STUDIES OF HAEMOPOIETIC STEM CELLS AND MICROENVIRONMENT IN CHRONIC IRRADIATED MICE

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Abstract

Mice were irradiated at a dose rate of 70 rad per day for 25 days. Changes in properties and functions of haemopoietic stem cells and microenvironment were observed through a period of 12 months after termination of continuous irradiation. It was shown that the radiation damage of haemopoietic stem cells played an important role in the radiation-induced damage of haemopoiesis. This was further supported by the fact that transplantation of syngeneic bone marrow cells immediately after termination of continuous irradiation at 70 rad per day for 25 days would greatly improve the haemopoietic function of the irradiated mice, including the total recovery of CFU-S and numbers in the bone marrow and cell counts in the peripheral blood.

Introduction

The haemopoietic organ is one of the most radio-sensitive organs and plays an important role in the development of radiation sickness. Within the haemopoietic organ there are haemopoietic cells and stromal cells which form the haemopoietic microenvironment. The haemopoietic stem cell is the primitive progenitor which has the potential to give rise to the various lines of blood cells including lymphocytes and is highly sensitive to ionizing radiation. The microenvironment is the internal milieu which provides the optimum conditions for normal haemopoiesis. It is possible, therefore, that the radiation damage to haemopoietic function involves both haemopoietic stem cells and their microenvironment.

For the elucidation of the underlying mechanisms involved in radiation damage and subsequent recovery of haemopoietic function, the mutual influence between haemopoietic stem cells and their microenvironment has been studied by many workers[1-6].

Based on our previous work we present in this paper the results of a long-term observation of the radiation effect on haemopoietic stem cells (CFU-S) and the microenvironment after 25 days of continuous irradiation at a low dose rate of 70 rad per day and peculiarities of recovery of radiation-damaged haemopoiesis elicited by the transplantation of normal bone marrow cells from the syngeneic animals.

I. Materials and Methods

Animals: Animals used in this work were inbred LACA or C57 mice. At the beginning of the experiments the mice were 9—11 weeks old.

Irradiation condition: In acute irradiation, gamma rays generated from a ^{60}Co source with a dose rate of 85—89 roentgen per minute were used. Under the condition of chronic irradiation animals were irradiated continuously for 22 hr and a total of 70 rad per day was given.

CFU-S assay: Groups of 15 recipient mice were irradiated with 800 rad and injected with a suitable number of bone marrow or spleen cells through the tail vein. Nine days later, the spleens were excised from the recipients, fixed in Bouin's solution and colonies formed on the spleen surface were counted under dissecting microscope[7].

Assay for self renewal ability of CFU-S: A group of 20 mice, given 800 rad irradiation, were injected with 3×10^4 bone marrow cells. Nine days later, 15 of the mice were killed and the spleen colonies were counted. On the 10th day, spleen cell suspensions from the remaining 5 mice were prepared to assay their CFU-S content by spleen colony technique. Thus, the average content of CFU-S in each spleen colony on the 10th day after transplantation can be calculated.

Differential count of bone marrow cells: Bone marrow cells were expelled from a femur and smeared on a clean glass slide. After being stained with Wright and Giemsa staining solution granuloid and erythroid cells were enumerated respectively by counting 500 nucleated bone marrow cells for each sample.

Karyotype analysis was done by the c-band staining techniques. The method of c-band staining is similar to that described in our previous paper[8], but with slight modification as follows: three hours after injection of colchicin (7 μg/g body weight), the bone marrow cells from test mouse was flushed into a 0.85% sodium chloride solution, treated in a hypotonic solution of 0.078 *M* potassium chloride at 37°C, then fixed in a 3:1 mixture of methyl alcohol and glacial acetic acid, finally mounted on slides. The slides were inserted into a 0.2 *N* hydrochloride solution for 20—30 min, followed by the treatment in a 5% barium hydroxide solution for another 20—60 min, and finally stained with Giemsa. More than 30 dividing cells in metaphase for each test mouse were arbitrarily chosen for analysis of sex chromosomes.

II. Results

1. *Comparison of the Radiation Effects on Haemopoietic Stem Cells After Acute or Chronic Irradiation*

Fig. 1 shows that one month after termination of 490 rad, either acute or continuous irradiation at a low dose rate of 70 rad per day, the number of femoral CFU-S approached the level found in age-matched normal mice. During the period of continuous observation up to 12 months, it was found that the content of CFU-S per femur in the normal mice increased slightly with the increase of age. In the irradiat-

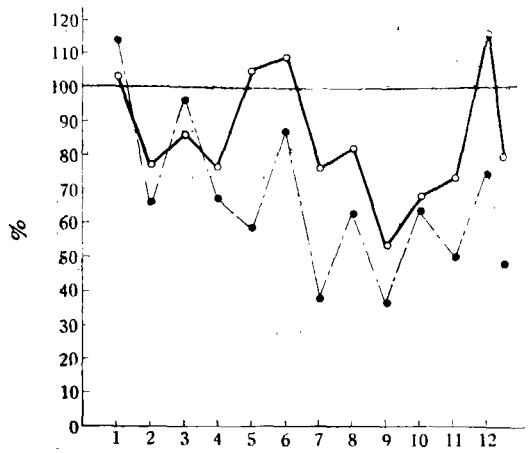

Fig. 1. Comparison of the femoral CFU-S after 490 rad acute irradiation or continuous irradiation at dose rate of 70 rad per day for 7 days (each experimental point was the average value from a group of 3 mice).

—— Normal control mice;
O mice after continuous irradiation;
● mice after acute irradiation.

ed mice, however, the content of femoral CFU-S was obviously lower than that of the normal mice of the same age. Although the total radiation doses in these two groups were the same, the biological effect of radiation on the CFU-S is slightly more serious in the acutely irradiated group than that in the chronically irradiated group.

The haemopoietic function of the bone marrow gradually recovered after the

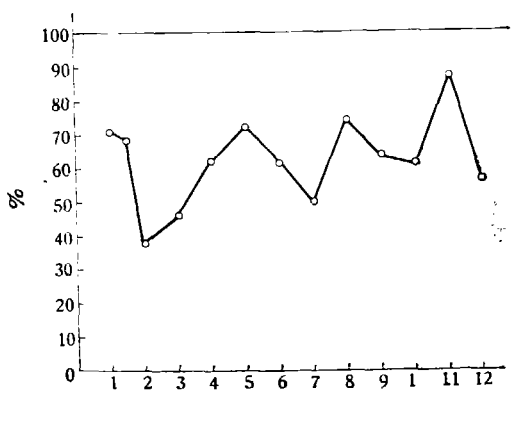

Fig. 2. Recovery of femoral CFU-S after termination of 25 days of continuous irradiation at a dose rate of 70 rad per day (each experimental point was the average value from a group of 3 mice).

—— Normal control mice.
O mice after continuous irradiation.

Table 1

Comparison of Self-renewal Ability of CFU-S After Termination of 25-day Continuous Irradiation at a Dose Rate of 70 Rad Per Day

Time After Termination of Continuous Irradiation (month)	CFU-S/Spleen Colony		
	(1)	(2)	(3)
1.5	21.1	9.6	—
2.0	18.6	3.5	—
3.0	11.9	12.7	4.3
4.0	20.8	9.3	6.7
5.0	18.7	7.0	10.5
6.0	13.7	4.0	2.6
7.0	—	—	—
8.0	11.5	4.9	2.1
9.0	6.3	4.2	3.6
10.0	8.4	16.5	3.0
11.0	—	—	—
12.0	21.4	4.6	2.1
Average value±S.D.	15.2±5.6	7.6±4.4	4.4±2.9

Note: (1) Normal control mice of the same age as other groups of animals. (2) Mice were irradiated at the dose rate of 70 rad per day for 25 days. (3) 2×10^7 femoral bone marrow cells (19.3 CFU-S/10^5) from the mice which have been 30 days after termination of 25-day continuous irradiation at a dose rate of 70 rad per day were transfused into a group of 800 rad acute irradiated mice.

termination of 25 days of continuous irradiation at a dose rate of 70 rad per day. A comparison between Fig. 1 and Fig. 2 shows that the recovery process of murine femoral CFU-S became slower as the accumulated radiation dose increased. Compared with normal mice of the same age, the CFU-S content in the irradiated femur

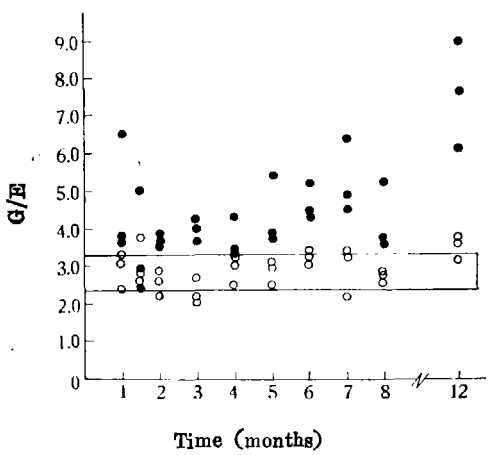

Fig. 3. Changes of G/E ratio in murine bone marrow after termination of continuous irradiation at dose rate of 70 rad per day for 25 days (mean value±S.D.).

○ Normal control mice

● mice after continuous irradiation.

remained at a lower level throughout the whole 12 months observation period. The accumulated data presented in Table 1 indicate that the deterioration in self-renewal ability of CFU-S is the determining factor which influences the full recovery of haemopoietic function after long-term continuous irradiation.

During the long observation period of 12 months after termination of 25 day continuous irradiation at low dose rate, the total number of both femoral nucleated cells and femoral granuloid cells recovered approximately to the pre-irradiation level, but the number of erythroid cells remained at a lower level than in the normal mouse of the same age. Therefore, the ratio of granuloid to erythroid elements in the femur, as shown in Fig. 3, is much higher than that in animals of the same age. This supports the view that haemopoietic damage resulting from the long-term continuous irradiation at low dose rate involves both the haemopoietic stem cells and the microvironment.

2. *Comparison of the Radiation Effects on Haemopoietic Stem Cells and Microenvironment*

The following experiments were designed for comparative studies of the radiation effects on haemopoietic stem cells and microenvironment.

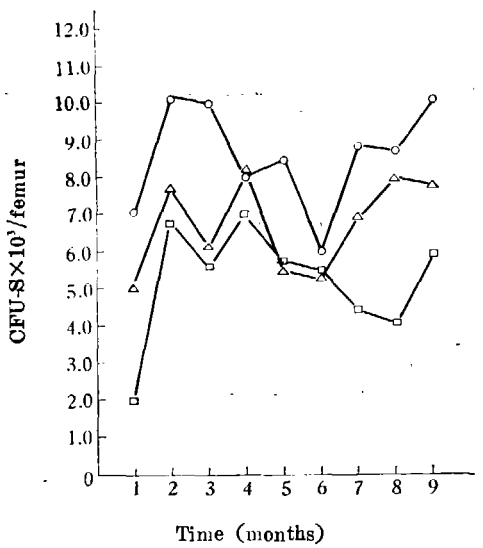

Fig. 4. Comparison of the recovery processes of femoral CFU-S content in three groups of experimental animals. ○, LACA mice were irradiated with a single dose of 800 rad and injected with 1×10^7 normal bone marrow cells (27.3CFU-S/10^5) from the same strain mice; △, mice 30 days after termination of continuous irradiation were given 800 rad of acute irradiation and injected with 1×10^7 normal bone marrow cells (27.3 CFU-S/10^5) from the same strain mice; □, 2×10^7 femoral bone marrow cells from the mice which have been 30 days after termination of 25 day continuous irradiation at a dose rate of 70 rad per day were injected into a group of mice irradiated a single dose of 800 rad.

First group: Mice were irradiated with acute irradiation of 800 rad and injected with 10^7 femoral bone marrow cells (27.3 CFU-S/10^5 bone marrow cells) from normal mice of the same age.

Second group: Mice 30 days after the termination of 25 days continuous irradiation at 70 rad per day, were given 800 rad of acute irradiation and injected with 10^7 femoral bone marrow cells (27.3 CFU-S/10^5 bone marrow cells) from normal mice of the same strain.

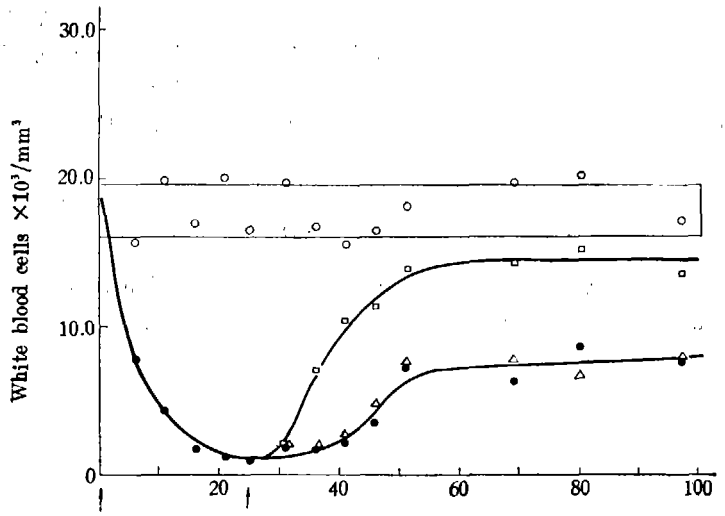

Fig. 5(a)

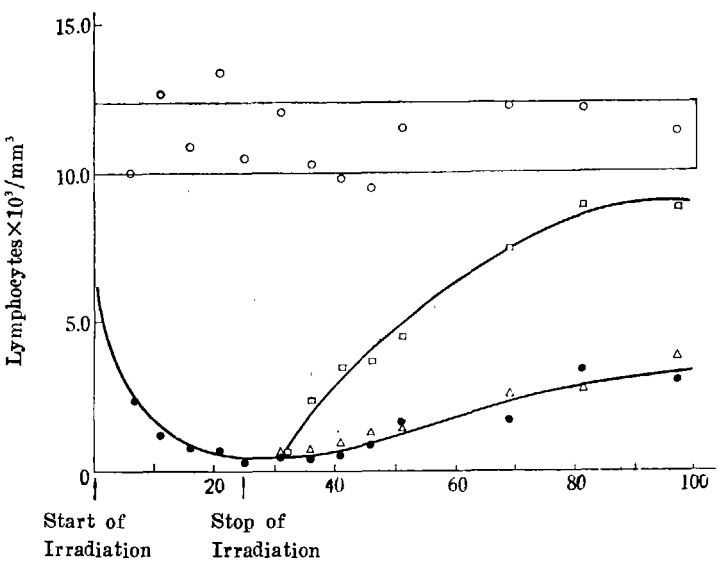

Fig. 5(b)

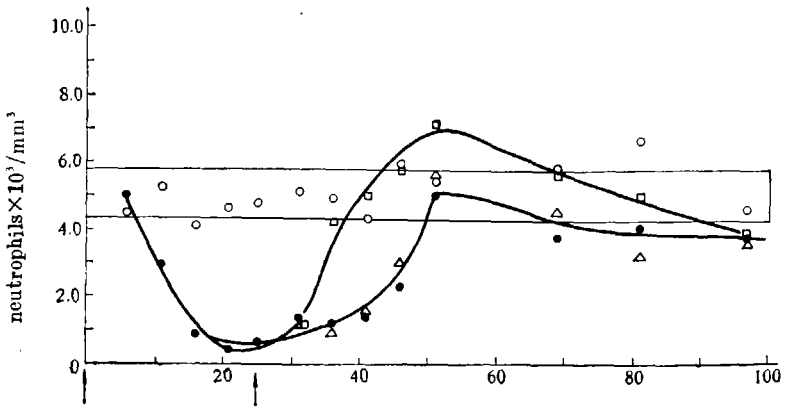

Fig. 5(c)

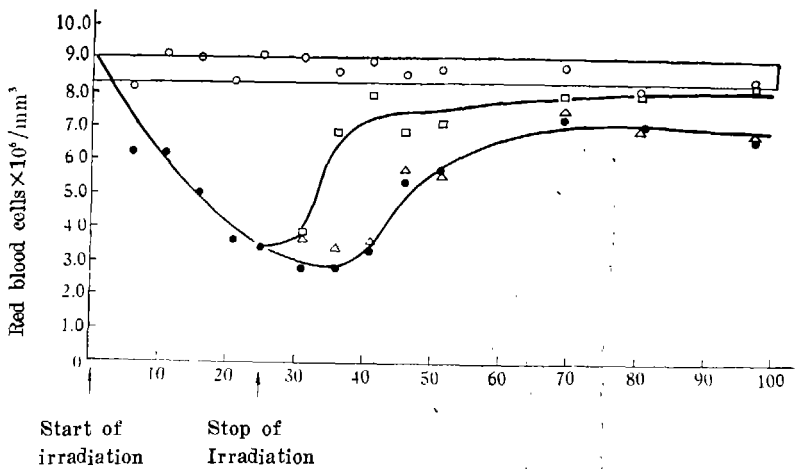

Fig. 5(d)

Fig. 5. Comparison of blood cell counts in mice after termination of 25 day continuous irradiation at a dose rate of 70 rad per day and transfusion of isogenic or allogenic bone marrow cells (▭ normal mean value±S.D.).

(a) White blood cells; (b) lymphocytes;
(c) neutrophils; (d) red blood cells.

○, Normal control mice; △, Chronic irradiated LACA mice after transfusion of 1×10^7 normal C57 bone marrow cells; □, chronic irradiated LACA mice after transfusion of 1×10^7 normal LACA bone marrow cells; ●, chronic irradiated control mice.

Third group: 2×10^7 femoral bone marrow cells (19.3 CFU-S/10^5 bone marrow cells) from mice at 30 days after the termination of 25 days continuous irradiation were injected into a group of recipients irradiated with 800 rad acute irradiation.

From Fig. 4 it is clear that the results of these experiments showed different re-

covery patterns of femoral CFU-S content. The difference in recovery curve between the first group (○) and second group (△) mainly reflects the deterioration of microenvironment in mice receiving 25 days continuous irradiation. However, the difference between first group (□) and third group (□) essentially reflects the deterioration of the proliferative ability of haemopoietic stem cells which have recovered in number of CFU-S to a sub-normal maximum at 30 days after termination of continuous irradiation. As shown in Table 1, when bone marrow cells at 30 days after termination of chronic irradiation were transplanted to a group of acutely irradiated mice (Table 1, group 3), the self-renewal ability of CFU-S in the recipients was less than CFU-S in mice after termination of chronic irradiation (Table 1, group 2).

From the experimental results as shown in, Figs. 3 and 4 and Table 1, it is reasonable, therefore, to conclude that under the given conditions of continuous irradiation the deterioration of both haemopoietic stem cells and microenvironment has profound effect in the display of radiation damage and its subsequent recovery of haemopoietic function.

3. *The Effectiveness of Bone Marrow Transplantation in the Treatment of Haemopoietic Deterioration After Low Dose Rate Irradiation*

During continuous irradiation at the low dose rate of 70 rad per day, the body

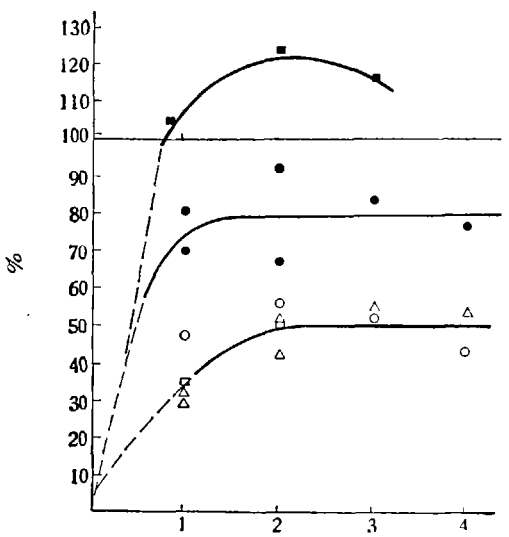

Fig. 6. Comparison of the recovery in femoral CFU-S numbers in mice after termination of 25 day continuous irradiation and transfusion of isogeneic or allogenic bone marrow cells.

——, Normal control mice; ○, chronic irradiated LACA mice after transfusing of 1×10^7 C57 bone marrow cells; ●, chronic irradiated LACA mice after transfusion of 1×10^7 LACA bone marrow cells; ■, chronic irradiated LACA mice after transfusion of 8×10^7 LACA bone marrow cells; □ and △, chronic irradiated control mice.

weight and peripheral blood cell counts of the irradiated mice declined progressively. In a course of 25 day irradiation the peripheral white cell count fell to a value of 858/mm³, lymphocyte count to 216/mm³. Animals would die if the irradiation period were further prolonged.

Figs. 5 and 6 show that in mice transplanted with 10^7 syngeneic normal bone marrow cells immediately after 25 day continuous irradiation, the recovery of the peripheral blood picture and femoral CFU-S as well as the body weight was much accelerated. For example, the content of femoral CFU-S reaches about 50% of that in normal mice of the same age during the observation period of 4 months after termination of chronic irradiation (Fig. 6). With bone marrow transplantation, however, femoral CFU-S content rapidly increased to 80% of normal mice of the same age and this level was maintained throughout the observation period as described above. From the results of the above experiments, it is apparent that the recovery of femoral CFU-S in chronically irradiated mice not only depends on the transplantation of normal syngeneic bone marrow cells, but also on the dose of bone marrow cells transplanted. When 8×10^7 normal nucleated bone marrow cells were transplanted immediately after the termination of chronic irradiation, the content of femoral CFU-S would reach, and even overshoot, the pre-irradiation level in the similar observation period of 3 months (Fig. 6).

"Take" or engraftment of the transplanted haemopoietic stem cells in irradiated

Table 2

Comparison of the Engraftment of the Donor Haemopoietic Cells in the Irradiated Recipient Mice (LACA, ♀) Which Were transplanted by 1×10^7 Normal Bone Marrow Cells From Isogeneic (LACA, ♂) or Allogeneic (C57, ♂) Mice Immediately After 25 Days of Continuous Irradiation at a Dose Rate of 70 Rad Per Day.

Time After Transplantation (days)	Source of the Transplanted Cells	Number of Cells Analysed	Type of Sex Chromosome	
			XY	XX
28	C57	30	1	29
		30	0	30
		30	0	30
47	C57	30	0	30
		30	0	30
60	C57	30	1	29
		30	0	30
		30	0	30
35	LACA	60	59	1
		30	29	1
		30	30	0
60	LACA	100	98	2
		30	28	2
		30	29	1
		30	29	1
		30	30	0

recipients as has been demonstrated by using chromosome c-band technique (Table 2) is the important basis in the recovery of haemopoietic function. This is further evidence to support the view as described in the preceding section that the haemopoietic stem cell is primarily concerned in the radiation damage and hence in the recovery of haemopoietic function following a long term of continuous irradiation.

After 25 days of continuous irradiation the immunological function as shown by the peripheral lymphocyte count in Fig. 5 was greatly reduced, but it still maintained the capacity to repress an allogenic bone marrow transplantation. Haemopoietic regeneration in LACA mice transplanted with C57 bone marrow cells was entirely of host origin (Table 2) while recovery was of donor origin when transplantation was with syngeneic bone marrow cells.

III. Discussion

Under normal conditions, the number of femoral CFU-S in the adult LACA mice tends to increase. This is somewhat different from the results reported by Croizat et al.[5] in that, following a long period of observation, the number of femoral CFU -S in normal C3H/He mice remains constant, but the content of GM-CFC in the same femur increases.

In our subsequent observations, as long as 12 months after termination of continuous irradiation, the number of femoral CFU-S was consistently lower than that of the normal controls of the same age. Nevertheless the bone marrow cellularity was close to the normal level after one month following termination of the chronic irradiation. One may raise the question, how is it possible to meet the need of accelerated haemopoiesis in the irradiated animals with less than a normal number of CFU-S? The following points may be worth mentioning: (i) the relative increase in the rate of proliferation and differentiation of CFU-S, (ii) the increase in amplification potential of the haemopoietic progenitors and precursor cells in haemopoietic organs, and (iii) the relative reduction of the releasing rate of blood cells from bone marrow into the peripheral blood[2,9,10].

After long-term chronic irradiation, the self-renewal ability of CFU-S decreases and this lasts throughout the whole observation period of 12 months. The change of self-renewal ability of CFU-S is a mirror image reflecting the degree of cellular damage, but the reversibility in this fall in self-renewal ability is very much weakened. There are two possibilities in explaining the damage to the haemopoietic stem cell population.

First, according to the findings of Brezain et al.[11] chromosome aberrations in haemopoietic cells of rats after continuous irradiation may remain as long as 90 days after cessation of irradiation. The cellular defects caused by irradiation, particularly those occurring in haemopoietic stem cells, are still the main factor influencing the proliferative capability of these cells[12].

Second, owing to the low content of CFU-S in the bone marrow after continuous irradiation, the enhancement of proliferation and differentiation of haemopoietic stem cells is the natural consequence to meet the need of blood cell supply. This prolonged

and unrested cycling activity of haemopoietic stem cells would result in the ageing of these cells with lower self-renewal abilities[6,13].

It has been suggested that haemopoietic stem cells are associated with a special feature of the haemopoietic microenvironment which has been called the stem cell "niche"[4,6]. After long-term chronic irradiation, a considerable number of haemopoietic stem cells are killed, and some of the haemopoietic stem cells and niches are damaged. In the recovery phase various combinations of haemopoietic stem cells and niches exist either in a normal or damaged state. In many cases these undoubtedly stunt the recovery of CFU-S population and their micro-environment. Thus, although the haemopoietic stem cells initially play the more important role, the late effect of radiation damage on haemopoietic stem cells and microenvironment develops simultaneously[14,15]. If the normal bone marrow cells were transfused immeniately after termination of continuous irradiation at a time when many niches would be empty, then it might be possible for the transfused haemopoietic stem cells to occupy the empty niches and perform their haemopoietic activity. Thus, transfusion of bone marrow cells to the irradiated mice is beneficial for the recovery of haemopoietic function, and might be helpful in the repair of damaged microenvironment. But, there is no evidence of engraftment of donor stroma cells in the lethally irradiated recipient mice[16].

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