Kinetic study of endogenous-spleen-colony-forming cells in irradiated mice

Y. Ueno
Department of Experimental Radiology, Faculty of Medicine, Kyoto University, Kyoto, Japan

照射マウスの内因性脾コロニー形成細胞の動態

京都大学医学部放射能基礎医学教室
上　野　陽　里

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60日令と90日令マウスに 400Rを照射し，その後4時間における 内因性脾 コロニー形成細胞の D_0 値とn値を観察した．照射30分，1時間，4時間後のn値は 1.0より小さかつたが，2時間後のそれは 8.0より大きかつた．逆に D_0 値は照射30分，1時間，4時間後に非照射マウスのそれに比べて大きく，2時間後では小さかつた．これらの結果について分析を行なつた．

Recently, it has been reported by the author (1968) that the endogenous-spleen-colony (ESC) count changes when a split-dose technique is used; that is, the count increases for about two hours after the first irradiation and then decreases for about two hours. This change appears to be similar to the change in the surviving fraction of mammalian cells cultured in vitro after irradiation by the split-dose technique. The change in the endogenous-spleen-colony-forming cells (ESC-forming cells) in vivo after irradiation may be explained by a repair and progression mechanism (Elkind, 1966). This explanation, however, requires the assumption that the number of ESC-forming cells and the proportion of ESC-forming cells which are fixed in the spleen, to all cells available in ESC-formation do not vary for about four hours after the first irradiation. In the present study, the change in the size of population of ESC-forming cells in mice after irradiation by the split-dose technique was analyzed.

Materials and Methods

Ninety-day-old and 60-day-old female mice of the dd/YF strain were given 30 days to become adapted to the conditions of our animal room. Mice were given water and diet (Funabashi Farm) ad libitum and aureomycin powder (4 g/l) was added to the water after irradiation. The time of irradiation was always 14:00 in order to avoid diurnal variations in the radiosensitivity of the ESC-forming cells, when the diurnal change in ESC was small (Ueno, 1968 and 1969a). Single total-body and didided total-body irradiations were carried out. The first dose of the divided irradiation was 400 R and the second dose was 200 R to 500 R. The interval between the two irradiations was 30 minutes to four hours. The physical factors were: 190 kvp, 24 mA, focus body axis distance 50 cm with an added filtration of 1.0 mmCu + 0.5 mmAl and a dose rate of about 63 R/min. The doses and dose rate were checked by a Victoreen Radocon Dosimeter during irradiation. To draw each dose-effect line, seven or ten radiation doses were used.

Surviving mice were sacrificed 10 days after the second irradiation, and the spleens were removed and

fixed in Bouin's solution. The ESC were counted seven days after fixation. The ESC counts were tested by Smirnov's test of rejection (Torii and Takahashi, 1954) and then added together and the mean values were calculated. For each radiation dose, five to twenty mice were used. The experiment was repeated twice. The 90-day-old mice were used in the first experiment and the 60-day-old mice in the second.

Results

The dose-effect lines of ESC counts in mice irradiated once and in four groups of fractionally irradiated mice, were calculated statistically, as shown in Table 1, under the assumption that the relationship between x and y is linear in the dose range used. All regression coefficients were statistically significant in both experiments. According to get exact relationship between x and y, the mathematical method was carried out in the present report. The D_0 and n values calculated by equations are shown in Table 2. For calculating D_0 and n values, it is assumed that irradiation with 400 R always results in lethal damage to ESC-forming cells. The dose-surviving fraction lines of the ESC count in mice after the first irradiation were calculated on the basis of the original values of 114 in the first experiment and 161 in the second experiment to get the n values of these dose-surviving fraction lines.

Table 1. Equations showing the dose-effect lines of endogenous-spleen-colony-forming counts

experiment	1	2
single irrad.	$y = 3.3754 - 0.0033x$	$y = 3.3669 - 0.0029x$
interval of 30 min.	$y = 2.6841 - 0.0022x$	$y = 2.6955 - 0.0024x$
interval of 1 hr.	$y = 2.3949 - 0.0019x$	$y = 2.2487 - 0.0020x$
interval of 2 hrs.	$y = 4.7780 - 0.0046x$	$y = 4.8101 - 0.0039x$
interval of 4 hrs.	$y = 2.5739 - 0.0019x$	$y = 2.5513 - 0.0018x$

x: total radiation dose (R)
y: logarithmic mean ESC count.

Table 2. n and D_0 values of ESC-forming cells in mice after irradiation in vivo with 400 R

	1st experiment		2nd experiment	
	D_0	n	D_0	n
single irrad.	220		150	
30 min. after irrad.	283	0.55	180	0.33
1 hr. after irrad.	328	0.45	220	0.18
2 hrs. after irrad.	136	8.50	110	11.00
4 hrs. after irrad.	329	0.60	240	0.42

Discussion

One of the disadvantages of in vivo experiments on ESC-formation is the impossibility of knowing directly the original count of marked cells in an unirradiated body, with a few exceptions (Hornsey, 1967). Using the hypothesis that the ESC can always be produced from a single ESC-forming cell (Becker et al., 1963), the D_0 value of ESC-forming cells in mice irradiated once can be measured directly from the D_0 value of ESC, but the n value cannot be measured directly. When the n values were calculated in the pre-

sent study, the author assumed that the survival fraction of ESC-forming cells irradiated with a first dose of 400 R was always less than 1.0, that was, 400 R was greater than the Dq value of unirradiated ESC-forming cells. The results of the author's calculation of the Dq value using 30 mammalian cells listed in Yamada's report (1968) show Dq values less than 400 R. The assumption is therefore reasonable. The intervals between two exposures are maximally four hours and there is a possibility to appear the changes caused by diurnal rhythmicity in the interval. However, comparing the present data to the author's previous data (Ueno, 1968), it is reasonable to conclude that the present data are hardly effected by the diurnal rhythmicity.

The n values lower than 1.0 with fractionated irradiations at intervals of 30 minutes, one hour and four hours, should be noted. There are four possible reasons for these low values. The first is that the original values of 114 and 161 used in the calculation are over-estimated values. The second is that surviving ESC-forming cells fuse with each other. The third is that the process of seeding is disturbed. The forth is that there are two fractions of surviving cells, one of which recovers while the other does not.

Considering the actual Dq values of various cells, as mentioned above, it is feasible to use the values of 114 and 161 as the original values as the base to calculate surviving fractions. However, the ESC count after irradiation with 400 R cannot be measured directly due to limitations in the experimental techniques, and the values of 114 and 161 are backward extrapolated values from the results of experiments using doses higher than 400 R. Therefore, there is a risk that the actual dose-ESC count relationship in the case of a single irradiation may be nonliner in the range of less than 400 R, which would cause an over-estimation.

The second possibility seems very unlikely at first glance, although it has been reported that one ESC-forming cell forms more than two colonies (Barnes, et al., 1968) and that some cells can fuse with each other after irradiation (Thompson and Suit, 1969). However, the possibility which the fused cells survives to form colonies in the spleen, is unlikely. This problem is refered in the author's other report (1970).

The third possibility is the disturbance in the seeding of ESC-forming cells probably from the bone marrow to the spleen caused by the second irradiation. The phenomenon described as a seeding is regulated by various factors which are analyzed mathematically by Vogel et al. (1968) and Matioli et al. (1968). The f fraction changes under certain conditions (Schooley, 1969). According to the author's data (1969b), the ESC-forming cells return to the bone marrow through the spleen for four hours after irradiation with 400 R. The fact of a loss of CFU from the spleen after irradiation was observed as well by Guzman and Lajtha (1970). The complex behavior of ESC-forming cells after irradiation may be disturbed. Thus, it is likely to change the population size of ESC-forming cells seeded in the spleen. If the population size is decreased by the second irradiation because of the suppression of seeding, the n value is naturally less than 1.0.

The fourth possibility is that there may be two fractions of ESC-forming cells, which has been suggested by Schofield (1970). One of them may be able to recover after the first irradiation with 400 R and/or is relatively radioresistant, while the other may be unable to recover and/or is relatively radiosensitive. Though numerous studies on the properties of ESC-forming cells have been reported, the problem remains to be solved. The two fractions may be erythropoietic and myelopoietic cells, two stages of one kind of cell with different radiosensitivities in the process of differentiation, or two phases of cell division (Sinclair, 1968, 1969 and Lazzio 1968). The population of exogenous SC-forming cells with DNA synthesis in the

bone marrow of adult mice is negligible (Becker, et al., 1965). If two phases of ESC-forming cells with different degrees of radiosensitivity exist, these phases may be mainly G_1 and G_2. The possibility that there are two periods with different radiosensitivities in the differentiation process of one kind of ESC-forming cell, cannot be ruled out be the results of this experiment.

The difference between the results of the first and second experiments appears to be due to the difference in the ages of the mice used: 90-day-old mice in the first experiment and 60-day-old mice in the second. The D_0 value of unirradiated ESC-forming cells was 220 R in the former and 150 R in the latter. The former experimental condition was same as the latter and was not accompanied with a hypoxic condition.

The present data show that there are various unknown factors affecting the kinetics of ESC-forming cells, especially their population size and radiosensitivity. The technique of exogenous SC is well known as a valuable one to know the cell kinetics of spleen colony. However, the results from the experiment using the technique of exogenous SC are not same as that from the experiment using the ESC. The cell kinetics of ESC are not able to be explained by the results of exogenous SC completely. This is one of reasons why the present experiment was carried out, even if it is a difficult problem. The fact that there is still no way to determine the initial count of ESC-forming cells has delayed the development of kinetic studies in ESC-forming cells. This problem needs further study.

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