Influence of Sex on Bone Marrow Transplantation in Mice With Induced Tolerance. (27036)

ALBERTO P. CRISTOFFANINI*, EARL V. LONG AND LEANDRO M. TOCANTINS
Charlotte Drake Cardeza Foundation, Jefferson Med. College, Philadelphia, Pa.

Eichwald and Silmser(1) and Hauschka(2) reported that female mice frequently reject male skin grafts, while males usually accept female grafts. This phenomenon which was thought to be related to the sex chromosomes has been shown to play a significant role in every strain of mice tested(3,4,5,6,7,8,). Furthermore, using tolerant rat mice combinations, Billingham and Silvers demonstrated that this sex factor is also present in heterologous combinations, and added evidence to relate it to the Y chromosome(9). Rejection or decreased receptivity by female mice of male material was later reported in iso and homografts of transplantable tumors(10) and also in isologous normal thymus transplants(11).

Lengerova and Chutna(12) reported the appearance of secondary disease in $C_{57}Bl$ males protected against lethal irradiation with isologous female bone marrow. Similar results were obtained by us in C_3H mice(13). They interpreted this secondary disease as being caused by the reaction of the female graft against the male host in a way equivalent to the "Eichwald and Silmser phenomenon," lending support to the graft-anti-host theory(14). During studies in our laboratory of the effect of induced "tolerance" on homologous bone marrow transplantation(15), it was observed that "tolerant" males behaved differently from "tolerant" females, when used as donors, or hosts, in homologous bone marrow transplantation experiments. This different behavior was thought to be related to the "Eichwald-Silmser phenomenon."

Material and methods. Newborn animals of A/Jax and C_3H strains† were injected, within 24 hours after birth with 2 to 5 $\times$ 10^6 bone marrow cells from C_3H and A/Jax adult females respectively, via the anterior fascial vein. The cells were obtained from marrow of a resected femur and suspended in 0.05 ml of 0.1% solution of Sequestrene in phosphate buffered saline at pH 6.5-7.2. The newborn were allowed to nurse under usual animal farm conditions and survivors were weaned at 30-40 days. At 6 weeks of age, a number of the injected C_3H animals were grafted with A/Jax skin (males with male skin and females with female skin). The animals which were used later for bone marrow transplantation experiments were irradiated and protected with marrow injections. The irradiation was carried out at 14-16 weeks of age by giving 800r in a rotatory lucite cage to groups of 10 animals, at 250kv, 15A, 4.0 mm. Al. filter, target distance 60 cm, rate 114-118r per minute. The adult animals which received marrow were given 10×10^6 cells in 0.5 ml of suspension, via tail vein, within 4 hours after irradiation. The animals were observed and weighed daily.

Results and discussion. Experiment 1. C_3H mice of both sexes, injected at birth with female A/Jax bone marrow, when grafted with A/Jax skin (males with male skin and females with female skin) uniformly rejected these skin-grafts; however, date of rejection was delayed as is shown in Graph 1. Examination of this data by Analysis of Variance (Appendix A) demonstrates that the injected animals rejected their grafts 3.7 days later than the non-injected controls ($P<0.001$) and there is no significant difference between sexes. These results are markedly different from those reported by Billingham and Brent(16,17) for this same strain combination. They have reported that 70% of their animals became at least partially tolerant and 43% of them were highly tolerant, while our animals as a group showed a slight degree of tolerance and none of them appeared to be highly tolerant. However, in their experiments, donors of the bone

* From Catedra "B" de Medicina, Prof. O. Avendano M. and Hospital Clinico J. J. Aguirre, Univ. de Chile; Research Associate in Medicine, C. D. Cardeza Foundation, Jefferson Med. Coll. Supported by a grant from the Division of Biology, U.S. Atomic Energy Commission.

† From Jackson Memorial Laboratories, Bar Harbour, Maine.

APPENDIX A. Analysis of Variance.

Source of Variation	Source of Squares	Degrees of Freedom	Mean Square	F	P (F)
Sex	2.74	1	2.74	.75	NS
Injection	179.43	1	179.43	48.89	.001
Sex-Injection Interaction	1.30	1	1.30	.35	NS
Individual Mice	213.04	58	3.67	—	—
Correction for Disproportion	34.26	—	—	—	—
Total	430.77	61	—	—	—

1. Effect or lack of effect of injection seems to be independent of sex of animals (no significant interaction between sex and injection).

2. No significant difference between sexes (in either non-injected or injected groups).

3. Highly significant effect of injection (regardless of sex). Injection associated with an average increase of 3.7 days in survival time of grafts.

marrow used to inject newborns were mostly males(17) whereas we have used females throughout.

Experiment 2. In a group of experiments to be reported elsewhere(15) we have observed a delay in the occurrence of secondary disease when C_3H females injected at birth with female A/Jax bone marrow cells were used as donors, or hosts, in homologous transplantation experiments. This was interpreted to mean that a slight tolerance (demonstrated in littermates by skin grafts), delays the immunological phenomenon which results in secondary disease.

In Graph 2 are plotted average body weight and survival curves of the following groups:

A) Eleven normal C_3H *males* lethally irradiated at 14-16 weeks of age and then protected with bone marrow from A/Jax *males* which at birth had received bone marrow cells from C_3H *females.* (tolerant donor, males).

B) Eleven normal C_3H *females* were lethally irradiated and then protected with bone marrow from A/Jax *females* which at birth had received C_3H *female* bone marrow(15). (tolerant donor, females).

C) Thirty male and female C_3H mice lethally irradiated and protected with male and female bone marrow respectively, from *normal non injected* A/Jax mice (males and females in this combination behaved identically, and are plotted together).

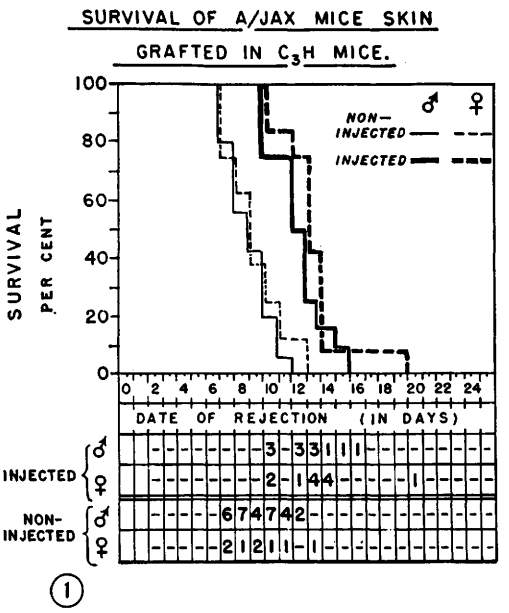
Paradoxically, the use of supposedly "tolerant" male donors, instead of increasing survival time of the male recipients, had the opposite effect: mean survival time of the Group A), 10.55 ± 6.4 days, is significantly shorter than the homologous bone marrow control C) (24.77 ± 12.3 days) ($P<0.01$), and much shorter than mean survival time of the females B) which received "tolerant" bone marrow (35.63 ± 13.9) ($P<0.001$). The survival curve of these males follows closely those reported in homologous experiments in which hypersensitized animals have been used(18,19,20).

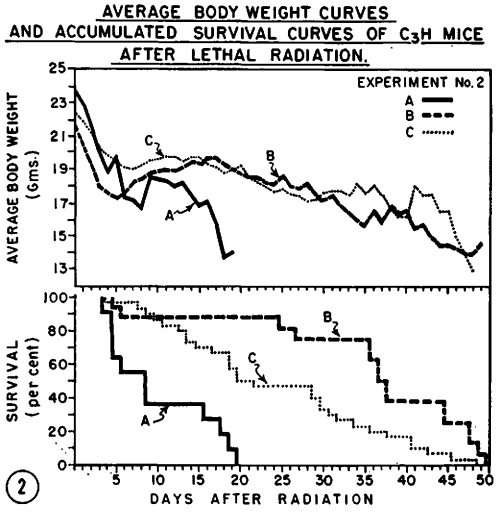
The difference between Groups A) and B) is sex, and the paradoxical effect in A) is obtained by male donors which received female material at birth. It can be speculated that injection of animals at birth produced a "chimera"; if so, the A/Jax male donors of group A) contain C_3H *female* cells which have been sensitized against their *male* host, and A/Jax male cells which have become tolerant to female C_3H cells. When the bone marrow from these animals is used to protect normal C_3H *males,* their A/Jax cells will not react immediately against C_3H (the reaction will rather be delayed since the animals are partially tolerant), but the already hypersensitized C_3H female cells will react against "male."

Experiment 3. In Graph 3 are plotted the average body weight and survival curves of the following groups:

D) Eight C_3H *males* which at birth had received bone marrow from A/Jax *female* donors, were lethally irradiated (at 14-16 weeks of age) and protected with A/Jax male bone marrow. ("tolerant hosts" males).

E) Thirteen C_3H *females* which at birth had received female A/Jax bone marrow (littermates of Group D), were irradiated and protected with normal A/Jax female bone marrow. ("tolerant hosts" female).





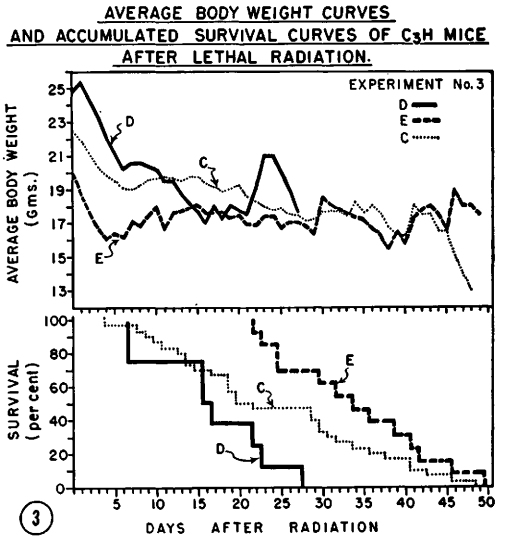


FIG. 1. Survival and date of rejection of grafts of A/Jax skin in C3H mice — injected at birth with A/Jax bone marrow—as compared with similar grafts in non-injected C3H mice. Data for both sexes are plotted separately.

FIG. 2. Average body weight curves and accumulated survival curves of C3H mice after lethal irradiation and protection. A=C3H males protected with bone marrow from A/Jax male mice—injected at birth with C3H female bone marrow—("tolerant" donor, males). B=C3H females protected with bone marrow from A/Jax female mice—injected at birth with C3H female bone marrow—("tolerant" donor, females). C=C3H mice (males and females) protected with bone marrow from normal non-injected A/Jax mice (males and females respectively).

FIG. 3. Average body weight curves and accumulated survival curves of C3H mice after lethal irradiation and protection. D=C3H males—injected at birth with A/Jax female bone marrow—protected with bone marrow from normal non-injected A/Jax males ("tolerant" hosts, males). E=C3H females—injected at birth with A/Jax female bone marrow—protected with bone marrow from normal non-injected A/Jax females ("tolerant" hosts, females). C as in Fig. 2, C.

C) The same homologous bone marrow control as in Exp. 2. Mean survival time for the males D) (17.00±7.4 days) is shorter than that of the homologous bone marrow controls, (24.77±12.3 days) but this difference does not reach statistical significance. However, when this mean survival time is compared with that of the "tolerant" female Group E), (34.23±9.1 days), the survival of the males is significantly shorter ($P<0.001$).

As in the previous experiment, it may be postulated that the injected animals are partial chimeras and that the female cells they carry are already hypersensitized against males. If this assumption is correct, this hypersensitization is responsible for the shortening of survival in the males.

Summary. Mice of A/Jax and C_3H strains were injected at birth with bone marrow cells from females of the opposite strain in an at-

tempt to induce "tolerance." Skin grafting experiments demonstrated that both males and females so treated did acquire a slight degree of tolerance. When the partially tolerant female mice were used as donors or hosts in homologous bone marrow transplantation experiments (with the opposite strain), the date of appearance of the secondary disease was delayed, an effect attributable to acquired tolerance. However, when partially tolerant males were used, survival did not increase, but actually decreased. The animals died in a very short time, as if hypersensitized. It has been proposed that the "tolerant" animals are chimeras; if so, the male animals may contain female cells already hypersensitized against "male," and these "hypersensitized" cells may be responsible for the short survival.

1. Eichwald, E. J., Silmser, C. R., *Trans. Bull.*, 1955, v2, 148.
2. Hauschka, T. S., *ibid.*, 1955, v2, 154.
3. Eichwald, E. J., Silmser, C. R., Wheeler, N., *Ann. N. Y. Acad. Sci.*, 1956-1957, v64, 737.
4. Prehn, R. T., Main, J. M., *J Nat. Cancer Inst.*, 1956, v17, 35.
5. Eichwald, E. J., Silmser, C. R., Weissman, I., *ibid.*, 1958, v20, 563.
6. Zaalberg, O. B., *Trans. Bull.*, 1959, v23, 433.
7. Krohn, P. L., *ibid.*, 1958, v5, 126.
8. Eichwald, E. J., Lustgraaf, E. C., Weissman, I, Stainer, M., *ibid.*, 1958, v22, 387.
9. Billingham, R. E., Silvers, W. K., Communications of Wistar Inst., University of Pennsylvania, April 1959.
10. Casey, A. E., Casey, J. G., Dowling, E. A., Hathaway, C. O., Wimberley, C. B., *Trans. Bull.*, 1958, v5, 106.
11. Hirsh, B. B., *ibid.*, 1957, v4, 58.
12. Lengerova, A., Chutna, J., *Fol. Biol. (Praha)*, 1959, v5, 24.
13. Cristoffanini, A. P., Long, E. V., Tocantins, L. M., *Blood* (Abst.), 1960, v15, 425.
14. Maleck, I., *Fol. Biol. (Praha)*, 1959, v5, 147.
15. Cristoffanini, A. P., Long, E. V., Tocantins, L. M., In preparation.
16. Billingham, R. E., Brent, L., *Phil. Trans. of Roy. Soc. London, S. B.*, 1959, v242, 439.
17. ————, Personal communication.
18. Barnes, D. W. H., Loutit, J. F., *Progress in Radiobiology*. Ed. by J. S. Mitchell, B. E. Holmes and C. C. Smith. 1956, p. 291, C. C. Thomas, Springfield, Ill.
19. Doherty, D. G., Communication to Bone Marrow Transplantation and Chemical Protection Conf., Oak Ridge, Tenn., Jan. 1959.
20. Cole, L. J., *J. Immunol.*, 1959, v83, 57.

Received September 5, 1961 P.S.E.B.M., 1961, v108

A Goitrogenic Rat Diet Producing Normal Body Weight Gain.* (27037)

FREDERIC W. NORDSIEK** (Introduced by Elmer L. Severinghaus)
Institute of Nutrition Sciences, Columbia University, New York

The first demonstration of low iodine goiter in the rat was reported by Levine, Remington and Von Kolnitz(1), and the diet they designed is, with minor modifications, still in general use today and has come to be known as the Remington diet. Attempts to improve the slow rate of body weight gain produced by this diet by adding animal proteins(2) have shown that such additions reduced the goitrogenic effect of the diet, making it less suitable for use in experimental goiter studies. Thus Parker *et al.*(3) observed good growth in rats fed a diet similar to the Remington diet with addition of DL-methionine, dried beef and "animal protein factor," but only the second generation of animals showed substantial thyroid enlargement, whereas growing rats consuming the standard Remington diet consistently develop goiter in 5 weeks.

The present paper describes a new low iodine goitrogenic rat diet, formulated without animal proteins, that supports a normal rate of body weight gain.

Materials and Methods. Brewers type dry

* This study was supported by grants from Nat. Inst. of Health and Nutrition Foundation.

** Present address: Memorial Sloan-Kettering Cancer Center, New York.