Bone Marrow Treatment of Mice Lethally Irradiated with Gamma-Rays under High Dose Rate (V)

The Long Term Preservation of Bone Marrow at −80°C

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Received January 18, 1964

The isologous bone marrow which had been kept in glycerol-Tyrode's solution at −80°C as long as 270 days protected well the lethally gamma-irradiated mice as well as the fresh IBM transplantation did, as far as 5 million of nucleated cells were injected.

The homologous bone marrow preserved as long as 90 days gave higher 21 days survival rates than those in the fresh HBM transplantation, as far as 5 million of nucleated cells were injected, and furthermore postponed the development of the secondary diseases as compared to the fresh HBM transplantation. The HBM which had been preserved more than 90 days and given in dose of 5 million nucleated cells, however, could not protect the lethally irradiated mice. When the number of the nucleated cells which were preserved and transplanted was increased up to 10 million, good early survival rates were observed in 120 days and 180 days preserved HBM transplantation. The supposed reasons for this favorable effect by freezing were discussed.

INTRODUCTION

The long term preservation of bone marrow cells is essentially necessary for the clinical application of bone marrow transplantation. Since Polge[1] reported the successful preservation of living cells with glycerol as a preservative additive, the application of this method to bone marrow cell preservation has been developed using glycerol in the solid carbon dioxide or liquid nitrogen, and the preserved isologous as well as homologous bone marrow transplantation in lethally irradiated mammals has been studied by many workers[2,3,4]. On the other hand, there have been some reports concerning tissue transplantation suggesting that there is a possibility that the cooling or freezing might facilitate taking of the homologous tissue graft[5,6], but there has been no report concerning the homologous bone marrow transplantation in lethally irradiated mice which was carried out from such a view-point, so far. These are the reasons why this type of experiments was undertaken.

MATERIALS AND METHODS

Dd/s strain mice supplied from the Kyoto University Animal Center were

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used as recipients and the isologous bone marrow (IBM) donors. Na2 mice were used as the homologous bone marrow (HBM) donors. They were two to two and a half months old mice weighing approximately 20 g at the time of experiments. The sexes of the donors and recipients were identical. The animals were fed whole wheat and dried fish, and were given tap water ad libitum.

All mice were irradiated in the Co^{60} gamma-irradiation facility belonging to the Institute for Chemical Research of Kyoto University[7]. The procedure for transplantation was described previously[8,9].

In order to preserve the freezed bone marrow cell suspensions, 15% glycerol-Tyrode's solution was used as one of the protective additives according to Tran[10].

The sealed glass ampules containing an aliquot of the suspension of bone marrow cells were freezed and kept at −80°C with ethanol-dry ice system in the vacuum bottle. The cooling rate was approximately 1°C per minute untill the temperature in the bath reached −20°C and then not beyond 10°C per minute untill −80°C. The ampules were immersed in warm water at 37°C for thawing, and then the bone marrow cell suspension was immediately used for the transplantation.

RESULTS AND DISCUSSIONS

1. Preserved IBM Transplantation

The results are shown in Table 1 and Fig. 1. In this Table, the control group consisted of the mice lethally irradiated with 900 r gamma-rays without bone marrow treatment and the fresh group means the mice lethally irradiated and treated with the fresh IBM. The number of the injected nucleated cells was 5 million. When the bone marrow cell suspension was preserved with ethanol-dry ice at −80°C as long as 270 days, the percentage of eosin-staining

Table 1. Survival rate of gamma-irradiated mice treated with preserved IBM.

Preservation period	No. of experiment	Percent staining with eosin (%)	Number of survival / Number of irradiated (at days)					% Survival (at days)			
			14	21	30	60	90	21	30	60	90
Control	10	—	1/49	0/49	—	—	—	0	—	—	—
Fresh	2	9	14/19	13/19	13/19	13/19	13/19	68	68	68	68
10 days	2	18	16/20	16/20	15/20	13/20	13/20	80	75	65	65
20 days	2	21	17/24	16/24	16/24	16/24	16/24	65	65	65	65
30 days	1	21	10/17	10/17	10/17	10/17	10/17	58	58	58	58
60 days	1	27	7/8	7/8	6/8	6/8	6/8	87	75	75	75
90 days	1	33	6/10	6/10	6/10	6/10	5/10	60	60	50	50
120 days	2	39	12/13	12/13	11/13	10/13	10/13	92	84	84	84
150 days	2	58	12/14	10/14	10/14	6/14	6/14	71	71	42	42
180 days	3	60	12/16	11/16	11/16	10/16	10/16	68	68	62	62
270 days	2	48	12/15	10/14	10/14	10/14	10/14	71	71	71	71

The number of nucleated cells injected was 5 million which was preserved at −80°C in 15% glycerol-Tyrode's solution.

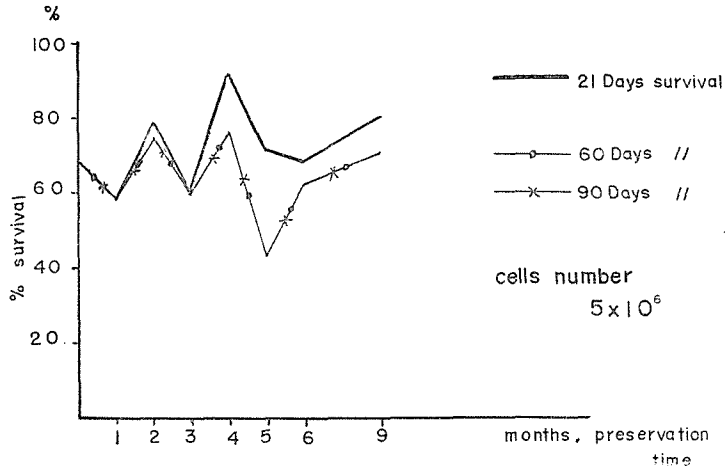

Fig. 1. Changes of percent survival in mice irradiated lethally and treated with preserved isologous bone marrow at −80°C.
The nucleated cell number was 5 million.
Preservation was carried out in 15% glycerol-Tyrode's solution for 10, 20, 30, 60, 120, 150, 180 and 270 days.

cells, which was thought to show the viability of the preserved cells, was 48%, while that of the fresh, non-preserved bone marrow cells was 9% in an average of 19 mice bone marrow cell preservation. It was found, however, that the survival rates at 21 days were 58 to 92% in every group. This means that the preservation of the freezed IBM as long as 270 days did not change the effectiveness of the protection against the irradiation under the lethal dosis.

Body weight decreased up to approximately 90% of the pre-irradiation level following the bone marrow transplantation in lethally irradiated mice, and it took 12 days in mice treated with 90 days preserved IBM, 19 days with 180 days preserved, 18 days with 270 days preserved, untill the body weight recovered to the pre-irradiation level, while it usually took 15 days is mice treated with IBM without preservation. Namely, a retarded recovery of body weight for 3 to 4 days was found in mice treated with the preserved IBM, as compared to mice treated with the fresh IBM. On the other hand, the most decreased body weight was found 6 to 10 days after the irradiation, and there was no difference in these days between the preserved IBM group and the fresh IBM group. These results show that such a condition of the freezed preservation could give the effect of treatment almost similar to that of the fresh IBM in lethally irradiated mice.

As to the freezing at 0° to 4°C, Urso[11] reported that the viability of the cells was lost in 6 days under this condition. Luyet[12] as well as Merryman[4] claimed that Tyrode's solution with 15% glycerol as a protective additive could protect the cells from the intracellular formation of the ice crystals, and Bender[13] also reported that the bone marrow cells preserved for one year at −80°C had still their viability. Our results also support these report. Further investigations of hematological and pathological findings in details will be

reported elswhere[14].

2. Preserved HBM Transplantation

The survival rates of Dds/S mice lethally irradiated with 900 r gamma-rays which were treated with the preserved HBM of Na2 donor mice are shown in Table 2 and Fig. 2, in which the number of the nucleated cells was 5 million. In this type of experiment, the survival rates at 21 days were 31%, 44% and 50% in 30 days, 60 days and 90 days preserved HBM transplantation, respectively. It should be noted that the early survival rates in the transplantation of the preserved HBM as long as 90 days gave better results than those in the fresh HBM transplantation group, although the number of the non-viable cells measured with the eosin-staining method increased from 10% in fresh bone marrow cell suspension to 25% in 90 days preserved bone marrow. The delayed death rates increased from 30 days to 60 days after the preserved HBM transplantation equally in every group, while those in the fresh HBM transplantation group were increasing till 90 days after the transplantation. The survival percentages, however, were higher in the preserved group than in the fresh group.

Table 2. Survival rate of gamma-irradiated mice treated with preserved HBM.

Preserved period	No. of experiment	Percent staining with eosin (%)	Number of survival / Number of irradiated (at days)					% Survival (at days)			
			14	21	30	60	90	21	30	60	90
Control	10	—	1/49	0/49	—	—	—	0	—	—	—
Fresh (without preservation)	5	10	27/65	17/65	11/64	6/60	3/60	25	17	10	5
30 days	2	13	8/16	5/16	3/16	2/16	2/16	31	18	12	12
60 days	4	17	18/25	11/25	8/25	6/25	5/25	44	32	24	20
90 days	2	25	6/10	5/10	4/10	2/10	2/10	50	40	20	20
120 days	2	26	1/13	0/13	—	—	—	0	—	—	—
150 days	1	33	0/8	0/8	—	—	—	0	—	—	—
180 days	2	51	3/14	0/14	—	—	—	0	—	—	—

The number of nucleated cells injected was 5 million which was preserved at −80°C in 15% glycerol-Tyrode's solution.

Therefore, this suggests the possibility that the preservation at −80°C of HBM in 15% glycerol-Tyrode's solution makes the recipient mice take easily and postpones the development of the secondary diseases. On the other hand, HBM transplantation preserved longer than 120 days showed no protection effect. This observation was also confirmed by the body weight change following the transplantation. As reported previously[8,15] body weight change in HBM transplantation can be classified into three types, namely, type a) being the almost same change as that of the irradiated and non-treated controls, type b) being the same as that of mice treated with IBM and type c) showing a rather slight degree of initial weight loss followed by a tendency to increase by 15 or 20 days and then followed by rapid decreases. The HBM transplanta-

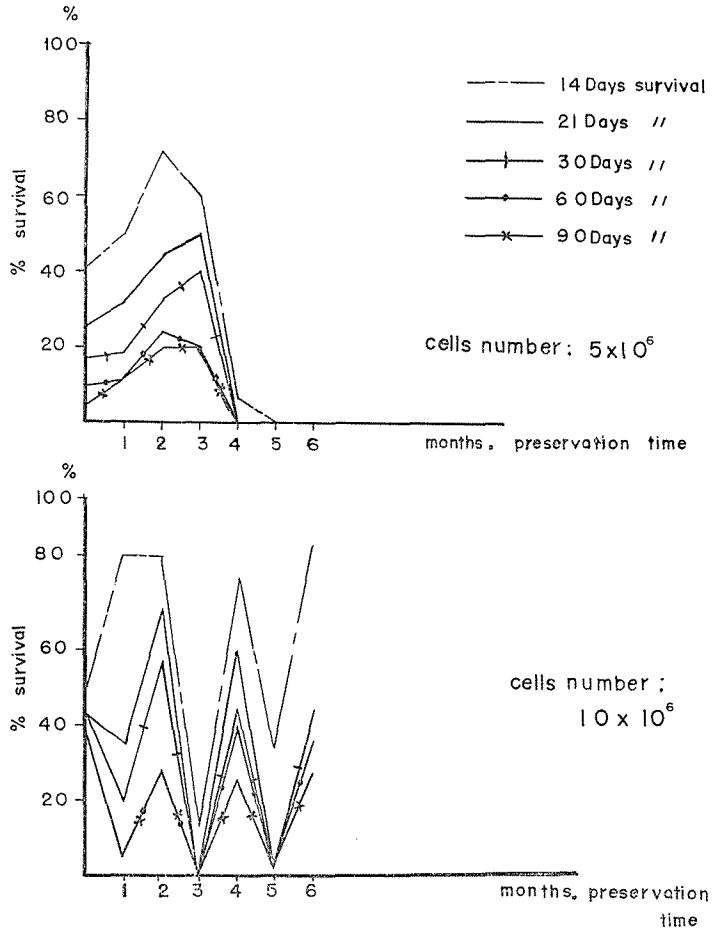

Fig. 2. Changes of percent survival in mice irradiated lethally and treated with preserved homologous bone marrow at −80°C.
The nucleated cell number was 5 or 10 million.
Preservation was carried out in 15% glycerol-Tyrode's solution for 30, 60, 90, 120, 150 and 180 days.

Table 3. Survival rate of gamma-irradiated mice treated with preserved HBM.

Preservation period	No. of experiment	Percent staining with eosin (%)	Number of survival / Number of irradiated (at days)					% Survival (at days)			
			14	21	30	60	90	21	30	60	90
Control	10	—	1/49	0/49	—	—	—	0	—	—	—
Fresh	1	10	8/16	7/16	7/16	7/16	6/16	43	43	43	37
30 days	3	13	17/20	7/20	4/20	4/20	1/20	35	20	20	5
60 days	1	17	6/7	5/7	4/7	3/7	2/7	71	57	28	28
90 days	2	25	2/15	0/15	—	—	—	0	—	—	—
120 days	2	26	12/15	9/15	7/15	6/15	4/15	60	46	40	26
150 days	3	33	9/26	1/26	1/26	1/26	1/26	3	3	3	3
180 days	2	51	9/11	5/11	5/11	4/11	3/11	45	45	36	26

The number of nucleated cells was 10 million which was preserved at −80°C in 15% glycerol-Tyrode's solution.

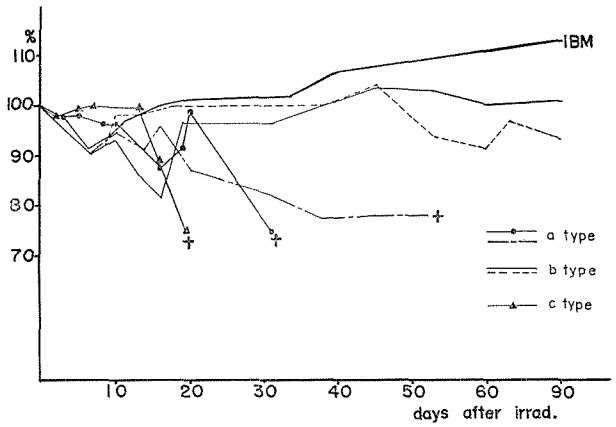

Fig. 3. Body weight changes in mice irradiated lethally and treated with preserved isologous as well as homologous bone marrow.
The nucleated cell number was 5 million.
Preservation was carried out at −80°C in 15% glycerol-Tyrode's solution for 60 days.

tion preserved longer than 120 days resulted in type c) body weight changes as shown in Fig. 3. Table 3 and Fig. 2 show the result when 10 million nucleated HBM cells were used. In this type of experiment, the survival rates at 21 days were 60% and 45% in 120 days and 180 days preserved HBM transplantation, respectively. The early survival rates in the preserved HBM transplantation as long as 180 days also gave better results so far than those in the fresh group, although those in 90 days and 150 days preserved HBM transplantation were quite disappointing. On the other hand, the delayed death rates showed almost the same in every group. It appeares that the preserved HBM transplantation also makes the recipient mice take easily as compared to HBM transplantation without preservation. As to the development of the secondary disease, however, conclusive results could not be obtained in this expriment.

Mathé *et al.*[16] claimed that there is a difference between the immunnologically competent cells and hematopoietic myeloid-restoring cells as to the sensitivities against the change of the temperature. This idea appears to support our observations that freezing facilitated taking and postpond the development of the secondary diseases. The further observations on this type of experiments and on the use of dimethylsulfoxide as a protective additive are now in progress and will be reported in near future[14].

ACKNOWLEDGEMENT

This work was supported by a grant-in-aid from the International Atomic Energy Agency (No. 138/R1/RB), to which thanks are due. Thanks are also extended to Mr. Rintaro Katano of the Institute for Chemical Research of Kyoto University for his kindness in frequently operating the Co^{60} irradiation facility.

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