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STUDIES ON TRANSPLANTATION OF BONE MARROW AND SPLEEN CELLS

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SUMMARY

The mechanisms of protection of radiation death by bone marrow transplantation and transplantation immunity using bone marrow cells and spleen cells were studied.

The results are summarized as follows;

1) Hematological and histological findings of blood forming organ in lethally irradiated mice with and without bone marrow transplantation revealed that the essential feature of prevention of acute radiation death by bone marrow transplantation is the rapid replacement of the destroyed marrow by transplanted normal hematopoietic tissue.

2) Optimal 30 days survival is seen with doses of about 1×10^8 bone marrow cells in heterologous transplantation, whereas in homologous approximately the same range of survival was obtained with doses of about 1×10^7 cells. Therefore, it can be said that the optimum dose of heterologous bone marrow cells that will protect lethally irradiated mice is about 10 times that of homologous.

3) Imprints of bone marrow and spleen from X-irradiated mice given rat bone marrow were stained for alkaline phosphatase-positive cells (rat bone marrow cells). With increase in X-ray dose exposed to mice, rat bone marrow cells persisted longer in mice, namely 3 to 4 days in 400 r and 8 to 10 days in 800 r. When it increased upto 950 r, approximately 30 to 40 per cent of donor cells were detected even after 8 days. This data confirmed repopulation theory as mechanism of action of the injected bone marrow.

4) Infusion of various doses of splenci cells of adult rat (2.5×10^7, 5×10^7, and 2×10^8 nucleated cells) could not keep lethally irradiated mice alive. However, when infused 2.5×10^7 of splenic cells from 4 hours newborn rat, 90 per cent of lethally irradiated mice survived over 60 days, yet no such protective effect was observed when used that from 72 hours infant rat. These results suggest that transplanted antibody forming cells in spleen from newborn rats acquired immunologic tolerance versus host.

I. INTRODUCTION

Lorenz, Uphoff, Raid and Shelton, successively demonstrated at first that homologous transplantation of bone marrow could be used to prevent radiation death. Since then, studies on bone marrow transplantation have bean pursued

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for the significance (it holds) in transplantion immunity. And Jacobson *et al.* recognized higher survival rate of mice exposed to X rays when splenic cells of young mice were transplanted. Makinodan *et al.* reported that the death rate within 30 days of homologous bone marrow transplantation is 39%, whereas 98% of death rate was found in case of homologous spleen transplantation. Schwartz also raported a similar finding and he accounted for the results by the action of antibody forming cells present in the transplanted splenic cells. We have studied on the mechanisms of protection of radiation death by bone marrow transplantation and also some aspects of transplantation immunity using bone marrow and splenic cells.

II. EXPERIMENTAL MATERIALS AND METHODS

A) The experimental animals consisted of Sprague-Dawley (isologous) rats, Donryu (homologous) rats, C_3H (isologous) mice and Swiss albino (homologous) mice. X-Ray irradiation was conducted with 300 KVP, 20 mam, using a 0.5 mm Cu 0.5 mm Al filter and the hvl was 1.09 mm Cu. The dose rate for mice was 48 r/min. at a distance of 40 cm.

B) The marrow was removed in ice cold Hank's solution from the femora and humeri of the animals and the cells were isolated, and then the suspension of cells was passed through white gold mesh (#80) to remove fat tissues and bone fragments, and the filtrate was centrifuged for 10 minuteds at 600 r.p.m. The cells obtained were then suspended in ice cold Hank's solution.

Spleen was excised and placed on the mesh which was dipped in the ice cold Hank's solution. Spleen was then teared on the mesh repeatedly. No appreciable cell clumping was found. The splenic cells weve resuspended in Hank's solution and the number of nucleated cells per cu. mm was determined. One ml of the suspension of bone marrow cells or splenic cells was injected into the tail vein of mice. This transplantation of cells of donor was made at the time of 24 hours after whole body X-ray irradiation.

III. TRANSPLANTATION OF BONE MARROW CELLS

Experimental results

A) Effects of X-ray irradiation on the hematopoietic organs following heterogous bone marrow transplantation

i) hematological findings

a) The peripheral blood findings of Swiss albino mice exposed to 950 r (lethal dose) whole body irradiation following transplantation of 1×10^8 Donryu rats bone marrow cells are shown in Fig. 1.

In the controls exposed to 950 r irradiation without bone marrow transplants the leukocytes showed severe decrease 24 hours after the irradiation, and to less than 1,000 cu. mm at the end of 48 hours. Thereafter, the decrease

continued and the death resulted on the 12th day after the irradiation. Blood platelets also showed conspicuous decrease on the 3rd or 4th day, and continued to do so till death occurred. The hematocrit value fell rather mildly when compared with the above two, and a sudden fall was noted from the 8th day and only on the 10th or 11th day fell to less than 5% till death occurred.

In animals with similar irradiation (950 r) following bone marrow transplantation there appeared first, as in case of the controles, decreases in leukocytes and erythrocytes as well as blood platelets, but the leukocytes began to increase on about the 7th day and continued to do so thereafter. But, recovery to the normal count was not seen even after one month. Blood platelets showed a tendency to increase, on about the 10th day, and recovery to normal value was seen at the end of one month. Recovery of erythrocyte count followed a course closely alike the platelets.

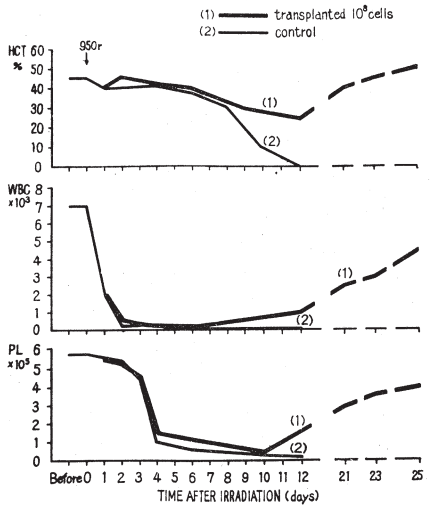

FIG. 1. Effect of bone marrow transplantation from Donryu rats on blood cells of Swiss albino mice exposed to 950 r.

b) Experiments with exposure to 400 r irradiation (Fig. 2).

In the non-transplantation group, a rapid fall in leukocyte count was noted, as in case of irradiation with 950 r, after the 2nd day, falling to a minimum count of less than 1,000 cu. mm on the 4th day, and showing a tendency to recover from the 5th day. But a return to normal was not seen even at the end of 40 days. The platelet count indicated a minimum value on the 4th day, which was however less marked than following irradiation with 950 r recovery to the normal value occurred on the 30th day. Decrease of the hematocrit value was extremely slight. In the

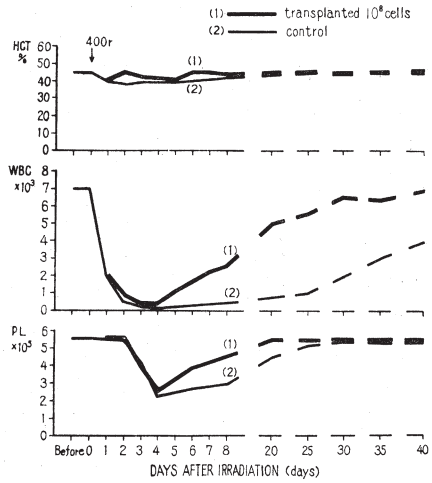

FIG. 2. Effect of bone marrow transplantation from Donryu rats on blood cells of Swiss albino mice exposed to 400 r.

group with 400 r irradiation following bone marrow transplantation there were noted decreases in leukocytes, platelets and erythrocytes as in cases of the controls, but recovery occurred early, and return to the normal values was seen on the 30th day in case of leukocytes, on the 20th day in case of platelets.

ii) Histological findings

In the group exposed to 950 r (lethal dose) destruction of marrow cells was very marked, evidenced first by high grade congestion of the bolld vessels of the bone marrow after 24 hours, and with decrease of bone marrow cells at the end of 48 hours there occurred conspicuous changes in the cyto-histological pattern, followed by disappearance of cells of the erythropoietic and granulopoietic series from the bone marrow on the 3rd day. Megakaryocytes however, remained up to the 3rd or 4th day after irradiation. In the spleen there appeared high grade congestion of the red marrow 24 hours after irradiation, as well as extreme decrease in cellularity, followed by loss of the germinal center 3 days later, and destruction with loss of the follicles and occurrence of fibrosis. The liver showed vacuolar degeneration of the hepatic cells 24 hours after irradiation.

In contrast to the above, in the group with 950 r irradiation following bone marrow transplantation, the bone marrow showed destructive changes almost similar to those seen in the controls on the 2nd or 3rd days, but the findings indicative of regeneration of marrow cells were already noted from the 5th day, recovery of bone marrow tissue accompanied by increase of cellular constituents from the 8th day, and recovery to almost the normal tissue pattern on the 12th day.

In this group with transplantation the pattern of regeneration was seen earliest in the spleen, on the 2nd day after transplantation, evidenced by appearance of groups of hyperchromic cells. These cell groups appeared in increasing numbers with passage of time and eventually come to envelope the redmarrow. On the 12th day after transplantation the picture of tissue reconstruction of the spleen was observed. And these cell groups were found in liver also. However, in the liver of the control groups with irradiation only the appearance of groups of hyperchromic cells was not observed at all.

The results obtained were of interest as they hinted the replacement of cells to be a mechanism of marrow transplantation. Also, from the blood and histological findings it become clear that in the present series of experiments on radiation hazard the direct cause of death can be said to be the destruction of tissues of the bone marrow and other organs. Such destruction of bone marrow tissue was, however, almost completely repaired histologically on about the 12th day in the group with bone marrow transplantation, and such a transplantation prevented from death due to acute radiation hazard.

B) On the cell dose in heterologous and homologous transplantation of bone marrow

a) Survival of C_3H mice irradiated with 950 r, followed by transplantation of Sprague-Dawley rat bone marrow cells in doses of 1×10^6, 1×10^7, 1×10^8, is shown in Fig. 3.

In the groups with no transplantation all animals died within 12 days after irradiation. However, even in the groups with transplantation doses of 1×10^6 and 1×10^7 all animals died within 20 days. When the dose was raised to 1×10^8 the death rate fell to 47% after the 12th day and this rate fell further to 37.5% when the transplantation dose was increased to 2×10^8.

b) The survival rate of Swiss albino mice irradiated with 950 r, followed by homologous transplantation of mice bone marrow cells in doses of 1×10^7, 5×10^7 and 1×10^8, are shown in Fig. 4.

It will be seen that in the group with no transplantation 70% of the animals died within 10 days and 90% within 19 days, but in the group transplanted with 1×10^7, 5×10^7 and 1×10^8 marrow cells the death rate was only about 40% after the 12th day.

From the results of the above two series of experiments it may be said that in case of heterologous transplantation there is need to transplant about 10 times the number of cells necessary in case of homologous transplantation.

c) Persistence of bone marrow cells transplanted.

Bone marrow cells of Donryu rats were transplanted to Swiss albino mice that had received whole body irradiation with 950 r, and at definite intervals

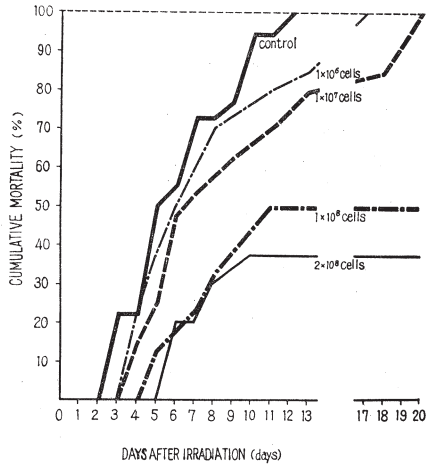

FIG. 3

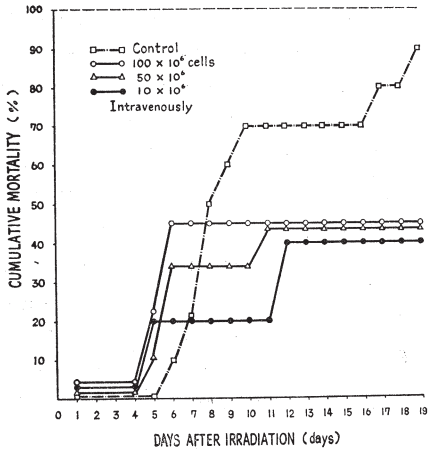

FIG. 4

FIG. 3. Effect of bone marrow transplanation of Sprague-Dawley rats on survival of C_3H mice exposed to 900 r.

FIG. 4. Effect of homologous bone marrow transplantation on survival of Swiss albino mice exposed to 950 r.

the animals were sacrificed and alkaline phosphatase positive cells (rat's bone marrow cells) in the marrow and spleen of the host animals were examined according to Gomori's method, and the results are shown in Fig. 5.

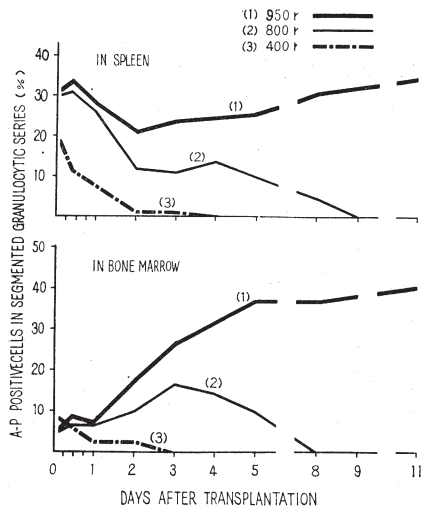

FIG. 5. Alkaline phosphatase-positive cells in spleen and bone marrow mice given rat's bone marrow.

In non-irradiated mice transplanted with rat bone marrow cells there appeared small numbers of alkaline phosphatase positive cells (transplanted rat cells) in the spleen and bone marrow 4-8 hours after the transplantation, but no such cells were recognized at all at the end of 24 hours.

In the groups that received irradiations of 400 r, 800 r and 950 r there were seen in the spleen 18%, 28% and 32% respectively of alkaline phosphatase positive cells, 4 hours after the transplantation of rat bone marrow cells, and in the bone marrow 8%, 5% and 5.5% of these cells respectively. In the group irradiated with 400 r a gradual decrease of these positive cells occurred, and they disappeared on the 3rd or 4th day. In the 800 r irradiated group they disappeared on the 8th or 9th day, while in the 950 r irradiated group a temporary decrease occurred on the 2nd day, followed by an increase and these alkaline phosphatase positive cells were seen in both bone marrow and spleen in proportions of 30-40% on the 11th day.

The results showed that with increase in dose of irradiation there was noted prolongation of persistence of the transplanted cells within the organs, indicating repopulation mechanism in the bone marrow transplantation resulted in protection of radiation death.

Also, with sublethal doses of 800 r and 400 r there was noted a relation between irradiation does and time for rejecting heterologous marrow cells. In other words, the activity of the host to reject heterologous cells recovers more rapidly the lower the irradiation dose, and prevents the persistence of the transplanted cells.

IV. TRANSPLANTATION OF SPLENIC CELLS

Experimental results

a) Into C_3H mice exposed to 900 r total body X-irradiation, 2.5×10^7, 5×10^7 and 2×10^8 splenic cells of adult Sprague Dawley rats were injected intravenously,

and the results are shown in Fig. 6.

It will be seen that in all the groups, irrespective of numbers of cells transplanted, the survival curve closely resembled that of the non-transplanted X-irradiated group, and all animals died within 13–14 days following irradiation.

b) Splenic cells of newborn Sprague-Dawley rats (4 and 72 hours after birth) were transplanted into C_3H mice exposed to 900 r total body X-irradiation, and the results are shown in Fig. 7.

It will be seen that when 2.5×10^7 splenic cells of rats 4 hours after birth were transplanted the survival rate on the 6th day is 90%, and remained so even on the 60th day after irradiation. In contrast when 2.5×10^7 splenic cells of rats 72 hours after birth were transplanted no effects were noted at all.

From the above results it seems that in case of heterologous splenic cell transplantation no shortening of the survival time will occur, as has been reported for cases of homologous spleen transplants when the transplanted cell number is increased. Also, no prolongation of survival period was seen when given heterologous splenic cells from adult animal as in case of bone marrow cell transplantation. These are believed to be due to the lack of hematopoietic ability in the spleen of adult rats.

And the above suggests that immunologically the incompatible antibody forming cells in the spleen from new born rats come to acquire immunologic tolerance for the antigens in the host. It would be much interesting that the above results were obtained in case of heterologous transplantation cells.

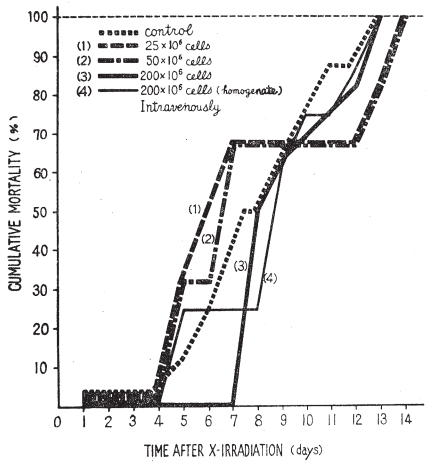

FIG. 6. Effect of spleen cells of Sprague-Dawley rats on survival of C_3H mice exposed to 900 r total-body X radiation.

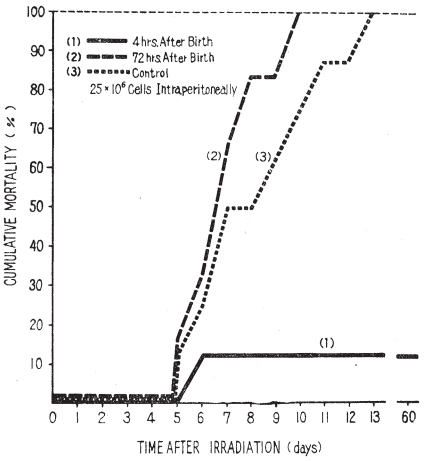

FIG. 7. Effect of transplanation of splenic cells from new born Sprague-Dawley rats on survival of C_3H mice exposed to 900 r.

V. DISCUSSION AND SUMMARY

1) Peripheral blood findings and histological data of blood forming organ

in lethally irradiated mice with and without bone marrow transplantation revealed that the essential feature of prevention of acute radiation death by bone marrow translantation is the rapid replacement of the destroyed marrow by normal hematopoietic tissue.

2) A dose-response relationship between the amount of bone marrow cells injected intravenously and survival after X-ray exposure has been studied in homologous and heterologous experiments. Optimal 30 days survival is seen with doses of about 10×10^7 bone marrow cells in heterologous transplantation, whereas in homologous approxiamately the same range of survival could be obtained with doses of about 10×10^6 cells.

Therefore, it can be said that the optimum dose of heterologous bone marrow cells that will protect lethally irradiated mice is about 10 times that of homologous.

3) Imprints of bone marrow and spleen from X-irradiated mice given rat bone marrow were stained for alkaline phosphatase-positive cells (rat bone marrow cells). Donor cells were detected in bone marrow and spleen of non-radiated mice given rat marrow cells but none were seen 24 hours after treatment.

With increase in X-ray dose exposed to mice, rat bone marrow cells persisted longer in mice, namely 3 to 4 days in 400 r and 8 to 10 days in 800 r. When it increased upto 950 r, approximately 30-40 per cent of donor cells were detected even after 8 days. This data confirmed repopulation theory as mechanism of action of the injected bone marrow which has already been reported by many authors by different ways. In addition, our data imply that degree and time of fatality of the treated animals depend on the dose or with sublethal doses, and that the immune mechanism recovers faster with subsequent rejection of foreign marrow.

4) Transplantation of splenic cells is one of the most interesting features in view of incompatible antibody-forming cells in spleen.

We have used this experiment in heterologous system, *i.e.*, Sprague-Dawley rat as a donor and C_3H mice as a host. Infusion of various doses of splenic cells of adult rat (200×10^6, 100×10^6 and 50×10^6 nucleated cells) could not keep lethally irradiated mice alive. However, when infused 20×10^6 of splenic cells from 4 hours newborn rat, 90 per cent of lethally irradiated mice survived over 60 days, yet, no such protective effect was observed when used that from 72 hours infant rat.

It is extremely of interst to note that our result strongly suggests that transferred incompatible antibody forming cells in spleen from new born rats acquired immunologic tolerance versus host.

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