Alteration of Homograft Reaction by A-methopterin in Lethally Irradiated Mice Treated with Homologous Marrow. (24450)

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Mice exposed to lethal total-body X irradiation which receive an intravenous inoculation of homologous marrow may survive acute irradiation damage. Later, they may succumb to a homograft reaction. Although it is generally accepted that there is an immune response, the problem of whether this reaction is initiated by host(1) or graft(2) has not been resolved. Methods of precluding this homograft reaction have been studied with little success(3). Increased long-term survival(4,5) may be readily obtained following use of embryonic hematopoietic tissue as a protective agent in place of adult marrow cells. This procedure is limited in usefulness since complete absence of homograft reaction (2) is restricted to certain strain combinations (unpublished data). Drug treatments as a method of modifying the homograft reaction have proved generally unfavorable(3). An attempt was made to apply the observations of V. H. Haas and M. Potter (personal communication), on inhibitory effect of A-methopterin on immune response in mice, to the problem of preclusion of homograft reaction in lethally irradiated mice treated with homologous marrow. This preliminary report shows alteration in homograft reaction in male and female mice by administration of folic acid antagonist, A-methopterin.* This alteration consists of an increase in survival in several groups and absence of a reaction in 2 groups.

Materials and methods. At 3 to 4 months of age mice were exposed to 800 r total-body X irradiation. This is a 30-day LD 98 dose for F_1 hybrid mice. Two X-ray tubes placed opposite to each other were operated at 200 kvp and 15 ma.; filtration was 0.25 mm cu. and 0.55 mm Al with 54 cm from each focal spot to center of mouse, with dose rate of 120 r/min. Previously described experimental procedures for marrow preparation and treatment were followed(6). Male and female mice from inbred strains or F_1 hybrids between 2 inbred strains were used in host-donor combinations which had elicited an early severe reaction under these experimental conditions. A-methopterin was administered by intraperitoneal injection at 2 dose levels: 3 mg/kg body weight and 1.5 mg/kg body weight at 48 hour intervals for 5, 6 and 9 treatments. Drug treatment was started at 14 days following irradiation and homologous marrow inoculation. At this time the animals survived acute irradiation damage, body weight is returning to preirradiation level and homograft reaction has not become apparent. The variation in drug treatment was an attempt to acquire maximum beneficial effects, with minimum drug toxicity in lethally irradiated animals.

Results. A group of 23 (C57BL $\times$ DBA/2)F_1 hybrid male mice were exposed to 800 r total-body X irradiation. Five animals served as irradiated controls and were all dead by 12 days. The 5 animals which received isologous marrow and the 13 animals which received parental-strain C57BL marrow all survived acute irradiation damage. Of the 13 mice which received C57BL marrow, 7 animals were treated with intraperitoneal injections of A-methopterin at a dose of 1.5 mg/kg body weight at 48-hour intervals for 9 treatments and 6 animals served as controls. Fig. 1 shows curves for body weights recorded every 48 hours for these 3 groups of mice starting at 14 days post-irradiation.

In this graft-host combination there is an early, severe reaction. Although this reaction appears to be uniformly severe in all mice treated, 11% of the treated mice survived the reaction(2). In the group which received only homologous marrow treatment, one animal survived the reaction and weights for this mouse are represented by the broken-line curve. The weight of this animal declined

* A-methopterin (Methotrexate) kindly supplied by Lederle Labs., Amer. Cyanamid Co., Pearl River, N. Y.

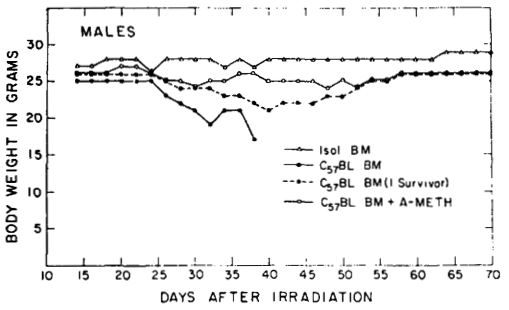

FIG. 1. Mean body wt of (C57BL × DBA/2)F_1 hybrid male mice receiving 800 r total body irradiation followed by isologous marrow inoculation, or parental-strain marrow, or parental-strain marrow and A-methopterin at a dose of 1.5 mg/kg × 9 q 48 hr. Wt curves start at 14 days post-irradiation when drug was first administered and continued until all mice were dead or recovery was established.

between 26th and 40th day representing the period when the reaction was grossly quite apparent. Eighteen days after lowest weight was recorded, this animal had fully recovered its weight loss. Average weights were recorded for all mice which did not survive the reaction and show a declining weight after 24 days until all animals were dead at 38 days.

In these hybrid male mice drug toxicity was noted near the end of series of drug treatments but there was quick recovery in most mice following suspension of treatment. One animal died on third day after drug was suspended and 2 animals failed to recover and died on days 50 and 56 following irradiation. These deaths were considered due to drug toxicity since the gross appearance of these animals was not that of a "typical" reaction in animals treated with homologous marrow following lethal irradiation. These animals appeared anemic, had enlarged spleens, and lymphoid tissue did not appear depleted as in animals dying of homograft reaction. The animals which survived drug treatment showed no secondary weight loss which might indicate a delayed homograft reaction. At 60 days post-irradiation these animals were grossly indistinguishable from those receiving isologous marrow, having already acquired the gray coat typical of black mice receiving high doses of irradiation. This group represents a 57% survival at 70 days.

While attempting to determine the dose of A-methopterin that would produce maximum beneficial effects and minimum toxic effects in these irradiated mice, several factors became quite apparent. Heavily irradiated animals do not tolerate A-methopterin as well as non-irradiated animals. One important factor may be the very short recovery period after irradiation and marrow treatment, as these animals barely regain their pre-irradiation weight when drug treatment is started. The previously observed(7) sex difference in toxicity to antifolic analogues was present, with females more sensitive than males to this drug treatment. It appears that to obtain maximum beneficial effects in suppressing the homograft reaction there must be some degree of toxicity. For example: In treating 10 (C57BL x DBA/2)F_1 hybrid males and 5 hybrid females with 3 mg/kg body weight of A-methopterin at 48-hour intervals for 5 treatments, females showed marked drug toxicity from which 4 of 5 mice recovered with no homograft reaction noted. On the other hand, 10 males showed no ill effects of this limited drug treatment, but 10 days following suspension of drug treatment, the beginning of a homograft reaction was noted. This reaction appeared quite "typical" with severe diarrhea, hunched appearance and ruffled coat. Although it appeared to be a severe reaction, 5 of these males recovered. It is not clear how much of this effect can be attributed to sex difference and how much to drug toxicity. It would appear that degree of depression of the reticuloendothelial system, as measured by toxicity, is important. There was toxicity noted and no discernible reaction (Fig. 1) at a total dose of drug less than in mice in which there was no toxicity but a reaction occurred, presumably because of insufficient drug treatment. The females which survived toxic effects of drug and the males which survived the reaction were still alive over 90 days post-irradiation.

In an attempt to reduce toxic effects of drug in female mice, 8 (C57BL x DBA/2)F_1 hybrid females were given A-methopterin at a dose of 1.5 mg/kg body weight at 48-hour intervals for 6 treatments. Drug toxicity was noted in these mice and weight loss occurred in the group receiving drug before it was observed in animals which received only ho-

mologous marrow. Although a severe homograft reaction was observed in drug-treated mice, all treated mice were still alive at 90 days after treatment. This reduced drug dose appears to be insufficient to suppress immune response in females although 9 treatments of the same dose proved effective in the male. CBA female mice irradiated, treated with C57BL marrow and given the modified drug treatment of 1.5 mg/kg body weight for 6 treatments gave results similar to those of the (C57BL x DBA/2)F_1 hybrid females.

Preliminary data are available on another hybrid combination, namely (C57BL x A)F_1 hybrid mice treated with C57BL marrow. In this case, the onset of reaction is later than in (C57BL x DBA/2)F_1 hybrids treated with C57BL marrow(2). When (C57BL x A)F_1 hybrid males were treated with 3 mg/kg body weight every 48 hours for 5 treatments starting at 14-day post-irradiation, drug treatment appeared deleterious. Although this observation must be confirmed, it would appear that drug treatment might lose its beneficial effect if administered too long before the expected onset of homograft reaction. Support for this belief is found in the work of R. Schwartz(8), who observed suppression of immune response in rabbits to bovine serum albumin as long as 6-mercaptopurine was administered. Upon suspension of drug treatment, the rabbits regained their ability to react against the foreign antigen. In the case of homograft reaction in irradiated mice treated with homologous marrow, it appears possible to permanently suppress this reaction as illustrated by male mice in Fig. 1 and female mice which received A-methopterin at 3 mg/kg body weight for 5 treatments at 48-hour intervals, or to reduce severity of the reaction as illustrated by increase in survival following the reaction in the other cases cited, if drug is administered during the time when the reaction would be apparent.

The mechanism of the action of A-methopterin in suppressing the homograft reaction in irradiated mice is obscure. This action appears to be of a fundamental nature since it is also possible to increase survival time of skin grafts by a similar treatment (unpublished data).

Although this is a preliminary report and further work is necessary, the results indicate that this may well be a profitable approach to the problem of preclusion of homograft reaction in irradiated mice treated with homologous marrow as well as other types of homograft reactions.

Summary. Mice which would normally succumb to a homograft reaction following lethal, total-body X irradiation and homologous marrow inoculation, may be spared if they are treated with folic acid antagonist, A-methopterin. Using different treatment schedules, varying degrees of effectiveness from increased survival following a typical reaction, to no detectable reaction, may be obtained. The relationship between drug toxicity and effectiveness in altering the homograft reaction is discussed.

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Received September 11, 1958. P.S.E.B.M., 1958, v99.