Hematopoietic Stimulation and Radiosensitivity in Mice*

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ABSTRACT

Differences in 30-day percent survival in dd/YF male mice after a
single dose of 600 R were observed after three kinds of hematopoietic
stimulation, i.e., fasting and refeeding, heavy breeding and low baro-
metric treatment. Erythropoietic activity was estimated by measuring
"Fe uptake for six hours into the femur and spleen after the stimula-
tion. Number of colony forming cells in the bone marrow was deter-
mained by adopting the transplantation method. Mice appeared to be
resistant when the hematopoietic activity is high and hematopoietic stem
cells in the marrow are reduced.

INTRODUCTION

It has been generally accepted that the sensitivity to whole-body lethal irradiation is closely related to that of cells in some specific systems, i.e., blood-forming cells for LD 50 (30) range and Lieberkühn’s crypt cells for the range of intestinal death. Till and McColluch1) showed that the percentage survival of groups of irradiated mice was directly related to their content of viable marrow cells. Alexanian et al.2) suggested the correlation between the number of hematopoietic stem cells and LD 50 (30) in case of the acquired resistance after 150 R whole-body irradiation. Kallman3) tried to explain the fluctuation of the whole-body radiosensi-

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tivity after conditioning dose by the change in the radiosensitivity of the appropriate cell populations. As cited above, all studies so far published on the sensitivity to whole-body irradiations in the dose range of so-called hematopoietic death concerned with the sensitivity of the hematopoietic stem cells to radiation. Yet many factors modifying radiosensitivity remain to be elucidated such as age\(^4\), some kind of vaccine\(^5\), endotoxin\(^6\), magnetic field\(^7\), acquired resistance\(^8\), colchicine\(^9\) and urethane\(^10\).

The purpose of the present investigations is to suggest a new possible cellular mechanism to modify the sensitivity of mammals against whole-body irradiation in the dose range of hematopoietic death. The results obtained so far are not extensive enough to verify every details of the proposed mechanism but seem to support it.

**MATERIALS AND METHODS**

Male mice of a closed colony stock, dd/YF, obtained from Funabashi Farms, Chiba were used at 60–70 days of age throughout the experiments. Animal pellets produced by Funabashi Farms and tap water were given *ad libitum* except otherwise mentioned.

Three types of hematopoietic stimulation were adopted to the animals as follows: 
*Experiment 1* (Fasting and refeeding): Mice were kept in individual cages were fasted for 48 hours. Tap water and 0.9% saline solution were given *ad libitum* during fasting. For refeeding the pellets were given *ad libitum* again.

*Experiment 2* (Bleeding): The blood of about 1/50 of the body weight was taken from the inner canthus using an injection syringe.

*Experiment 3* (Low barometric treatment): Animals in individual cages were transferred to a low barometric chamber of 450 mmHg for 8 hours everyday. In the chamber fresh potato was given instead of water. Control animals were kept in a similar chamber but at normal pressure for the same time period as treated.

Animals were divided into groups of 25 to 30, and exposed to X-ray of 600 R in air immediately, 24 hours, 48 hours, 72 hours or 96 hours after the stimulation in case of (i) and (ii) and immediately after the treatment for 3 or 6 days in case of (iii) respectively. Every series included one group of control animals without any stimulation treatment. Some of the animals were lost by accidental death during low-barometric treatment.

Similarly animals were divided into groups of 5 to 10 and sacrificed at the same time as the irradiation. Bone marrow cells were taken from their femur with a definite volume of Puck's solution and the number of nucleated cells was determined under a hemocytometer. The cells of \((2.4 \pm 0.1) \times 10^6\) were injected into the tail vein of the recipient mice as described by Till and McCulloch\(^11\). The recipients were irradiated with X-rays of 900 R 24 hours before the injection and sacrificed 10 days after the injection. Number of nodules in the spleen was counted after fixation in Bouin's solution. Total number of colony forming cells per femur in relative
value was calculated from the above values.

Groups of five mice were treated in the same way as for the irradiation. One hour before the time corresponding to irradiation, except for the low barometric treatment, 0.5 $\mu$Ci of ferrous citrate $^{59}$Fe (Spec. Act. 12.8 mCi/mg Fe) was injected. Six hours after intraperitoneal injection of $^{59}$Fe, animals were sacrificed. $^{59}$Fe uptake was measured by determining the activity of $^{59}$Fe in the spleen and in the left femur cleaned from muscle tissue by using a well-type scintillation counter. The spleen was washed after preparation, in water in order to eliminate the possible contamination of the organ surface by intraperitoneal injection of $^{59}$Fe. Results are presented as percentage of the $^{59}$Fe uptake in experimental animals to that of control animals.

RESULTS

Percent survival, mean body weight, average number of nodules per spleen and average number of colony forming cells per femur at the time of irradiation for three experimental systems are shown in Figs. 1, 2 and 3 respectively. In all series about 50 percent survival was obtained in control animals irrespective of the difference in body weight. Thus the control data in three series of experiments were pooled for statistical test.

In the first experiment (Fig. 1), the percent survival decreased immediately after the termination of fasting and increased gradual-

Fig. 1. Results of Experiment 1 (Fasting and refeeding). A: percent survival for 30 days after 600R whole-body X-irradiation. Vertical bar indicates standard deviation. B: Mean body weight. Vertical bar indicates standard error of mean. C: Mean colony count in the spleen of heavily irradiated recipient transplanted with $2.4 \times 10^7$ nucleated bone marrow cells taken from the treated animals. Vertical bar indicates standard error of mean. D: Percent colony forming cells per femur calculated from the mean colony count and total number of nucleated cells per femur.
ly reaching maximum at 48 hours. These decrease and increase were statistically significant. Body weight decreased remarkably but recovered gradually till 96 hours after the initiation of refeeding. Bone marrow transplantation experiment was repeated twice, and similar pattern of change was observed. Pooled data are shown in Fig. 1. Spleen colony count was slightly increased after fasting till 24 hours but decreased remarkably at 48 hours then recovered to normal level. Total number of colony forming cells per femur rather increased after fasting, gradually decreased to the minimum at 48 hours and increased thereafter, but not statistically significant.

In the second experiment (Fig. 2) the percent survival increased significantly immediately after bleeding and remained high till 96 hours. The body weight decreased gradually after bleeding throughout the experiment. Spleen colony count
decreased after bleeding as well. But the decrease was significant only at 96 hours. Total number of colony forming cells per femur decreased after bleeding, reaching minimum value at 48 hours and increased thereafter. Oxygen tension in the muscle was measured after bleeding using a Clark's type microelectrode inserted in it. As shown in Fig. 4 oxygen tension in tissue decreased markedly immediately after bleeding probably due to anemia and increased gradually up to the normal level in 48 hours.

In the third experiment (Fig. 3), the percent survival increased significantly after the 3 day hypoxia treatment and no death for 600 R was observed after the 6 day treatment. Though there occurred a slight decrease of body weight by giving potato for water for 6 days, there was no change in percent survival. Decrease in body weight was much higher in low barometric treatment. Spleen colony count remained rather constant during the experiment but total number of colony forming cells per spleen decreased by about 50%.

Results of $^{59}$Fe uptake are shown in Figs. 5, 6 and 7. After fasting (Fig. 5) $^{59}$Fe uptake to the spleen decreased markedly and started to restore after 24 hours reaching at the normal level at 72 hours. Changes in the bone marrow were rather mild showing elevated uptake at 48 and 72 hours but remained at the normal level at
other time. After bleeding (Fig. 6) $^{59}$Fe uptake to the spleen and bone marrow showed a marked increase reaching maximum at 48 hours. But the increase was more marked in the spleen than in the bone marrow. After the low barometric treatment (Fig. 7) changes in $^{59}$Fe uptake was rather complicated. It increased in the bone marrow but decreased in the spleen after 3 day treatment. But after 6 day treatment $^{59}$Fe uptake to the bone marrow and spleen elevated to the same extent above the normal level. Six hour uptake as was done in the present experiment is generally assumed to represent the erythropoietic activity in each organ. Thus higher uptake than control means the elevated erythropoiesis as compared with control.

**DISCUSSION**

In the present study the modification of radiosensitivity against single whole-
body irradiation was demonstrated after hematopoietic stimulation. The colony forming cells in the present study can be assumed as hematopoietic stem cells. Though the hematopoietic activity as a whole was not measured, the changes in the activity can be reasonably assumed in each of three series of experiment.

It was reported by Porteus et al., that erythropoietic activity as measured by $^{59}$Fe uptake decreased markedly to 5-50 percent of normal value by fasting for 70 hours. Depression continued for 30 hours after the food was replaced and then the activity returned to normal level in 30 to 40 hours. It may be generally accepted that the depression of cell proliferation is a general result of starvation in mammals. And there is a possibility that cell proliferation increases over normal level after its cessation in order to compensate the temporary shortage induced by it.

In the present case it may be assumed that hematopoietic activity in general is depressed by starvation and restored to or above normal level 48 hours after refeeding. Erythropoiesis in the bone marrow was in good accord with this assumption though that in the spleen was depressed markedly and restored to the normal level a little later. Hematopoiesis may have a similar pattern to erythropoiesis. The curve of total number of colony forming cells may reflect the hematopoietic activity. The resting cells in the stem cell pool may be mobilized after hemopoietic stimulation. Thus the number of colony forming cells in the bone marrow may be decreased temporarily, thus result in reciprocal change to hemopoietic activity. The curve observed seems to be in good accord with the presumed activity in this respect. Radioresistance as measured by percent survival seems to change in parallel with the hemopoietic activity. Body weight appears to have correlation to radioresistance with exception of the last two points.

In the second experiment the induction of severe anemia by bleeding was demonstrated by measuring oxygen tension in tissue. Thus the restoration by increased hemopoiesis can naturally be expected which probably corresponds to the increase of oxygen tension. Erythropoietic activity as measured by $^{59}$Fe uptake was in good accord with this expectation. Colony forming cells decreased corresponding the hemopoietic activity till 48 hours and increased gradually since then after the oxygen tension returned to normal level. Radioresistance remained high for whole period of the experiment after heavy bleeding. Body weight decreased slowly but continuously till 96 hours and was rather low contrary to radioresistance of the animal.

In the third experiment the elevated erythropoietic activity could be easily recognized with deep red color of various tissues when autopsied. Bruce and McCulloch reported the increase in in vitro $^{59}$Fe incorporation into the spleen and bone marrow of mice treated with hypoxia (10.5±0.5% oxygen). In the present experiment the spleen colony count remained rather constant but total number of colony forming cells decreased gradually throughout the experiment. Erythropoiesis was markedly increased in the bone marrow reaching maximum at 3 days but slightly depressed in the spleen. But since erythropoiesis as a whole may be in-
increased at 3 as well as 6 days, the hematopoietic activity in the whole-body may remain high throughout the treatment. According to Bruce and McCulloch\textsuperscript{13} erythropoietic stimulation resulted in a marked decrease in colony forming cells, although this decrease was observed only in the spleen and not in femoral marrow. There may be some strain difference since strain difference in response to radiation of the spleen and bone marrow has been demonstrated by Tsuchiya and Hayakawa\textsuperscript{49}. Anyway, the radioresistance of mice increased after hypobaric treatment though the body weight decreased remarkably by the treatment.

As a common feature of the results of these three experiments it may be suggested that the radiosensitivity against whole-body irradiation decrease with the increase in hematopoietic activity. The number of stem cells seems to be rather decreased at the same time. At least no increase was observed in any case after hematopoietic stimulation. The suggestion appears to be contradictory to the ordinary theory in two respects, \textit{i.e.}, i) the cell population should be radiosensitive when its proliferative activity is high, and ii) the radiosensitivity of the animal depends on the number of stem cells in issue. However, there seem to be two possible explanation for the reason why animals become resistant after hematopoietic stimulation. First, hematopoietic stimulation may induce cell differentiation resulting in the decrease of stem cells and the increase of functional cells. Animal may survive the critical period after single irradiation when they have a large reservoir of differentiating as well as functional cells which are resistant to radiation at the time of irradiation.

Second, the earlier the recovery process starts, the higher the survival of animals. But the stimulation to recovery after irradiation may be mediated by reduced peripheral cells induced by radiation thus the recovery may occur too late. Under hematopoietic stimulation, the recovery will starts early enough for better survival. Smith \textit{et al.}\textsuperscript{18} reported that the repopulation of colony forming unit in the spleen and bone marrow after 400 rads of \textit{γ}-irradiation was observed earlier in the mice pretreated with endotoxin than in untreated.

According to Till and McCulloch\textsuperscript{11} $D_0$ of bone marrow stem cells is below 100 rads. Thus after 600 R surviving fraction may be $10^{-3}$–$10^{-4}$. On the other hand, change in the number of stem cells induced by hematopoietic stimulation is 0.6–0.3 at most. The latter difference may be neglected as compared with very low survival. Biological significance of surviving stem cell may be only for repopulation after the animal has survived its critical stage of functional cell depletion.

Radioresistance after hemopoietic stimulation has been demonstrated by Eskuche and Hodgson\textsuperscript{16} using erythropoietin as a stimulus and $^{59}$Fe uptake in the femur as a measure of erythropoietic activity in fasted rats. Barnothy\textsuperscript{7} reported that the radiation mortality had reduced through magnetic pretreatment. Leucocytosis was induced by this treatment. The situation seems to be quite similar to that in this study. Acquired radioresistance induced by low dose irradiation may be explained similarly.
The measurement of the hematopoietic activity as a whole is a very difficult problem. Iron uptake can only indicate the level of erythropoiesis. Determination of total number of stem cells in the body may be practically impossible, because they may be distributed in various parts of the body and relative number may vary from time to time. However, the fact that similar results were obtained in three different situations of hematopoietic stimulation may suggest that the radiosensitivity is closely correlated with the hematopoietic activity at least as far as hematopoietic death is concerned.

REFERENCES


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