The Effect of Presensitization of Parental Donors on Graft-Versus-Host Disease in Irradiated F_1 Hybrid Mice[1] (35864)

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The phenomenon of graft-versus-host (GVH) disease has been studied extensively in mice, with parent strain hemopoietic cell donors and irradiated or unirradiated F_1 hybrid recipients. An excellent general review of GVH reactions has been made by Simonsen (1).

GVH disease induced in $1C3F_1$ hybrid mice after irradiation and injection of parental spleen or bone marrow cells was noted previously to be mild compared to that occurring in some other strain combinations. However, presensitization of the parental donor by spleen cells of the F_1 or of the reciprocal parent results in a severe GVH reaction under the same conditions (2–5).

In the experiments reported here, presensitization of parental donors at various times produced a rapidly heightening effectiveness of GVH inducing capability in the early days after sensitization, with a prolonged persistence of this capability.

Materials and Methods. The mice used in these studies were young adult (12–20 weeks old) $1C3F_1$ (101/Cum × C3H/Anf) F_1, 101/Cum, and C3H/Anf males and females obtained from Cumberland View Farms, Clinton, Tennessee. Spleen cell recipients ($1C3F_1$) were exposed to 500 R whole-body X-radiation by means of a General Electric Maxitron 300 operated under the following conditions: 300 keV; 20 mA; inherent filtration, 0.1 mm Al; 3 mm Al added filtration; 0.45 mm Cu, HVL; 93-cm target-to-mouse distance; dose rate in air, ~100 R/min. Mice were restrained during radiation exposure in a perforated Lucite cage rotated horizontally in the radiation field at 6 rpm. Within 2 hr after irradiation, some mice were given intraperitoneal (ip) injections of C3H spleen cells equivalent to 1/10 of the donor spleen nucleated cell content (~15×10^6 cells). Male and female donors were presensitized by ip injections of 101 spleen cells equivalent to 1/10 of the cell content of the same sex at 1–7 days, 28 days, or 7–24 months prior to use as donors. Four groups of experimental controls were arranged: (i) sensitization controls were nonsensitized C3H spleen cell donors at ages approximating those of sensitized donors; (ii) syngenic spleen cell donor controls consisted of two groups of recipients given a similar injection of syngenic spleen cells from either young or old donors; (iii) injection controls were nonirradiated $1C3F_1$ mice given an ip injection of sensitized C3H spleen cells; (iv) radiation controls were noninjected $1C3F_1$ mice exposed to 500 R X-rays. The experimental and most of the control groups were repeated several times.

Spleen cell suspensions were prepared by gentle homogenization of donor spleens in a glass homogenizer and diluted to volume with cold Tyrode's solution. All mice were observed for signs of illness and time of death. Some mice from each group were examined histopathologically after Zenker or Bouin fixation of major hemopoietic organs and viscera.

Results. The experimental results are summarized in Tables I and II. Radiation controls in this study showed a 30-day mortality of 16%. In previous experiments using $1C3F_1$ mice, an exposure to 500 R whole-body X-radiation was often an $LD_{0/30}$. The injection

[1] Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

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TABLE I. The Effect of Time of Donor Presensitization on Mortality and Average Survival in $1C3F_1$ Mice Receiving C3H Spleen Cells After 500 R X-Ray Exposure.

No. of mice	Elapsed time since donor sensitization[a]	Mortality (%) at day			Av survival (days)
		30	60	120	
89	1–3 days	3	6	18	65
40	4 days	10	33	46	42
16	5 days	38	56	69	30
24	6 days	60	80	94	28
19	7 days	79	84	100	26
43	28 days	81	100	100	22
56	28[b] days	93	100	100	18
50	7–11 months	76	92	96	26
70	12–24 months	46	79	87	34

[a] Donor sensitization consisted of an ip injection of 101 spleen cell suspension equivalent to 1/10 of a spleen.

[b] Donors were 12–17 months old at time of sensitization.

of syngenic spleen cells after a 500 R exposure to the recipient did not result in any significant mortality. Spleen cells from either young or old unsensitized C3H donors produced a mild GVH disease with low 30-day mortality. Mortality was mild to moderate at 120 days after treatment with young C3H spleen cells but higher with older (12–23 months) cells.

Presensitization of the C3H spleen donors by 101 reciprocal parent spleen cells (same sex) at various times before use altered the mortality pattern, shortened the average survival, increased signs of illness, and produced severe GVH tissue lesions. This effect was noticeable to a mild degree even in unirradiated recipients of presensitized C3H spleen cells. The time that elapsed between presensitization and use of the donor was also important. If only 1–3 days passed, no effect of sensitization was seen. However, at and after 4 days, the effectiveness in inducing GVH disease increased rapidly, with increasing mortality and shortening of average post-treatment survival. By day 7, the donors appeared to be nearly fully sensitized, causing reactions nearly as severe as those induced by donors used 28 days after sensitization. Donors were also used at 7–24 months after sensitization and still were capable of inducing severe GVH disease even at the end of that period, although mortality fell and average survival lengthened somewhat as the elapsed postsensitization time increased. The average life-span of untreated C3H mice is about 23 months, while that of the $1C3F_1$

TABLE II. Mortality and Average Survival of $1C3F_1$ Mice Receiving Unsensitized Parent or Isogenic Spleen Cells After 500 R X-Ray Exposure.

No. of mice	Exposure (R)	Donor	Donor age	Mortality (%) at day			Av survival (days)[b]
				30	60	120	
44	500	—	—	16	16	18	27
31	500	C3H	12–16 weeks	3	19	26	64
66	500	C3H	12–23 months	14	23	44	69
37	500	$1C3F_1$	12–16 weeks	0	3	5	69
58	500	$1C3F_1$	12–17 months	0	0	7	99
41	0	C3H[a]	12–16 weeks	5	20	32	66

[a] Donors were sensitized 28 days prior to use by injection of reciprocal parent spleen cells as in Table I.

[b] Of those dying during the experimental period.

hybrid is near 29 months.

Discussion. Since the earlier observation in this laboratory that presensitization of parental donors enhanced subsequent GVH disease in irradiated F_1 recipients (2), we have studied several aspects of this disease. The use of presensitized parental bone marrow cells in lethally irradiated recipients has been found to enhance the resulting foreign bone marrow type of GVH disease (3). Further studies and comparisons with other parent-to-F_1 strain combinations have been reported in terms of histopathology (4) and effect of radiation dose (5). There have been a number of other reports concerning the effectiveness of presensitization on parent donors [*e.g.*, Bain and Alton (6), Fox (7)] using both irradiated and unirradiated recipients.

The two parent mouse strains (101, C3H) of the $1C3F_1$ hybrid used in these experiments are the same at the H2 locus ($H\text{-}2^k$). In this strain combination the GVH disease that results from irradiation and injection of parental strain spleen cells is mild unless the donor is presensitized by injection of reciprocal parent spleen cells. Results are similar regardless of which parent strain is used as the donor, but we have confined this report to the use of the C3H donor because of more thorough studies with this combination. Apparently some histocompatibility antigens other than H2 are responsible for this presensitization. Donor presensitization seems to be effective only after at least 3 days, is near maximum effectiveness at 7 days, and only gradually falls off through the life of the donor. Sensitization is still active 24 months after treatment. This implies that the transplanted cells responsible for this disease either have a long life and long immunologic memory or give rise to generations of offspring capable of reacting in the same way. Various authors have reported the immunological capabilities and long life-spans of certain small lymphocytes thought to act in this capacity (8–11). The observation that the effect of presensitization of donors may persist for a long time has also been made under other circumstances; however, in the experiments of Billingham *et al.* (12), presensitization by skin graft was somewhat delayed and much longer lasting than presensitization by spleen cells, a result somewhat at unexplained variance with our findings.

Summary. The GVH disease in $1C3F_1$ hybrid mice following 500 R X-irradiation and injection of normal parent strain spleen cells is mild in terms of mortality, signs, and tissue pathology. Presensitization of the donor with spleen cells of the reciprocal parent strain, however, results in a highly lethal GVH disease. The timing of presensitization before donor use is an important factor in the severity of the resulting GVH disease. Presensitization of the donor seems to require more than 3 days, is near maximum effectiveness at 7 days, persists at this level for many months, and gradually falls off through the life of the donor. After 24 months, sensitized donors still are more effective in producing GVH disease than controls of comparable age.

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Received May 19, 1971. P.S.E.B.M., 1971, Vol. 138.