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

Application of Antifolic "Methotrexate" for Suppression of Immune Response

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1) The early administration of folic acid antagonist, Methotrexate, for lethally gamma-irradiated mice treated with HBM is highly effective for the suppression against the development of the homologous disease, but its effect is still less than that of the IBM treatment.

2) The early administration of Methotrexate for the lethally gamma-irradiated mice treated with the homologous spleen cells is not enough effective for the suppression of the homologous disease.

3) The probable mechanism as to the suppression of the immune response by the administration of Methotrexate was discussed.

INTRODUCTION

Mice exposed to lethal total body irradiation which receive an intravenous inoculation of the homologous bone marrow (HBM) may survive acute irradiation death. Later, the majority of them may die from so-called homologous disease. It is generally accepted that there is an immune response between host and graft. Therefore, in order to inhibit the homologous disease, the use of immunological tolerated fetal liver as a protective agent in place of adult HBM has been studied, but its effect seems to be not always enough[1~4]. Drug treatment as a method of modifying the homologous disease has been tried. Uphoff[5] reported that the use of folic acid antagonist, Methotrexate, might spare mice that would normally succumb to a homograft reaction following lethal total body X irradiation and HBM treatment. Ferrebee *et al.*[6~8] also reported that an early administration of Methotrexate prevented the delayed foreign marrow reaction. We have been working also several years for the suppression of the homologous disease. The results obtained by the use of Methotrexate up to the present time are to be reported here.

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MATERIALS AND METHODS

Dd/s and Na2 strain mice were used as recipients and donors. They were supplied from the Kyoto University Animal Center. A Co^{60} gamma-irradiation facility which belongs to the Institute for Chemical Research of Kyoto University was used in the present experiment. The conditions of irradiation were described elswhere[9,10]. Lethal irradiation in this study means approximately 900 r irradiation which is 100% lethal to mice within 30 days. We have LD100 dose of gamma-rays to mice in less than half a minute. Methotrexate (1.5 mg per kg body weight) was injected intraperitoneally 4 times every other day, biginning 1 day after the total-body irradiation and HBM transplantation. In one group, Methotrexate was given 8, 10, 12 and 14 days after the irradiation and HBM transplantation. Non-irradiated mice which were injected Methotrexate with the same dose and duration did not show any signs of toxicity due to this drug. There were found no changes in the peripheral blood pictures and body weight[11].

The number of the nucleated cells of inoculated bone marrow and spleen cell suspension was 10×10^6, respectively.

RESULTS AND DISCUSSIONS

Eight types of experiments were carried out, as summarized in Table 1.

In the experiment 1, Methotrexate was given at 1, 3, 5 and 7 days to mice irradiated and treated with HBM. In this group of experiment, the survival rates were 78% at 21 days and 73% at 30 days. It should be noted that they

Table 1. Survival of gamma-irradiated mice treated with homologous bone marrow and homologous spleen cells.

Type of Experiments	Number of survival / Number of irradiated (at days)						% Survival (at days)			
	7	14	21	30	60	90	21	30	60	90
1) HBM+MT* (1,3,5 and 7 days after irrad.)	22/22	20/22	17/22	16/22	10/22	7/22	78	73	45	32
2) HBM+MT** (8,10,12 and 14 days after irrad.)	16/16	12/16	9/16	9/16	4/16	4/16	51	51	25	25
3) HSC+MT (1,3,5 and 7 days after irrad.)	26/32	13/32	10/31	8/30	3/29	0/29	33	26	10	0
4) HSC+MT (1,3,5 and 7 days after irrad.)	31/32	31/32	19/32	14/32	7/32	7/32	59	44	22	22
5) HBM	6/10	3/10	3/10	2/10	1/10	1/10	30	20	10	10
6) HSC	17/20	13/20	7/20	2/20	1/20	1/20	35	10	5	5
7) ISC+MT (1,3,5 and 7 days after irrad.)	8/8	7/8	7/8	7/8	7/8	7/8	88	88	88	88
8) ISC	8/8	8/8	8/8	7/8	7/8	7/8	100	88	88	88

Number of nucleated cells was 10×10^6.

* MT: 1.5 mg/kg of Methotrexate given at 1, 3, 5 and 7 days after Irradiation.

** MT: 1.5 mg/kg of Methotrexate given at 8, 10, 12 and 14 days after Irradiation.

HBM: Homologous bone marrow. HSC: Homologous spleen cells.

ISC: Isologous spleen cells.

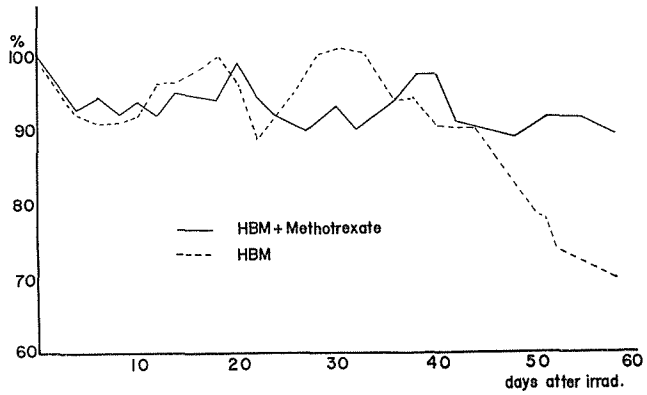

Fig. 1. Body weight changes in mice irradiated lethally and treated with homologous bone marrow and Methotrexate.
Body weight change to preirradiation level was represented in percentage.

were much better than those of 30% at 21 days and 20% at 30 days in HBM treatment alone in experiment 5. Body weight changes in both group are shown in Fig. 1. They reached almost pre-irradiation level approximately 20 days after the total-body irradiation and, then decreased later again. The mice treated with HBM alone, however, showed more rapid decreasing tendency than the mice treated with HBM and Methotrexate did, although the survival rates in the latter group also decreased to 45% at 60 days. These results may suggest the possibility that an immune response was suppressed by early administration of Methotrexate for about 30 days, though it occurred in some mice later.

Ferrebee *et al.*[6] recently discussed that, if the reaction of immunnologically competent cells in the marrow infusion can be restrained during a short interval, either these cells pass through their life-span and die without injuring the host, or they and their progeny acquire tolerance to their environment without first having injured it and themselves by reaction. Our results can be also explained with this idea.

Uphoff[5] reported that the secondary weight loss which might be caused by the homograft response was suppressed by the administration of Methotrexate every other day 9 times beginning at 14 days after the total-body irradiation. But the other workers[6] reported that the survival rates of mice treated with HBM and the delayed administration of Methotrexate were 90% at 10 days and 20% at 30 days. As shown in experiment 2, when Methotrexate was given at 8, 10, 12 and 14 days after irradiation, the survival rates were 51%, 51% and 25% at 21, 30 and 60 days after the irradiation, respectively. These results are not better than those in experiment 1, but a little better than those in HBM treatment alone.

It is well known that the spleen cells can protect the mice irradiated lethally, but it is also believed, so far, that there are many lymphoreticular cells in the spleen of mice, but almost nothing of them in the bone marrow. These facts led us to presume that the effect of Methotrexate must diminish

when the number of the transplanted immunological competent cells increases, and the following experiments were undertaken. Isologous spleen cell transplantation with and without Methotrexate are shown in experiments 7 and 8, respectively. The survival rates in both groups were almost the same. Therefore, it goes without saying that this dose of Methotrexate used has not any marked toxicity at all in lethally gamma-irradiated mice, while Uphoff[5] suggested that Methotrexate might be more toxic in the lethally irradiated mice than in normal mice. In the homologous spleen cell plus Methotrexate treated group, body weight began to decrease again about 20 days after the irradiation and did not show any tendency of recovery, as shown in Fig. 2. As described

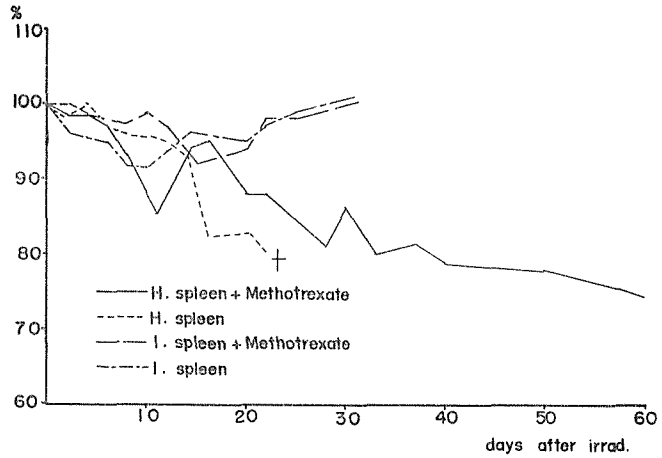

Fig. 2. Body weight changes in mice irradiated lethally and treated with homologous spleen cells and Methotrexate.
Body weight change to preirradiation level was represented in percentage.

above, the immunological competent cells seem to be reactive in the early stage of recovering after the gamma-irradiation, and it is probable that, the more the number of them is, the stronger the immune response occurs. When Methotrexate with the dose of demonstrating no toxicity is administered to mice treated with HBM transplantation, it is thought to be highly effective for the suppression against the development of the homologous disease, but the effect is still less than that of the IBM treatment. These results show the limitation of the usefulness of Methotrexate. Further investigations in order to prevent the immune response are awaited.

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REFERENCES

(1) D. W. H. Barnes, P. L. T. Ilbery and J. F. Loutit, *Nature*, **181**, 488 (1958).
(2) D. E. Uphoff, *J. Nat. Cancer Inst.*, **20**, 625 (1958).
(3) K. A. Porter and R. Moseley, *Brit. J. Exptl. Path.*, **39**, 128 (1958), **40**, 273 (1959).
(4) M. Hama, Unpublished data.
(5) D. E. Uphoff, *Proc. Soc. Exp. Biol. Med.*, **99**, 651 (1958).
(6) H. L. Lochte, Jr., A. S. Levy, D. M. Guenther, E. D. Thomas and J. W. Ferrebee, *Nature*, **196**, 1110 (1962).
(7) A. L. Lochte and E. D. Thomas, *Blood*, **20**, 635 (1962).
(8) E. D. Thomas and J. W. Ferrebee, *Blood*, **20**, 635 (1962).
(9) M. Yamagishi, *This Bulletin*, **37**, 440, 453, 461 (1959).
(10) M. Yamagishi, K. Adachi, S. Shirakawa, M. Yamaba and H. Uchino, *This Bulletin*, **40**, 42 (1962).
(11) K. Adachi, in preparation.