DISTURBANCE OF THE REACTIONS OF THE HEMOPOIETIC SYSTEM IN THE LATE PERIOD AFTER IRRADIATION

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(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 4, pp. 38-42, April, 1962
Original article submitted April 22, 1961

After exposure to ionizing radiation, changes occur in the functional state of the organism and in its ability to react to certain stimuli [1, 3, 7, 8, 10]. In particular, the leukocyte reaction is modified in response to stimuli such as trauma, electric shock, intramuscular injection of milk, intravenous injection of glucose, etc., [2, 4,5,6,9, 11]. Most workers, however, have limited their observations to the period of development of radiation sickness. It therefore was of undoubted interest to study the reactions of the hemopoietic system in animals at late periods after exposure to ionizing radiation.

EXPERIMENTAL METHOD

Radiation sickness was caused by irradiation with x-rays from an RUM-3 apparatus in the following conditions: voltage 180 kv, current 20 ma, filters 0.5 mm Cu and 0.5 mm Al, dose rate 85-96 r/min, distance from anticathode 30 cm. Dose of irradiation 500 r. Irradiation as above led to death of 35% of the animals in the course of the 30 days after exposure. Investigations were conducted on 236 rats.

A leukocyte reaction was elicited by injection of Filatov's serum (1 ml into each rat, intramuscularly) 90, 180, or 270 days after irradiation. In these experimental animals we investigated the leukocyte count and formula and the composition of the bone marrow 24, 48, 72, and 96 hours after injection of the stimulus. Two groups of unirradiated rats served as controls: the first group consisted of sexually mature rats weighing 180-200 g and aged 6-10 months, and the second group was made of old rats, aged over 18 months. Marrow films for analysis of myelograms were made immediately after decapitation of the animals. In addition, the nucleated cells were counted, for which purpose the whole of the marrow was collected from the tibia and quickly weighed on torsion scales. Weighed samples were diluted with Ringer-Locke fluid in proportions of 0.1 ml fluid to 1 mg marrow. The thoroughly mixed suspension was drawn up into a white cell counting chamber with 3% acetic acid.

The cells composing the bone marrow were classified as follows: cells of the erythroblastic series (proerythroblasts, erythroblasts), cells of the granulopoietic series (myeloblasts, promyelocytes, myelocytes, metamyelocytes, stab cells and polymorphonuclear neutrophils), basophils, eosinophils, lymphocytes, and reticulo-endothelial cells. For convenience of description, all the young cells of the myeloid series (myeloblasts, promyelocytes, metamyelocytes) were combined into one group. Our numerical results were treated statistically.

EXPERIMENTAL RESULTS

Injection of Filatov's serum into control rats aged 6-10 months caused in the overwhelming majority of animals a significant increase in the leukocyte count 24 hours after its administration ($P = 0.002$). The slight fall in the leukocyte count 48 hours after injection was not significant ($P = 0.1$). The leukocyte count returned to its initial level only after 3 days (see Fig. 1a). Similar results were obtained from a study of the leukocyte reaction in the old rats (see Fig. 1b). The leukocyte reaction in the animals of both control groups was expressed as an increase in the numbers of both the adult neutrophils (with a marked nuclear shift to the left) and the lymphocytes.

The percentage of young cells of the leukoblastic series in the bone marrow of the first group of control rats was raised ($P = 0.001$) 24 hours after the injection of Filatov's serum, and the relative number of adult neutrophils

was slightly diminished or remained unchanged. It must be pointed out that the absolute number of cells in the bone marrow was also increased.

The changes discovered in the marrow in the period of leukocytosis at the periphery were evidence of the intensified leukopoietic function of the hemopoietic system. Similar changes were also found in the older rats, the only difference being that 48 hours after injection of Filatov's serum the stimulation of the myeloid series of the marrow was much more clearly defined.

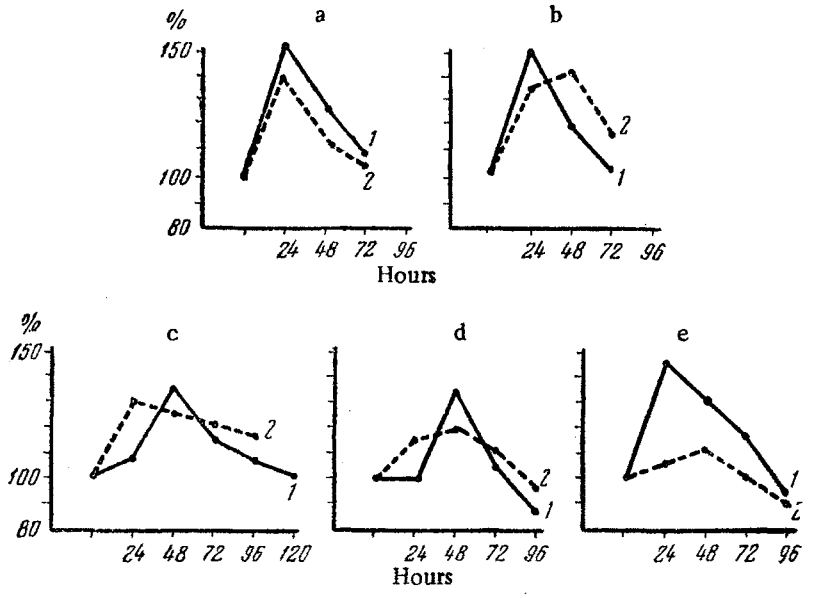

Fig. 1. Changes in the leukocyte reaction (1) and the number of young myeloid cells in the bone marrow (2) of rats after injection of Filatov's serum. a) Control rats (aged 6-10 months; b) control rats (aged over 18 months); c) 3 months after irradiation; d) 6 months after irradiation; e) 9 months after irradiation.

Ninety days after irradiation the rats appeared quite healthy and their weight did not differ from that of the controls. In the morphological composition of the peripheral blood there was only a slight increase in the number adult neutrophils. The injection of Filatov's serum into the animals of this group did not lead to an increase in the leukocyte count 24 hours after injection (see Fig. 1c). However, the differential leukocyte count showed a slight shift to the left, whereas the relative proportions of adult polymorphonuclear neutrophils and lymphocytes were unchanged (before injection 24.6 and 70.3%, after injection 25.5 and 69.9% respectively). After 48 hours the leukocyte count in the peripheral blood of the rats of this group rose sharply ($P = 0.002$) on account of both polymorphonuclear neutrophils and lymphocytes. The leukocyte count was still maintained at a high level ($P = 0.05$) 72 hours after injection of the serum, but in contrast to the previous time, this was entirely on account of lymphocytes. Only 120 hours after the injection of Filatov's serum was the initial leukocyte count observed and the normal qualitative composition of the white blood restored.

Thus 3 months after exposure to radiation, the injection of foreign protein caused a modified leukocyte reaction; the modification took the form of delay in its development and protraction of its course. The percentage of young regenerative forms of the white series in the bone marrow of these animals rose from 27.6 to 37 only 24 hours after the injection. After 48 hours the percentage of young myeloid cells remained increased (33.6%), and even 72 hours after the injection of foreign protein the myeloid series in the bone marrow was still in a state of stimulation (33.7% of young myeloid cells). Consequently, 24 hours after the injection of a foreign protein irritant into the irradiated animals a difference was observed between the reactions of the marrow and the peripheral blood. After 48 hours the reaction of the marrow and the peripheral blood was quadratic. Subsequently, the increased leukocyte count was maintained in the peripheral blood, but this increase was evidently independent of the marrow, for it was due exclusively to lymphocytes.

Six months after irradiation some animals were retarded in weight, while other rats had begun to lose their fur. The leukocyte count in the peripheral blood was normal, but the differential count showed the appearance of immature forms of neutrophils. Filatov's serum caused no increase in the leukocyte count in the irradiated animals, in contrast to the controls, 24 hours after its injection, and it did not alter the relative proportions of the cells in the blood formula. The leukocyte reaction appeared only 48 hours after the injection (see Fig. 1d), and took the form mainly of an increase in the number of lymphocytes (from 8031 to 12,139), the leukocyte count fell 72 hours after the injection and the normal blood formula was restored. In spite of the absence of a leukocytosis in the blood stream, the bone marrow of the irradiated rats showed a reaction 24 hours after the injection of Filatov's serum. The number of young regenerative cells of the white series rose from 24 to 28.2% ($P = 0.002$). The myeloid series of the bone marrow was also in a state of stimulation 48 hours after the injection, but the development of a leukocytosis in the peripheral blood was due entirely to an increase in the number of lymphocytes.

Thus, just as in the preceding period, the considerable delay in the development of the leukocytosis was evidently caused by inhibition of maturation and release of the myeloid cells from the marrow. Meanwhile, in the irradiated animals, an obvious decrease in the ability of the marrow to react was observed. The number of young myeloid cells in the marrow of the rats receiving an injection of Filatov's serum 6 months after irradiation reached 116% after 24 hours, whereas in control animals the figure was 143%. The diminished ability of the marrow to react was also shown by the fact that the leukocytosis in the peripheral blood was due entirely to an increase in the number of lymphocytes.

The animals which survived for 270 days after irradiation showed an increased leukocyte count 13,012 compared with 5771 in control rats of the same age). The differential count revealed both immature neutrophils and myelocytes. Injection of Filatov's serum against the background of this initial state of the peripheral blood caused a maximal increase in the leukocyte count after 24 hours ($P = 0.05$); after 48 hours a slight decrease was observed, and after 72 hours it returned to its original level (see Fig. 1e). Consequently, the leukocyte reaction 270 days after irradiation was nearer to the control picture. In contrast to the control animals of the same age, however, the leukocyte reaction in the irradiated animals was due wholly to an increase in the number of lymphocytes, and the proportion of neutrophils was actually lower. The slight change in the relative proportion of young myeloid cells in the marrow of the animals of this group was not significant. This condition of the marrow of the irradiated animals after injection of a protein irritant, in which a peripheral leukocytosis was due entirely to lymphoid elements, indicated the suppression of the power of the marrow to react. It must be stressed that in control animals of the same age excitation of the myeloid series in the marrow was found not only 24 hours, but also 48 hours after the injection of Filatov's serum.

Consequently, the injection of a protein irritant into animals surviving radiation sickness, against the background of a relatively sound state of hemopoiesis (3 months after irradiation), causes a disturbance in the ability of the hemopoietic system to react. The leukocyte reaction in response to the injection of a foreign protein is slow to develop and is protracted in its course. This disturbance of the response reaction is evident associated both with inhibition of the maturation of the marrow cells and with delay in their release into the blood stream. In the later periods after irradiation (9 months) the character of the leukocyte reaction approximates to that in the control animals. In the marrow, however, at this period no response reaction can be observed. At this late period after irradiation the ability of the marrow to react is disturbed to such an extent that in response to the injection of this protein irritant recourse is made to the less seriously affected hemopoietic organs—the spleen and lymph glands,—the reaction of which accounts for the development of the leukocytosis in the peripheral blood.

SUMMARY

A study was made of the reactivity of the hemopoietic system of animals at a remote period following the x-ray irradiation. Experiments were conducted on albino rats. Leukocytic reaction was provoked by the administration of Filatov's serum in 90, 180 and 270 days following the irradiation (RUM-3, x-ray unit, dose rate 85-80 r/min, distance from the anticathode—30 cm, irradiation dose—500 r).

Administration of protein stimulus to the animals with radiation sickness (against the background of relative favorable state of hemopoiesis—3 months after the irradiation), a disturbed reactivity of the blood system was provoked. Development of the leukocytic reaction in response to the administration of foreign protein is retarded and prolonged. Disturbed response is evidently connected both with the inhibited maturation of the cells in the bone marrow and their retarded exit to the peripheral blood.

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