Modification of Hematopoietic Cell Transplantation in Mice Irradiated with Gamma Rays under High Dose Rate. (III)

Alteration of Midlethal Dose Killing Effect

Kazushige ADACHI*

The First Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University (Director: Prof G. WAKISAKA)

Received January 31, 1966

Midlethal dose killing effect was altered by preimmunization, delayed transplantation, passive transfer of antiserum and transplantation of chimeric donor.

INTRODUCTION

In the previous reports[1,2], it was demonstrated that suppression of homograft response in homologous hematopoietic cell transplantation could partially be obtained but not completely after gamma-irradiation under high dose rate. Therefore, in order to suppress the homograft response, further experiments are needed. As for the midlethal dose killing effect (MLD effect) as previously described, methotrexate altered the effect and the preimmunization of host mice also prevented its lethal effect after 700r irradiation and homologous spleen cell (HSC) transplantation. In this study, it is to be reported whether MLD effect can be altered by the preimmunization which is given at various pre-irradiation days, by the delayed inoculation of HSC which is given to host mice at various post-irradiation days, by the passive transfer of anti-donor-host-serum to host mice or by the transplantation of chimeric donor.

MATERIALS AND METHODS

Dd/s and Na2 strain mice were used as irradiated hosts and donors, respectively. Only female mice were used. Both the donors and hosts were 2 to 2 and half months old. The method of obtaining spleen cell suspensions, and the conditions of irradiation were described previously[1,2]. Preimmunization was given with homologous viable spleen cell suspension which contained $5-10\times10^6$ nucleated cells or with various semihomogenized donor tissues 7 days, 2 days or 30 minutes before irradiation and HSC transplantation. "Homologously immunized" host materials were obtained by killing the mice immunized by the homologous donor tissue 7 days previously. Anti-donor-host-serum was obtained from the 7 days previously "homologously immunized" mice of the host strain.

* 安達 百成

RESULTS

I. Effect of Various Preimmunizations on 700r Irradiated and HSC Transplanted Host

A) **Survival rate.** As shown in Table 1, MLD effect was altered by the preimmunization with spleen, liver, kidney and thymus obtained from Na2 mice 7 days before 700r irradiation and HSC transplantation, but it could not be altered by the preimmunization with unrelated AKR spleen cell suspension in Dd/s mice

Table 1. Effect of various preimmunization of 700r irradiated Dd/s host against transplantation of Na 2 spleen cells.

Exp.	Antigen	Preimmunization before irrad.	No. of mice	% survival (at days)					
				7	14	21	30	60	90
1	None	None	34	91	82	68	24	6	6
2	Na2 spleen	7 days before	20	100	100	100	100	100	100
3	Na2 thymus	〃	8	100	100	100	100	75	63
4	〃 liver	〃	10	100	90	90	80	80	80
5	〃 kidney	〃	9	100	100	100	88	88	88
6	AKR spleen	〃	9	55	33	33	33	33	33
7	Na2 spleen	2 days before	23	96	96	96	91	34	22
8	〃	30 minutes before	38	100	92	87	82	24	22
9	〃	4 weeks before	16	100	56	31	31	31	25

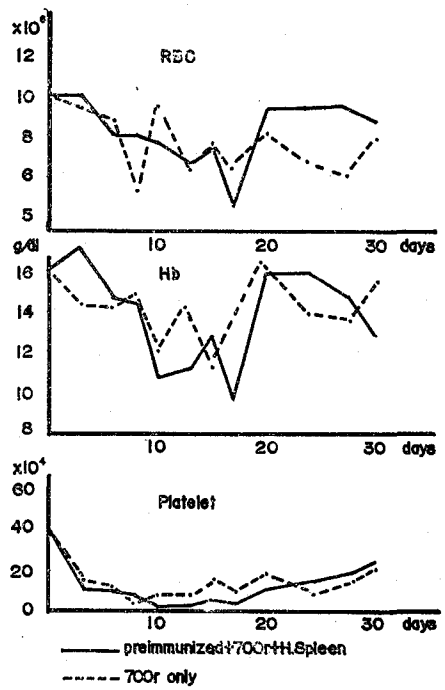

Fig. 1. Changes of RBC, Hb and platelet count.

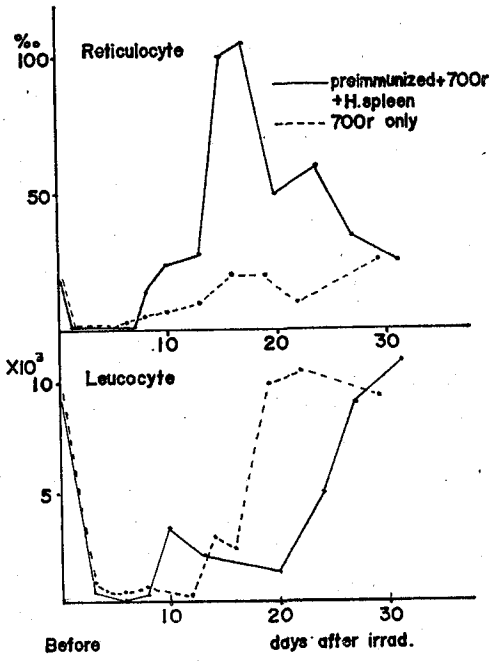

Fig. 2. Changes of leucocyte and reticulocyte count.

infused with Na2 spleen cells. When the host mice were immunized by the donor tissues 2 days before irradiation anb HSC inoculatiou, MLD effect was altered as shown in Exp. 7, i.e., survival rate was 91% at 3) days, though it decreased to 22% at 90 days. The 30 minutes previous immunization also altered MLD effect as shown in Exp. 8, i.e., survival rate was 82% at 30 days, but the 4 weeks previous immunization did not alter MLD effect as shown in Exp. '9.

B) **Hematological findings.** When the host mice were immunized 7 days previously as shown in Figs. 1 and 2, changes of erythrocyte and platelet count and hemoglobin content in the preimmunized and HSC treated mice were almost the same as in the 700r alone irradiated controls. But, as for leucocyte and reticulocyte count, there was a definite difference between the preimmunized mice and the controls, i.e., leucocyte count in the former showed an initial increase up to 3000 at 10 days, followed by a decrease by 20 days and increase to the normal level by 30 days, while it in the latter did not show initial increase and began to increase after the 14 th day and became normal by 20 days. Reticulocyte count in the former increased at 8~10 days up to 20~30‰ and rapidly became 100‰ at 15 days and then decreased after the 20 th day, while it in the latter did not show crisis and reached the normal level by 30 days.

C) **Histological findings.** There were no marked differences between the preimmunized mice and the 700r alone irradiated controls. In both groups, even thymic recovery started at almost the same days.

II. Effect of HSC Administered at Various Post-irradiation Days to 700r Irradiated Host

As shown in Table 2, inoculation of HSC at 3, 5 or 7 days after irradiation did not induce early mortality, but more than half of such treated host mice died after the 30 th postirradiation day, i.e., survival rates were 40~57% at 90 days. When the mice were treated with HSC at 12 days after irradiation, all of them survived beyond 90 days.

Table 2. Effect of delayed transplantation of Na2 spleen cells to 700r irradiated Dd/s host.

Exp.	Transplantation days after irrad.	No. of mice	% survival (at days)					
			7	14	21	30	60	90
1	3 days after	21	100	100	100	100	81	57
2	5 〃	20	100	100	100	90	70	40
3	7 〃	20	100	95	85	85	50	50
4	12 〃	20	100	100	100	100	100	100

III. Effect of Preinoculation with "Homologously Immunized" Lymphocytes or Spleen Cells of the Host Strain

As shown in Table 3, the preinoculation with "homologously immunized" viable spleen cells of the host strain altered MLD effect and delayed death was not observed as shown in Exp. 1. When killed immunized tissue was preinoculated, delayed death occurred as shown in Exps. 2 and 3. Preinoculation with spleen

Table 3. Effect of preimmunization with Na2 immunized Dd/s tissues of 700r irradiated Dd/s host against transplantation of Na2 spleen cells.

Exp.	Antigen	No. of mice	% survival (at days)					
			7	14	21	30	60	90
1	Na2 immunized viable Dd/s spleen cells	19	100	100	95	95	84	84
2	Na2 immunized Dd/s liver	20	100	100	75	60	35	35
3	Na2 immunized and killed Dd/s spleen	20	100	95	95	80	60	50
4	Na2 immunized viable Dd/s lymph node cells	20	95	90	80	75	75	35
5	Spleen cells of Dd/s which 7 days previously adopted Dd/s spleen cells immunized whith Na2 tissues.	17	88	88	88	88	82	82

* Preimmunization was given 7 days before irradiation.

cells, which were obtained from the mice of the same strain which were inoculated 7 days previously with viable spleen cells obtained from the "homologously immunized" host strain mice, could alter MLD effect as shown in Exp. 5.

IV. Effect of Spleen Cells of Chimeric Donor to 700r Irradiated Host

As shown in Table 4, transplantation of spleen cells of the "homologously preimmunized" host mice which survived beyond 90 days after 700r irradiation and HSC inoculation altered MLD effect, but later delayed death occurred. When spleen cells of the "homologously preimmunized" host mice were used as donor cells at 21 days after irradiation and HSC inoculation, they also could alter MLD effect, but delayed death occurred after the 30 th post-irradiation day.

V. Effect of Anti-donor-host-serum (Antiserum) to 700r Irradiated and HSC Treated Host

As shown in Table 5, antiserum altered MLD effect but did not prevent delayed death, i.e., survival rates were 63% at 30 days and 32% at 90 days.

Table 4. Effect of chimeric donor to 700r irradiated dd/s host.

Exp.	Chimeric donor	No. of mice	% survival (at days)					
			7	14	21	30	60	90
1	Mice survived beyond 90 days in Exp. 4 of Table 3.	19	100	100	100	84	58	47
2	Mice survived beyond 90 days in Exp. 7 of Table 1.	12	100	88	67	58	50	50
2	Mice sacrificed at 21 days in Exp. 2 of Table 1.	21	100	100	100	100	81	48

Table 5. Effect of antiserum to 700r irradiated Dd/s host treated with Na2 spleen cells.

Exp.	Serum	Route of injection	No. of mice	% survival (at days)					
				7	14	21	30	60	90
1	Antiserum 0.02 c.c.	i.v.	38	92	87	68	63	45	32
2	Normal dd/s serum 0.02 c.c.	i.v.	20	85	85	70	30	20	20

* Antiserum means anti-Na2 Dd/s serum.

DISCUSSION

MLD effect was almost prevented by various preimmunizations to the host mice 7 days before 700r irradiation and HSC transplantation. In order to preimmunize, homologous viable cells or semihomogenates of liver, kidney, spleen and thymus were used for preimmunization and the effects of their ability to prevent MLD effect were almost equal to each other. Several workers reported[3,4] that the transplantation antigen was found in most of the tissues in mice. These data also suggest that all of these tissues have the same transplantation antigen. Futhermore, when the host mice were immunized with the donor tissues 7 days before irradiation and HSC inoculation, their leucocyte count increased temporarily, followed by secondary decrease, and then increased gradually after the 21th irradiation-day. These data may be undertood as a simple graft rejection as Uphoff's implication[5], but these spleen cells of host mice at 21 days after irradiation and HSC inoculation, when transplanted to 700r irradiated mice of the same host strain, killed more than half of such treated mice after the 30th irradiation-day, i.e., survival rates were 100% at 30 days and 48% at 90 days. If delayed death were caused by graft against host reaction, these data might indicate that preimmunization could not completely reject the grafted cells by 21 days in these strain combinations, and a temporary take of HSC should be considered instead of the simple graft rejection. Similarly, preimmunization 2 days or 30 minutes befere irradiation and HSC transplantation prevented early death, but not delayed death. As for these results, the possible implication appears too complex to clarify, but the spleen cells of the host mice which survived beyond 90 days after 700r irradiation and HSC transplantation, when they were used as donor, killed half of the 700r irradiated mice of the same strain by 90 days. Therefore, it is suggested that preimmunization 2 days or 30 minutes before did not reject the infused HSC completely and later delayed death ocourred as a result of graft versus host reaction. Injection of anti-donor-host serum in place of preimmunization also altered MLD effect. This means, at least, participation of serum antibody on homograft response. As previously reported[2], the cellularity of bone marrow and thymic recovery of the 700r alone irradiated mice became nearly normal by 10 days after irradiation. Therefore, it is reasonably thought that transplantation of HSC 12 days after irradiation did not kill host mice, i.e., survival rates were 100% at 30 days and 90 days. These data may mean the simple graft rejection. But transplantation of HSC 3 days, 5 days or 7 days after irradiation killed about half of the host mice in 90 days, especially after the 30th irradiation-day, i.e., delayed death was observed. Uphoff also reported[5] that 20 hours delayed transplantation of homologous bone marrow altered MLD effect, though delayed death was observed. In the present study when the host mice were inoculated with the spleen cells of the "homologously immunized" host strain mice 7 days before irradiation and HSC transplantation, MLD effect was nearly abolished. Furthermore, preinoculation with the spleen cells, which were obtained from the mice of the same strain 7 days previously inoculated with the

viable spleen cells of the "homologously immunized" host strain mice, could alter MLD effect and delayed death did not occur. However, when the spleen cells from the "homologously immunized" host strain mice were killed by three quick freezing and thawings and were used for preimmunization, delayed death could not be prevented but early death did not occur. Therefore, inoculation of immunized viable lymphocyte or spleen cells may transfer transplantation immunity to other mice, but the possibility of transfer of transplantation antigen should be born in mind at the same time.

ACKNOWLEDGMENTS

It is a great pleasure to thanks Professor G. Wakisaka, M. D. and Dr. H. Uchino, M. D. and Dr. M. Yamagishi, and Dr. M. Hama for their valuable suggestions and advices in carrying out the study here reported. Thanks are also extended to Mr. R. Katano, Institute for Chemical Research of Kyoto University, for his kindness in frequently operating the Co^{60} irradiation facility.

This work was supported by a grant-in-aid from the International Atomic Energy Agency 138/RB (1962-1964), to which thanks are due.

REFERENCES

(1) K. Adachi, *This Bulletin*, 44, 89 (1966).
(2) K. Adachi, *This Bulletin*, 44, 103 (1966).
(3) L. A. Manson, G. V. Foshi, T. Dougherty, and Joy Palm, *Ann. N. Y. Acad. Sci.*, 120, 261 (1964).
(4) Salah Al-Askari, D. C. Dumonde, H. S. Lawrence, and L. Thomas, *Ann. N. Y. Acad. Sci.*, 120, 261 (1964).
(5) D. E. Uphoff, *J. Nat. Cancer Inst.*, 30, 1115 (1963).