tissue observed at the later stages is different in various splenic structures. In particular, on day 21 the content of small lymphocytes in lymphoid nodules without germinal centers attained 60% of the control level, in the red pulp and PALS this parameter was 57 and 55%, respectively. The intensity of destructive processes in lymphoid nodules without germinal centers surpassed 2-fold that in the red pulp and PALS. The appearance of lymphoid nodules with germinal centers on day 15 postirradiation implies a slight activation of reparative processes in the spleen, but no mitotic figures in the germinal centers were found up to day 21. The number of small lymphocytes in the marginal zone of lymphoid nodules constitutes 70%, while the number of plasma cells is 6-fold below the control values. It should be also noted that the number of neutrophils and megakaryocytes in the red pulp considerably increased during the observation period. This is probably due to migration of these cells from the blood and bone marrow (it was previously shown that cell structure of the bone marrow is recovered by 90% to the 15th day postirradiation [2]).

Thus, γ-radiation in a single dose of 6.9 Gy induces similar and cyclic changes in the thymus and spleen. The recovery is more pronounced in the thymic (70-90%) than in splenic (55-70%) structures.

The study was supported by NAS (grant 15-10110).

REFERENCES

1. E. E. Bril', N. M. Draznin, I. B. Livshits, *et al.*, *Radiation endocrinology* [in Russian], Minsk (1971).
2. Z. K. Vymyatina, in: *Sanogenic and Pathogenic Effects of Ecological Factors on the Organism* [in Russian], Cholpon-Ata (1995), pp. 90-91.
3. N. A. Gaidamakin, V. G. Petrushin, V. S. Shashkov, *et al.*, *in: Problems of Space Biology* [in Russian], Ed. N. M. Sisakyan, Moscow (1965), pp. 430-436.
4. D. E. Grigorenko, *Arkh. Anat.*, **101**, No. 7, 9-13 (1991).
5. G. P. Gruzdev, *Damage to Hemopoietic Tissues in Acute Radiation-Induced Pathology* [in Russian], Moscow (1968).
6. Yu. B. Deshevoi, B. B. Moroz, K. V. Sudakov, et al., *Byull. Eksp. Biol. Med.*, **119**, No. 4, 349-353 (1995).
7. L. M. Erofeeva, *Arkh. Anat.*, **101**, No. 11-12, 25-28 (1991).
8. L. M. Erofeeva and M. P. Sapin, *Byull. Eksp. Biol. Med.*, **119**, No. 1, 96-100 (1995).
9. M. A. Kornev, O. S. Kul'bakh, and T. N. Nad'yarnaya, in: *Sanogenic and Pathogenic Effects of Ecological Factors on the Organism* [in Russian], Cholpon-Ata (1995), pp. 30-31.
10. E. F. Lushnikov and V. M. Zagrebin, *Arkh. Pat.*, **69**, No. 2, 84-89 (1987).
11. T. A. Menyavtseva, in: *Proceedings of Tomsk University* [in Russian], Tomsk (1972), pp. 106-110.
12. N. P. Savina and A. A. Yarilin, *Immunologiya*, No. 4, 39-43 (1994).
13. M. P. Sapin and L. E. Etingen, *Human Immune System* [in Russian], Moscow (1996).
14. I. Audoim and I. Daabold, *Am. J. Pathol.*, **6**, No. 2, 85-98 (1986).