Effect of Embryo Irradiation on Development of the Thymus and Formation of the T-Lymphocyte Population

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Irradiation of pregnant mice causes destruction of the embryonal thymus and delays its colonization with lymphocytes, T-helper maturation, and elimination of T-lymphocyte precursors. After birth splenic colonization with lymphocytes, particularly with T helpers and T suppressors, is decreased.

Key Words: *thymus; T lymphocytes; gamma-irradiation*

The effects of ionizing radiation on T lymphocytes of adult animals and the regularities of their postradiation repair are sufficiently well studied [3,5]. Albeit to a lesser extent, the effects of radiation on the formed thymus are known, too [9,13,15], and it has been demonstrated that radiation injury to the thymus may trigger rapid aging of the immune system [10,16]. On the other hand, the effects of radiation during intrauterine development, when dramatic events take place in the thymus connected with mass cellular transpositions and proliferation, formation of T-lymphocyte subpopulations, and colonization of peripheral lymphoid organs with these cells, have hardly been studied. Yet these processes, which occur in the period of early thymic ontogenesis and are crucial for the subsequent existence of the immune system, may be vulnerable to radiation.

In the present research we investigated the effects of embryonal irradiation on the formation of the T-cell population in the thymus and peripheral lymphoid organs.

MATERIALS AND METHODS

Experiments were carried out with CBA mice weighing 18-20 g bred at the Stolbovaya breeding center of the Russian Academy of Medical Sciences. The presence of pregnancy was assessed from the appearance of a vaginal plug. The mice were irradiated with a Stebel' device with ^{137}Cs as the source of γ-radiation.

Thymectomy was carried out under ether narcosis via a median incision of the sternal manubrium using an aspirator [11]. The completeness of thymus removal was monitored visually during autopsy. In order to obtain B-mice, 2-month-old animals were subjected to thymectomy, 20 days later irradiated in a dose of 8 Gy, and on the same day intravenously injected 2×10^7 bone marrow cells treated with monoclonal antibodies to Thy-1.2 antigen and rabbit complement. After 30 days B-mice were used for thymus transplantation. The thymus was transplanted through a median incision in the skin of the back and posterior abdominal wall under the renal capsule without fixation of any kind. Control mice were subjected to sham thymectomies and thymus transplantation.

Cell suspensions were prepared by forcing out the organ (thymus, spleen) in a homogenizer into Eagle's medium. The cell concentration was estimated by scintillation in Goryaev's chamber. Cell viability was assessed by staining with 0.15% eosin solution. For the identification of cell markers the following monoclonal antibodies were used: G4 (anti-Thy-1.2), RL-172 (anti-L3T4) (a gift from B.

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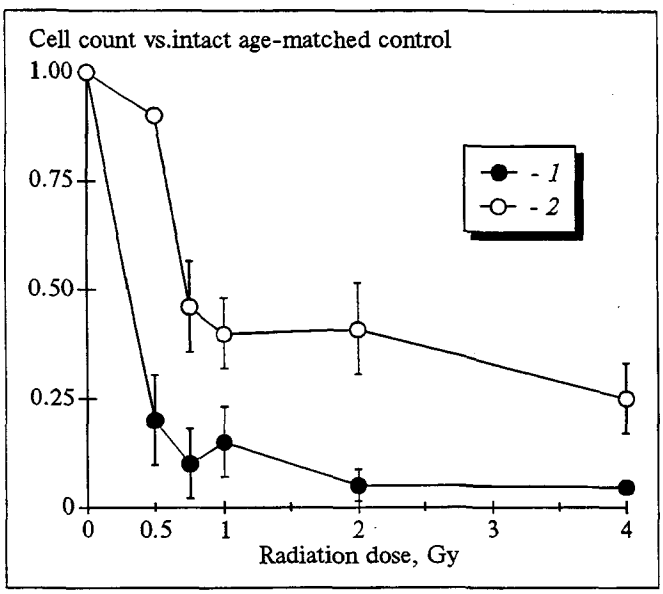

Fig. 1. Dose–dependent changes of thymic cells on day 4 after irradiation. *1*) 2–3–month mice; *2*) newborn mice irradiated on day 17 *in utero*.

D. Brondz, Russian Cancer Research Center, Moscow), and commercial (Becton Dickinson) antibodies to Lyt-2. Antiserum to SC-1 antigen was prepared by immunizing rabbits with murine brain homogenate; the resultant serum was depleted with thymocytes [8].

Membrane markers were detected by indirect immunofluorescence in suspensions of unfixed cells using fluorescein isothiocyanate-labeled IgG Fab-fragment from asinine antiserum against murine immunoglobulin (N. F. Gamaleya Institute of Epidemiology and Microbiology). For detection of SC-1 antigen the cytotoxic test with guinea pig antiserum as the source of complement was employed. Dead cells were detected by eosin staining.

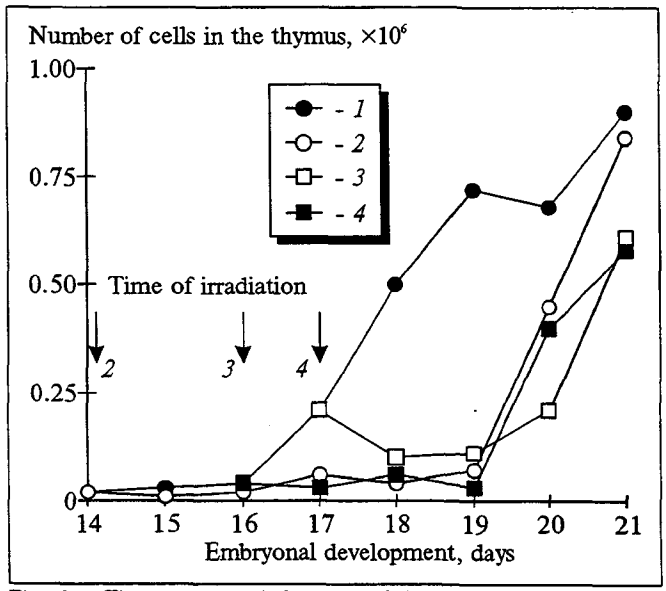

Fig. 2. Time course of thymic cellular composition increase in mouse embryos in health and after intrauterine irradiation (2 Gy). *1*) intact embryos; *2–4*) irradiated embryos: *2*) on day 14; *3*) on day 16; *4*) on day 17 of development.

The count of antibody-producing cells in the spleen was assessed by local hemolysis in liquid medium [7]. Thymocyte helper activity was assessed in an adoptive system: a mixture of sheep red cells (3×10^8), bone marrow cells (1.5×10^7) treated with antibodies to Thy-1.2 and complement to eliminate T cells, and thymocytes (1.5×10^7) was intravenously injected to lethally irradiated recipients; control mice were injected a similar mixture without thymocytes. The difference in the counts of antibody-producing cells (APC) in the spleens of experimental and control mice was the measure of T-helper activity.

Formation of hemopoietic colonies in the spleen was assessed by the exocolonies method [14]. For estimation of thymocyte helper activity a mixture of 10^5 cells of bone marrow SC-1$^-$ fraction and 10^4 tested thymocytes (without thymocytes in the control) was injected to lethally irradiated recipients. The difference between the number of colonies in the spleens of experimental and control mice was the measure of thymocyte helper activity [2].

The results were statistically processed by variational statistics methods using Student's *t* test.

RESULTS

Adult mouse thymocytes are known to be heterogeneous in terms of radiosensitivity (Fig. 1). The radiosensitivity constituent of curve *1* corresponds to cortical thymocytes (their Do is 0.5 to 1.0 Gy according to various authorities), whereas the radioresistance constituent reflects the death of mature medullary thymocytes and of subcapsular T-lymphocyte precursors (TLP), which are characterized by a relatively high radioresistance [13]. Curve *2* on Fig. 1 indicates that two groups of cells contrasting in radiosensitivity exist in the fetal thymus during the last period of embryonal development, but the share of radioresistant cells in fetuses is much higher than in adult animals. This is most probably due to a higher content of CD4$^-$CD8$^-$TLP in the embryonal thymus [12]. The reduced thymocyte count observed 1 day after embryo irradiation in the period from 14 to 19 days in dose 2 Gy indicated that the fraction of redioresistant thymocytes was higher at the beginning of this period (about 25%) than at the end (5-15%).

Irradiation of the thymus in adult animals triggered repair processes which led (in two stages)

TABLE 1. Effect of Irradiation (2 Gy) on Antibody Production of 30-Day Mice Immunized with Sheep Red Cells

Animal group	Splenic cell count, mln	APC count per spleen	APC count per mln splenocytes
Intact	180±30	10,700±1420	59±8
2 Gy on day 17 *in utero*	63±15*	24,600±320**	390±13**
2 Gy on day 5 of life	107±7	10,120±1120	95±11

Note. Asterisks show reliable differences vs. intact mice: one – $p<0.05$, two – $p<0.001$.

to a complete or partial recovery of thymocyte count and composition [3,5]. In embryos postradiation regeneration occurs in parallel with the normal processes of thymus colonization and a change of subpopulations in it. Figure 2 demonstrates changes in the time course of thymocyte counts in fetuses of various age under the effect of irradiation on days 14, 16, and 17 of development. In health the most intensive increase of the total count of thymocytes is observed between the 16th and 19th day of embryogenesis. Radiation exposure delays this process by 3 days and it is realized between the 19th and 21st days, the period of irradiation being unimportant here. Hence, irradiation of the embryonal thymus not only causes death of a certain number of thymocytes, but influences thymus "maturation" as well, but by the time of birth this process is as a rule over even after intrauterine irradiation.

Since the effect of intrauterine irradiation on the total number and time course of thymocytes is so pronounced, one might expect marked changes in the subpopulation composition of these cells. However, assessment of this parameter by scintillation of cells carrying T-cell markers (Thy-1), their subclasses (L3T4, Lyt-2), and TLP (antigen SC-1 common for hemopoietic tissue and brain) [6,8] revealed noticeable shifts only in the SC-1$^+$ cell counts (Fig. 3). In health the count of these cells in the thymus is quite high up to day 20 (on the eve of birth); at birth they are virtually undetectable (though present in small quantities, as shown by analysis of thymocyte fractions [1]). In the event of intrauterine irradiation on days 14 and 17 high numbers (20%) of these cells are detected in the newborns' thymus and even on day 2 of postnatal life (3%). Thus, irradiation delays SC-1$^+$ TLP elimination from the thymus (due to emigration or maturation) but virtually does not change the content of cells carrying the CD4 and CD8 markers.

More pronounced consequences of intrauterine irradiation are observed when assessing thymocyte functions in newborns (Fig. 4). The exposure undermines the formation of thymic T cell helper function in antibody production reactions (i.e., class 2 T helpers) to a greater extent than in adult animals and somewhat reduces thymocyte capacity to respond to PHA (irradiation of adult animals enhances this response; the data are not presented).

Since radiation exposure was found to affect TLP content in the newborn thymus, changes in characteristic thymocyte activity could be expected. It was previously shown that relatively mature TLP with the SC-1$^+$Thy-1$^+$ membrane phenotype, present mainly in the thymus, function as colony-forming cell helpers during the formation of hemopoietic splenic colonies [1]. Assessment of this TLP activity in newborns irradiated *in utero* revealed its increase after irradiation on day 14 (but not 17) of development (Fig. 4); no such effect was observed after irradiation of adult mice. An enhanced thymocyte proliferative response to thymic peptides, which is characteristic of younger SC-1$^+$Thy-1$^-$ TLP [2], is not observed after intrauterine irradiation (no data presented). Hence, in the thymus of newborn animals radiation exposure blocks SC-1$^+$Thy-1$^+$ TLP characterized by hemopoiesis helper activity.

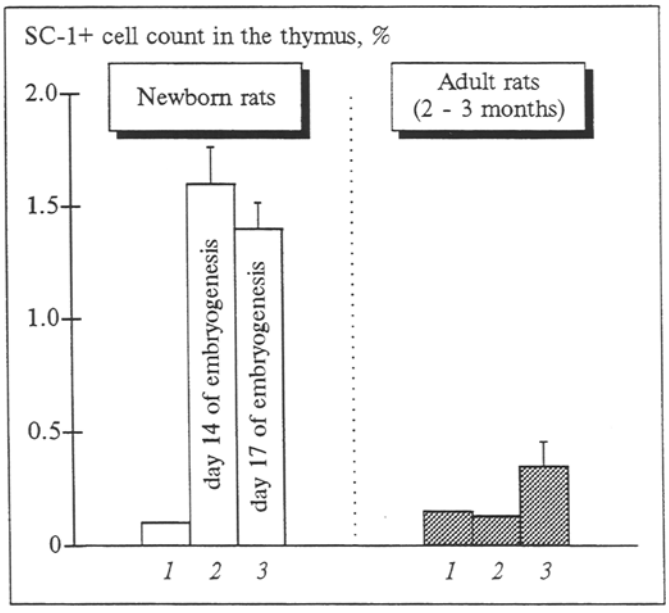

Fig. 3. SC-1$^+$ cell content in the thymus of newborn and adult mice after irradiation with a dose of 2 Gy. *1*) intact animals; *2*) irradiated 7 days before examination; *3*) irradiated 4 days before examination.

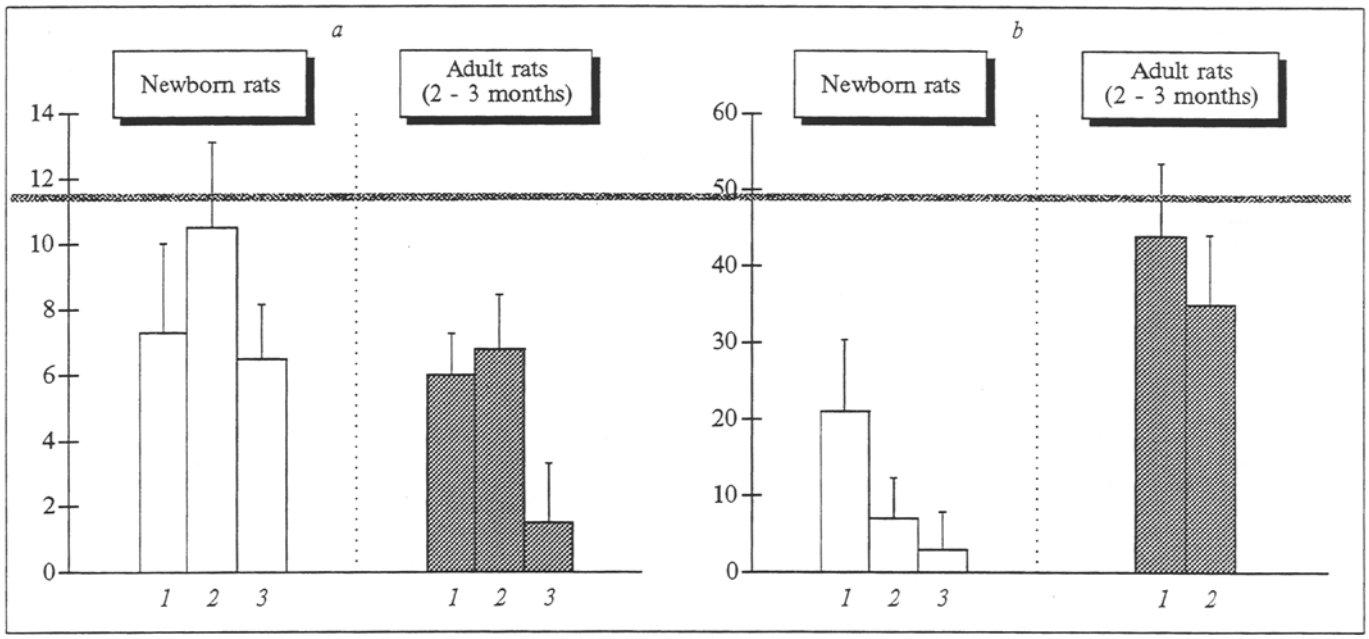

Fig. 4. Helper activity changes in reaction of splenic TLP colony formation (*a*) and hemopoiesis (*b*) in the thymus of mice irradiated with a dose of 2 Gy in the embryonal and postnatal period. *A*) newborns irradiated *in utero*; *B*) adult (2–3 months) mice. *1*) intact (control); *2*) 7 days postirradiation (*A*: day 14 *in utero*); *3*) 4 days postirradiation (*A*: day 17 *in utero*). Ordinate: *a*) increment of number of hemopoietic colonies in the spleen of bone marrow SC–1$^-$ cell recipients during simultaneous administration of thymocytes (thymocyte helper activity in splenic colony formation); *b*) increment of APC count per 10^6 splenocytes in bone marrow and sheep red cell recipients simultaneously administered thymocytes (thymocyte helper activity in humoral immune response).

Since thymus development in the embryonal period determines the formation of the peripheral T-lymphocyte population, it may be expected that intrauterine irradiation will appreciably influence the development of the thymus-dependent component of the immunity system as a whole. Moreover, the thymus determines the postnatal development of T lymphocytes, which may be disturbed as a result of radiation injury of the thymus stroma.

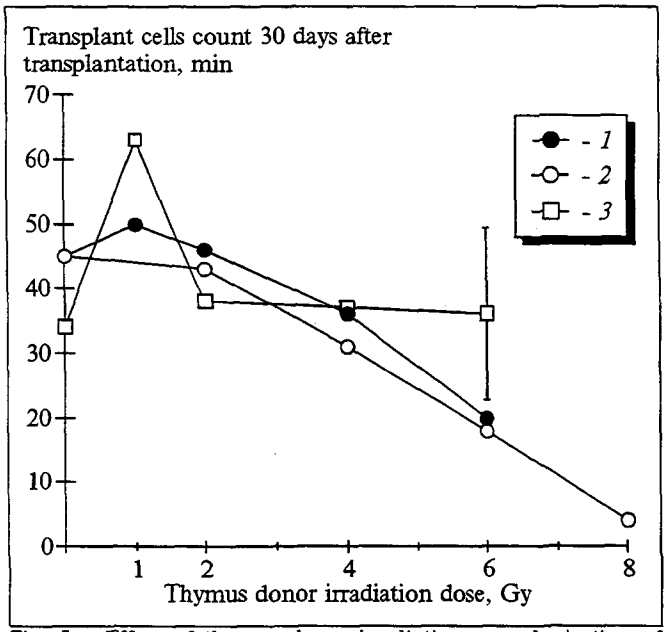

Fig. 5. Effect of thymus donor irradiation on colonization of its transplants 1 month after transplantation under the renal capsule. *1*) irradiation on day 17 of embryonal development; the thymus was removed at birth; B–mice were recipients of thymic lobes; *2*) newborns were irradiated and lobes of their thymus transplanted to B–mice on the same day; *3*) irradiation on day 17 *in utero*, newborn thymus transplanted to sham–operated irradiated bone marrow repaired mice.

Figure 5 shows the effect of intrauterine irradiation on splenic cells during the 1st month of life. Radiation exposure on day 14 of intrauterine development led to a stable defect of colonization of the spleen with lymphocytes which showed no tendency to be eliminated over the course of follow-up. Irradiation at a later period (day 17) at first did not have a noticeable impact on the formation of the spleen, but later a trend toward destruction of the organ was observed. Apparently, spleen colonization during this period was determined by descendants of the precursor cells, which suffered from intrauterine irradiation most of all. Judging by the virtually unchanged relative T-cell content during the first month of life of mice irradiated *in utero*, it may be assumed that the primitive precursor cells common to T and B lymphocytes suffer most of all from radiation in such a case.

Analysis of membrane markers showed that intrauterine irradiation caused no substantial disorders in the subpopulation composition of splenic T lymphocytes in the postnatal period. However, the

immune response of such mice (at the age of 1 month) to sheep red cells was markedly increased (Table 1), and this, together with the reduced cellular composition of the spleen, indirectly indicates underdevelopment of suppressor cells, most likely, of T suppressors.

The data on the status of the spleen in mice irradiated *in utero* do not permit us to determine which cells or structures of the fetus are injured, leading to the changes observed. In order to detect changes in the peripheral component of the immunity system which are related to radiation injury of the thymus, and notably of its stroma, we transplanted the thymus of newborn mice irradiated *in utero* or immediately after birth to intact syngeneic recipients or B-mice (i.e. thymectomized, irradiated, repaired with bone marrow cells free from T lymphocytes). One to two months after transplantation the cellular composition of the transplant was examined, and in recipients, B-mice, the status of the thymus-dependent component of the immune system. By this time the thymic transplant was colonized at the expense of precursor cell migration from recipient bone marrow. The appearance of T cells in such mice meant manifestation of stromal activity of the thymic transplant.

Figure 6 shows the relationship between colonization of the transplanted thymus stroma and the host irradiation dose. The curves demonstrate that the capacity of the thymic stroma to attract precursor cells is similarly impaired if irradiation is carried out immediately after birth or on day 17 of intrauterine development. At the same time it was shown that results of assessment of thymus colonization in "euthymic" and nude mice did not coincide: after exposure to 1 Gy, colonization of the transplanted organ in euthymic mice was sharply increased, while after exposure to 6 Gy it was decreased to a lesser extent than after transplantation to B-mice. An increased cell count after transplant exposure to 0.5-1 Gy was observed in the recipient's own thymus as well. The cause of this phenomenon is not clear; it is apparently related to some humoral factor originating from the irradiated stroma of the transplanted thymus.

An impaired capacity of the thymic stroma to be colonized with thymectomized host cells is observed after exposure to a dose of 6 Gy. The same dose induces a significant decrease of the T-cell count in the spleen of B-mice that are irradiated thymus recipients. The accumulation in the spleen of $CD4^+$ T helpers suffers even after exposure to 1 Gy (Fig. 7), this being in line with the concept of a closer relationship between T helpers and

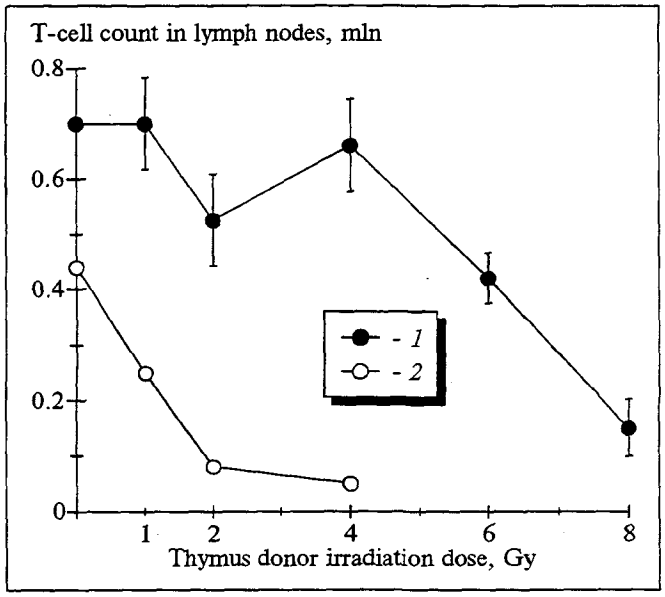

Fig. 6. Effect of thymus donor irradiation on colonization of recipient lymph nodes with T cells 1 month after transplantation. Newborn mice were irradiated in the mentioned doses; lobes of their thymus were transplanted under the renal capsule of B-mice; T-cell counts were assessed after 30 days in mesenteric lymph nodes.

the thymus and of a greater radiation injury of microenvironmental factors responsible for T-helper development [4].

The same degree of radiosensitivity is detected when assessing the formation of humoral immunity T helpers in B-mice, recipients of irradiated

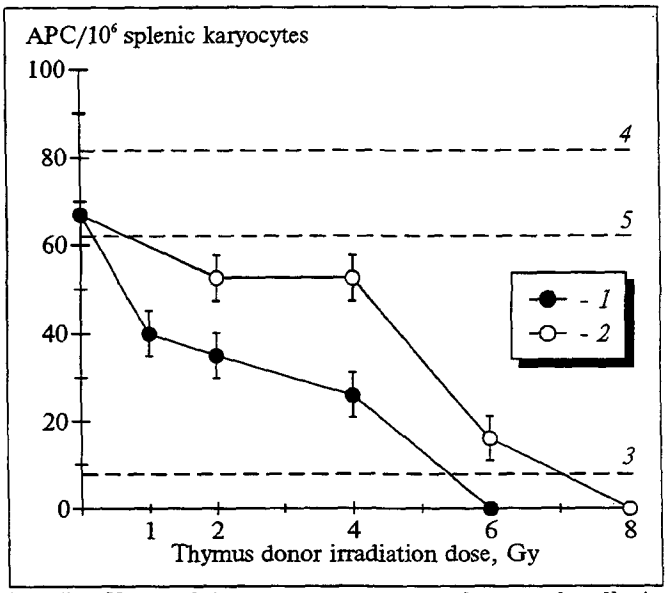

Fig. 7. Humoral immune response to sheep red cells in irradiated thymus recipients 1 month after transplantation. Experimental conditions as in Fig. 6. *1*) B-mice recipients of thymic lobes from newborns irradiated *in utero* on day 17; *2*) B-mice recipients of thymic lobes from newborns irradiated immediately after birth; *3*) B-mice without thymus transplants; *4*) sham-operated irradiated mice treated with bone marrow; *5*) similar mice transplanted intact thymus lobes.

thymus, by their capacity to produce antibodies to sheep red cells. B-mice without transplants are devoid of such a capacity and its appearance after thymus transplantation is connected with the development of T helpers in the transplanted thymus. T-helper formation in irradiated thymus recipients is suppressed if the animals are irradiated immediately after birth with a dose of 2 Gy or after irradiation *in utero* on day 17 with a dose of 1 Gy.

The data presented indicate a profound effect of intrauterine irradiation on the formation of the thymus and thymus-dependent component of the immune system. The defects connected with disordered formation of T-cell functional subpopulations and colonization of the peripheral part of the immune system are the most stable in such cases. Results of experiments with irradiated thymus transplantation give grounds for considering that these defects are caused by radiation injury not of the lymphocytes proper, but of the thymic microenvironment factors responsible for the development of T cells.

A practical result of this conclusion is the need to monitor the development of the thymus-dependent component of the immune system in children born of mothers exposed to radiation during pregnancy, as well as to develop methods of preventing and treating T cell immunodeficiencies in such children by thymic fetal tissue transplantations partially compensating for the defective stromal elements of the thymus.

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