THE LEUKOCYTIC REACTIONS OF IRRADIATED MICE TO TRANSFUSION WITH HETEROLOGOUS BLOOD

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Many authors have studied the acute (i.e., developing in the next few hours or days) leukocytic reaction to various stimuli in animals suffering from acute radiation sickness or having undergone it. Investigations have been made of the leukocytic reactions that arose in irradiated animals in response to certain physiological actions, under the influence of various traumatic factors, with parenteral injection of pharmacological preparations and other chemical compounds, and also with injection of such foreign-protein type stimuli as bacteria and their products [1, 7, 10, 11], vaccine [5], serum [9], milk [2, 4-6, 8, 9] and peptone [3].

At the same time, we did not encounter any works devoted to studying the acute leukocytic reactions due to heterohemotransfusion in irradiated animals. Meanwhile, a study of this subject would be expeditious not only from the point of view of further amassing data on the functional characteristics of leukopoiesis distributed by irradiation, but also from the aspect of ionizing radiation's effect on biological tissue incompatibility in general, and the complications of hemotransfusion in particular.

EXPERIMENTAL METHOD

Five series of experiments were set up on 52 white male rats (weighing 156-310 grams on the day of transfusion): one series on unirradiated animals and four on irradiated with respective intervals after the irradiation of 6 hours, 3 days, 4 weeks and 8 weeks. In each series there were 3 groups of experiments: one with transfusion of heterologous blood, and two controls (transfusion with physiological saline and chronological control). The number of animals in each group varied from 2 to 10.

Total irradiation with a dose of 450 r ($LD_{50/30}$) was carried out on the RUM-3 apparatus (voltage of 180 kv, current intensity of 15 ma, 0.5 mm Cu and 1 mm Al filters, dose output in air of 59 r/mm at a focal distance to the center of the body of 30 mm).

For the transfusion, we used ultraheparinized dog blood (0.1 mg of heparin per ml of blood, and the corresponding amount per ml of physiological saline in the control experiments). Transfusion was performed under sterile conditions, using 2 ml per 100 grams of body weight, into the femoral vein at a rate of 0.1 ml/sec. During the transfusion, the rat was fixed to the table with the abdomen upward.

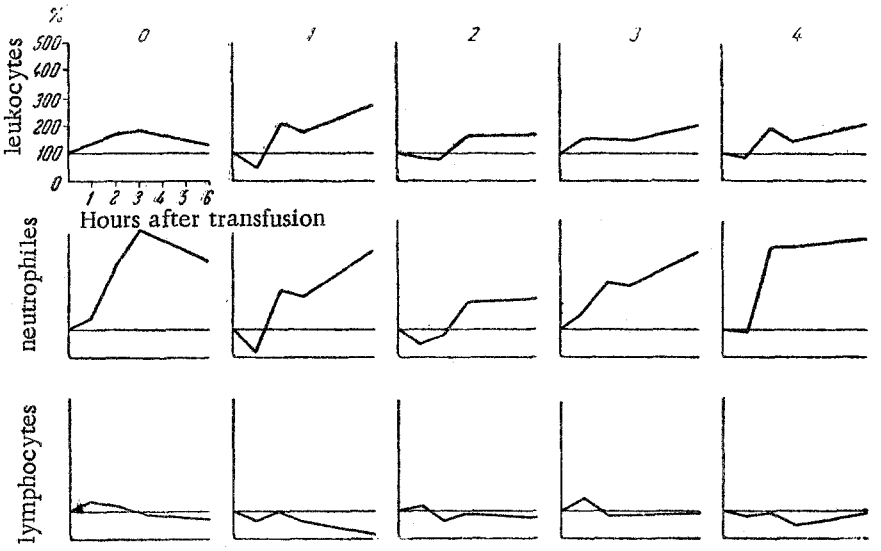
The leukocytic reactions were studied according to peripheral blood indices (total leukocyte count and leukocyte formula, according to the widely accepted method, with subsequent calculation of the absolute concentration of neutrophiles and lymphocytes). The blood samples for the analyses were taken from incisions in the caudal vein. Investigation of the blood was performed at an average of 54 ± 6 minutes before the transfusion, and 1, 2, 3 and 6 hours after it. In the series employing the irradiated animals, the initial normal level of leukocytes was also determined prior to the irradiation.

The results of the experiments were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The Leukocytic Reactions in the Unirradiated Rats. Transfusion of the heterologous blood was lethal in 3 of the 10 trials (time for death–8, 15 and 68 minutes). In the rat that died on the 68th minute, a

leukopenia (74%) was observed 8 minutes prior to its expiring, due to neutro- and lymphopenia*. In all surviving rats a leukocytosis developed, reaching levels of 139-313% in the different trials. In this case, the peak of the reaction for most of the animals was noted 3 hours after the transfusion. The leukocytosis was mixed in character (neutrophilo-lymphocytic), but the neutrophilia was manifested more markedly than the lymphocytosis, which shifted, in the majority of cases, to a lymphopenia by the end of the experiment. In one rat, the elevation in the number of leukocytes was preceded by a leukopenia (89%, 1st hour of observation). In this case, the leukocytosis was purely neutrophilic in nature, with an associated progressive lymphopenia.



The leukocytic reactions in the unirradiated (0) and irradiated (1-4) rats to administration of heterologous blood. 1 – 6 hours after irradiation; 2 – 3 days after irradiation; 3 – 4 weeks after irradiation; 4 – 8 weeks after irradiation.

Analysis of the results of the 10 control experiments showed that the rapid development of neutrophilia and slower production of lymphopenia, seen with heterohemotransfusion, are not specific for the latter, since these changes were also observed in the control trials. However, the leukocytic reactions to the heterologous blood were characterized by a greater rise in the number of neutrophiles, and the presence of a lymphocytosis stage preceding the lymphopenia. These two characteristics, taken together, also caused the development of a regular and clearly manifested leukocytosis in the presence of the heterohemotransfusion, which was absent from the control experiments.

A graph illustration of the leukocytic reactions associated with heterohemotransfusion is presented in the figure; for purposes of more effective visualization, the comparisons were made with mean data, expressed in percents (excluding the animals that died).

The leukocytic reactions in the irradiated rats developed in the setting of a profound change in the organism's reactivity, as indicated by the marked hematological shifts arising under the influence of the irradiation (see table).

Six hours after the irradiation, the heterohemotransfusion exerted a lethal effect on 1 out of 4 rats, on the 187th minute. In this trial, a rising neutrophilo-lymphocytic leukocytosis was observed, reaching 264% by the 180th minute. In the surviving rats of this series, the leukocytic reactions to the blood transfusions were characterized by the presence of a regular phase of leukopenia, down to 46-33% (in the unirradiated rats, a leukopenia was observed in 1 animal out of the seven, one hour after the transfusion, while in the irradiated rats of this series it was seen in all 3 animals; $P < 0.05$**), and by equally frequent development of a neutropenia at that time (in the unirradiated rats – in 2 trials

*Here and below, the terms "neutro- and lymphopenia (cytosis)" and their percent expression designate changes in the absolute concentration of the corresponding type of cell per mm^3 of blood.

**Here and below P represents the standard error of the difference, and analysis of the data by the method of alternative variation was performed according to Table 36 of A. Ya. Boyarskii's Manual, "Statistical Methods in Experimental Medical Investigations" [in Russian], Moscow, 1955.

out of 7; in this series—in all 3 trials; $P < 0.05$). In addition, after the transfusion we noted a tendency toward lymphopenia, more manifest than in the unirradiated animals, which was shown by the control experiments to be related to the continued postradiation drop in the lymphocyte count. The leukocytosis that arose after the leukopenia was basically neutrophilic in nature, and of an inert character, reaching its maximum (164-484%) only by the end of the experiment (in the unirradiated rats a reaction peak at the 6th hour was observed in 1 animal out of the 7; in this series—in all 3 animals; $P < 0.05$).

The State of the White Blood Cells in Irradiated Rats before Transfusion (M_2) in Comparison with the Blood Picture Prior to Irradiation (M_1)

Index			Interval following Irradiation			
			6 hours	3 days	4 weeks	8 weeks
Lymphocytes	Absolute concentration per ml of blood	$M_2:M_1$ (in %)	46	5.3	59	118
		$P_{M_2-M_1}$	< 0.01	< 0.01	< 0.01	< 0.05
Neutrophiles		$M_2 : M_1$ (in %)	204	18	146	197
		$P_{M_2-M_1}$	< 0.02	< 0.01	> 0.05—< 0.1	< 0.01
Leukocytes		$M_2 : M_1$ (in %)	17	2.7	44	100
		$P_{M_2-M_1}$	< 0.01	< 0.01	< 0.01	> 0.9

Three days after the irradiation, transfusion of heterologous blood yielded a lethal outcome in 1 rat of 6 (on the 12th minute). The leukocytic reactions to the heterologous blood in the surviving animals differed by the presence of a leukopenic phase, down to 86-53%, which was more prolonged than in the foregoing series (1st hour of observation: the presence of a leukopenia in the unirradiated rats—in 1 case out of 7, in rats of this series—in 4 cases out of 5; 2nd hour of observation: in all 7 unirradiated rats and in all rats irradiated 6 hours before the transfusion—leukocytosis, in 4 rats out of the 5 in this series—leukopenia; corresponding to $P < 0.05$). As in the foregoing series, there was an obligatory neutropenia one hour after the transfusion (in the unirradiated rats the neutropenia was observed in 2 animals out of 7, in this series, in all 5 animals; $P < 0.05$). The leukopenia and neutropenia, however, were less profound than in the foregoing series of experiments (respective mean indices for the 1st hour of observation: for the leukopenia—75 ± 4% and 40 ± 4%, $P < 0.01$; for the neutropenia—60 ± 8% and 25 ± 6%, $P < 0.02$). The posthemotransfusion dynamics of the lymphocytes were observed to be close to that seen in the unirradiated recipients. The elevation in the total number of leukocytes (up to 132-444%), arising after the leukopenia, was either neutrophilic or mixed in origin.

Four weeks after the irradiation, no deaths were observed as a result of the heterohemotransfusion, and the posttransfusion changes in the level of the neutrophiles, lymphocytes and total leukocyte count were analogous to those noted in the unirradiated animals (neutrophilia, transient lymphocytosis, leukocytosis up to 184-234%). The leukocytosis at the 1st hour of observation had mixed origin, while in the subsequent hours it was neutrophilic in character. In contrast to the leukocytic reactions in the unirradiated rats, the leukocytosis and neutrophilia in this series was observed for a more extended length of time (leukocyte and neutrophile peaks in all 3 animals of this series fell in the 6th hour of observation; out of 7 unirradiated rats, the leukocyte peak was observed at the 6th hour in 1 animal, and the neutrophile peak—in 2; corresponding to $P < 0.05$).

Eight weeks after the irradiation, transfusion of the heterologous blood was carried out with lethal complications. As in the animals that received the heterologous blood 6 hours or 3 days after the irradiation, the posttransfusion leukopenia developed by the 1st hour of observation more often in this series than in the unirradiated rats (among the unirradiated rats the leukopenia was seen in 2 cases out of 7, while in this series, it was seen in all 3 rats; $P < 0.05$). This leukopenia, reaching 89-75% in the different trials (an average of 83 ± 4%), was less profound than in the animals that were transfused 6 hours after the irradiation ($P < 0.01$), and did not essentially differ in their depth from the leukopenia seen in the series where transfusion was performed 3 days after the irradiation ($P > 0.2$). In this case, the drop in the leukocyte count occurred primarily as a result of a lymphopenia, which was character-

istically present in the 1st hour of observation in this series of experiments (in the unirradiated animals, one hour after the blood transfusion, lymphopenia was noted in 2 rats out of 7, while in the animals of this series – in all 3 rats; $P < 0.05$). The dynamics of the lymphocytes showed a predominant tendency toward decreasing, and in one animal the lymphopenia reached a depth of 22%; a stable lymphopenia was also noted in all the control rats. In contrast to all the foregoing series, the hemotransfusion leukocytosis arose exclusively from neutrophiles; its maximum magnitude was equal to 153-354% in the different trials.

Thus, the leukocytic reactions to intravenous injection of heterologous blood are still retained after irradiation, despite the profound disturbance of leukopoiesis inherent in acute radiation sickness. Along with this, it was possible to demonstrate a series of qualitative peculiarities, characterizing the direction, dynamics and interrelationships of the reactions to heterohemotransfusion on the part of the myeloid and lymphoid components of the white blood cells in irradiated recipients.

SUMMARY

Heterologous blood was transfused to intact and previously irradiated rats, the periods between irradiation and transfusion being 6 hours, 3 days, 4 and 8 weeks. For 6 hours after the transfusion a study was made of the dynamic reaction of leukocytes, neutrophiles and lymphocytes in the peripheral blood of these animals. Some postirradiation peculiarities attending posttransfusional acute leukocytic reactions were revealed.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
