Radiosensitivity of bone marrow cells in the mouse strains with different LD50*

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LD_{50} の異る系統の二十日ネズミの骨髄細胞の放射線感受性

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LD_{50} (30日) の異る二十日ネズミの2つの系統RFと C57BL/6 について，その骨髄細胞の放射線感受性を2つの方法で調べた．先ずこの2系統よりの骨髄を予め 800R全身照射した CF#1 に移植し，7日目の脾重を調べた．移植すべき骨髄を donor の中で照射 (100R) したときには，RFの方が C57BL/6 より影響が有意に大きかつたが，recipient に移植後照射したときにはその差が見られなかつた．次に2系統の動物に 400, 500, 600, 700Rの全身照射を行い，9日目にその脾重と脾のコロニー数を測つた．この場合には予期とは反対にRFの方が C57BL/6 より大きい値を示した．LD_{50} は C57BL/6 の方が大きいので，第一実験の成績は全身の感受性と骨髄細胞の感受性が平行することを示すが第二実験のそれはこの点反対である．これを照射による細胞増殖への刺激という点で説明することを試みた．

Introduction

It has been generally accepted that the principal cause of death of mammals following a single exposure of whole-body irradiation in the range below 800 rads is marrow failure. Furthermore it has been shown by McCulloch and Till (1) that the percentage of survival of groups of irradiated mice is directly related to their content of viable marrow cells. In mice it has been wellkown that there are several strains with different radiosensitivities,i.e., with different LD 50 for thirty days. It is the purpose of this paper to describe experiments in which the colony forming ability of bone marrow cells in mice of strains with different LD 50 was measured with two different techniques,i.e., a transplantation method and an endogeneous colony-forming method. The correlation between radiosensitivity of mice to whole-body irradiations and that of their bone marrow cells will be discussed.

Materials and Methods

The progenies of random matings of mice from C57BL/6, CF#1 and RF strains maintained by brother-sister mating in the laboratory have been used throughout the experiment,fed laboratory chow (Funabashi Farms) and given tap water *ad libitum*. The LD 50 of C 57BL/6 and RF mice for a single

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exposure was determined a few years ago (2), i.e., 625 R for C57BL/6 and 540R for RF. High sensitivity of RF mice as compared with C57BL/6 mice has been observed in other experiments thereafter (3).

Irradiations were given with a deep therapy X-ray machine 200 KVp, 25 mA with a filtration of 1.0 mm Cu and 0.5 mm Al. Mice were housed individually during irradiation in a cylindrical plastic box, 16 cm in diameter and 3 cm in height, containing eight radical compartments. A Radocon Model 575 dosimeter probe was placed in one of the compartments, and the total dose was checked at every exposure. The dose rate was about 65 R/min. at 50 cm from the target.

Two series of experimnts were carried out:

A) Transplantation experiment

For the transplantation of bone marrow cells C56BL/6 and RF mice of about 40 days of age were used as donors and adult CF# 1 mice as recipients irrespective of their sex. Donor mice were killed by cervical dislocation. Bone marrow cells taken from the femur of several mice were mixed in Puck's saline A and about 5.2×10^6 cells were transfered intravenously into the recipient mouse irradiated with 800 R 24 hours before according to the method by McCulloch and Till (1). The transfered cells were irradiated with 100 R in the donor immediately before killing the donors or in the recipient fifteen minutes after the transfer. The recipient mice were killed by cervical dislocatin seven days after the transfer and their spleen weights were measured.

B) Endogeneous colony formation

Twenty to twenty five adult mice of C57BL/6 and RF were given single radiations of 400, 500, 600 and 700 R respectively. Nine days after irradiation surviving mice were sacrificed by cervical dislocation and the spleens were removed. After measuring their weights, the spleens were fixed in Bouin's fixative and the number of nodules was counted two or three days thereafter.

In order to reconfirm the difference in radiosensitivity the thirty day percent survival was determined after a single exposure with 650 R to whole body for both strains of mice after the completion of the above two experiments.

Results

Mean spleen weights of the recipients after the bone marrow transplantation are shown in Table 1. Comparing the two strains of mice, difference in spleen weight was statistically significant only when the bone marrow cells were irradiated in the donors. The mean spleen weight of the recipient transplanted with nonirradiated bone marrow cells from C57BL/6 mice was actually greater than that from RF but the

Table 1. Comparison of radiosensitivity of bone marrowcells by transplantation experiment.

Donor	Dose		No. of	Spleen weight (mg)
	in donor	in recipient	recipient	
RF	100 R	0	9	41.8±2.01 *
C57BL/6	100 R	0	8	60.0±7.39 *
RF	0	100 R	8	40.6±3.47
C57BL/6	0	100 R	10	38.5±2.85
RF	0	0	21	62.5±5.28
C57BL/6	0	0	18	79.2±7.98

* Difference is statistically singnificant at 5 percent level.

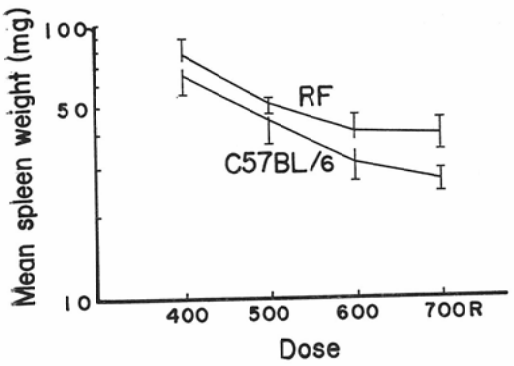

Fig. 2. Endogeneous colonies in the spleen of RF and C57BL/6 mice

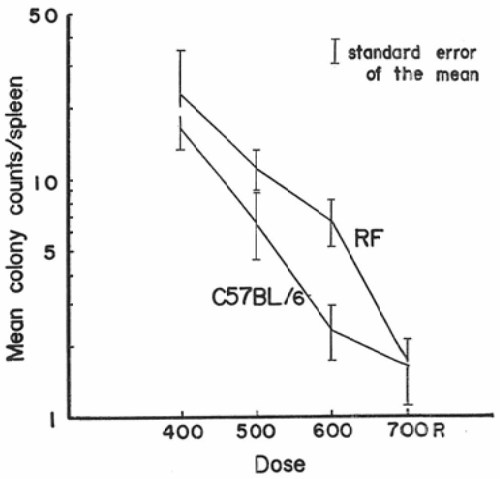

Fig. 1. Mean spleen weight nine days after irradiation

difference was not statistically significant. The mean spleen weight of CF# 1 mice eight days after 800 R whole-body irradiation was 23.7 ± 2.06 mg. The increase above this value in the transplanted mice may probably be due to the growth of the transplanted cells in the spleen.

Mean spleen weights and number of nodules per spleen after graded dose of X-irradiation in RF and C57BL/6 mice are shown in Fig. 1 and Fig. 2. The both values for RF were actually higher than those for C57BL/6 at almost all dose levels so far studied. However the differences were statistically significant only at 600 R for colony counts and at 700 R for spleen weight respectively.

Results of the studies on radiosensitivity against single whole-body irradiations in RF and C57BL/6 mice of the same breeding stock respectively are summarized in Table 2. The results indicate that their relative radiosensitivity remains rather constant for these five years.

Table 2. LD50 and per cent survival after single irradiations in RF and C57BL/6 mice maintained in the authors laboratory.

	RF	C57BL	Date
LD50 (30)	540R	625R	1960
600R	15.1%	78.8%	1961
650R	13.3%	77.8%	1965

* The dosimeter used in the last experiment was different from that in the other two.

Discussion

The results of transplantation experiments may indicate that the difference in radiosensitivity to whole-body irradiations between the mice of the two strains coresponds to the difference in radiosensitivity of their bone marrow cells. But the latter difference may depend on the internal milieu in the body but not on the characteristics of the cells, since the difference in response to radiation was demonstrated only when the cells were irradiated in the donor but not in the recipient.

For determining the radiosensitivity of bone marrow cells of mice, Drásil (4) used the spleen weight

of the recipient mice after transfering the cells. The method was revised by Till and McCulloch (5) who counted the number of colonies in the spleen of the recipient. Studies on radiosensitivity and repair of mouse bone marrow cells have been reported by them (6, 7, 8) by using this technique. However, since a consistent result has not been obtained by colony counting in the present transplantation experiment due to some unknown reasons, the changes in spleen weight of the recipient were taken as a measure of radiation response of the transplanted cells. Similarly Bennett *et al.* (9) have assayed the proliferative competence of the marrow by measuring splenic uptake of ^{131}IUdR in the recipient in stead of counting the colonies.

The results of endogenous colony-formers are quite unexpected if the difference observed would be real.

Till and McCulloch (7) obtained dose-effect curves by the same method in male and female C57BL/6j. The slope was quite similar to that of dose-percent survival curve of transplanted colony-formers by their method. According to their assumption that the relation between dose and mean number of colonies per spleen for endogenous colony-formers represents the effects of radiation on the cells themselves, and that the endogenous colony formers originate from the bone marrow, it would be expected that the curve for C57BL/6 mice is always above that for RF mice.

Higher spleen weight in RF mice before as well as after irradiation than in C57BL mice was also reported by Hayakawa *et al.* (10). But, maximum per cent reduction in spleen weight after whole-body irradiation of 350 R was larger in RF mice than in C57BL mice. Contrarily, recovery of the spleen weight was more remarkable in the former strain than in the latter, and overshoot of recovery was observed in the former alone.

These facts may suggest that the number of endogenous colonies per spleen does reflect the damage of the cells by radiations incompletely. Endogenous colony-formation may probably be dependent in part on some stimulating factors for cell multiplication such as erythropoietin produced to repopulate depleted cell populatons, since the colonies are observed only when the animals are sublethally irradiated. In radiosensitive animals the same dose of radiation may result in high stimulating effect for cell reproduction because of large destruction appropriate cell populations as compared with in radioresistant ones. A linear approximation of dose-effect relation in RF mice appears to be shifted parallelly about 70 R to higher dose level from that in C57BL/6. Doses to C57BL/6 mice 70 R higher than to RF may result in the same response of colony formers as in RF mice. This dose difference is in good accord with the difference in LD50. It may be speculated that the survival of cells in RF mice was elevated by high stimulation for cell reproduction as compared with C57BL/6 mice, though the survival itself is lower in RF mice. Such a stimulating effect may be diminished when the cellular damage is too heavy to recover as in the case of 700 R. Further study would be required to test the above hypothesis.

Summary

Radiosensitivity of bone marrow cells of the strains of mice with different LD50 (30 days), RF and C57BL/6, has been studied with two different techniques. In the first experiment, bone marrow cells from the two strains of mice were transfered to CF# 1 mice previously exposed to 800 R of X-rays and the spleen weight of the recipient was measured 7 days after the transfer. Difference in spleen weight was statistically significant, higher in C57BL than in RF, when the bone marrow cells were irradited in the donor but not when irradiated in the recipient. In the second experiment, spleen weights and nodules

after graded dose of X-irradiation were measured in the two strains of mice. The both values for RF were actually higher than those for C57BL/6 at almost all dose levels. Since LD50 is higher in C57BL/6 than in RF, the results of the first experiment may indicate that the radiosensitivity of bone marrow cells are in accord with that of whole-body but those of the second one appears to be contrary to the expectation. The apparent contradiction may be explained by assuming the difference in the level of stimulating factors for bone marrow cell multiplication induced by radiation.

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References

1. McCulloch, E.A. and J.E. Till (1960). The radiosensitivity of normal mouse bone marrow cells, determined by quantitative transplantation into irradiated mice. Radiation Res. 13:115—125.
2. Unpublished data.
3. T. Tsuchiya, J. Hayakawa, S. Muramatsu, H. Eto, and T. Sugahara (1963). Radiosensitivity and thyroid function in mice. Radiation Res. 19:316—323.
4. Drasil, V (1960). Effect of radiation on bone marrow cells at various temperatures *in vitro*. Folia Biol. 6:359—364.
5. Till, J.E. and E.A. McCulloch (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiation Res. 14:213—222.
6. McCulloch, E.A. and J.E. Till (1962). The radiosensitivity of cells from normal mouse bone marrow to gamma radiation *in vitro* and *in vivo*. Radiation Res. 16:822—832.
7. Till J.E. and E.A. McCulloch (1963). Early repair processes in marrow cells irradiated and proliferated *in vivo*. Radiation Res. 18:96—105.
8. Till J.E. and E.A. McCulloch (1964). Repair processes in irradiated muose hematopoietic tissue. Annals of N.Y. Acad. Sci. 114:115—125.
9. Bennett, M., G.M. Shearer, A.C. Upton and G. Cudkowicz (1964). Factors affecting the expansion of the stem cell pool in transplantd mouse bone marrow. Nature, 204:351—353.
10. Hayakawa, J., S. Muramatsu, J. Yamada and T. Tsuchiya (1964). The splenic and thymic weight response to whole body X-irradiation in three strains of mice. Nippon Acta Radiol. 24:370—376.