Homographs in Thymectomized, Irradiated Mice: Responses to Primary and Secondary Skin Grafts

Abstract. *Adult thymectomized, irradiated mice restored with syngeneic bone marrow exhibit an accelerated response toward second-set skin homografts, although the response to the primary grafts is impaired. Such mice have a long-lasting impairment (up to 223 days) of the primary response to allogeneic grafts (but not to xenogeneic grafts) and show the greatest deficiency of response toward grafts sharing the same H2 locus as the host. Thymectomy in the adult mouse is ineffective in altering a state of homograft sensitivity induced prior to irradiation.*

It is established that mice thymectomized at birth develop into immunologic "cripples" (*1, 2*). Adult mice, on the other hand, are apparently unaffected by thymectomy alone, but after exposure to radiation and restoration with syngeneic bone marrow cells, they do exhibit impaired responsiveness to homografts (*3, 4*). In addition, Miller *et al.* (*3*) have shown that adult thymectomized-irradiated mice show no primary or secondary hemagglutinin response to sheep erythrocytes. However, whether or not the thymus plays a role in second-set responses to homografts is not known. We have studied the response toward second-set skin grafts, the permanency of the immunological impairment, and the influence of antigenic difference between graft and host in thymectomized-irradiated adult mice.

Three strains of mice, mostly males, were used: (C57L × A)F_1 (hereafter designated LAF_1), C3H/HeJ, and CBA/J. All mice were thymectomized (or sham-thymectomized) at an age of 9 to 12 weeks, and 2 to 3 weeks later they were exposed to a single lethal dose of 250 kv (peak) x-rays (880 rad for the LAF_1; 840 rad for the C3H and CBA strains). Within 3 hours after irradiation each mouse received intravenously 5 to 8×10^6 syngeneic bone marrow cells derived from normal adult donors. One month later tail skin grafts were implanted according to the method of Bailey and Usama (*5*). The second-set grafts were put on from 56 to 196 days later, as indicated.

Typical data are shown in Fig. 1 (group I) for LAF_1 mice grafted 30 days after irradiation with BALB/cJ, SWR/J, and rat (Sprague-Dawley) skin. The allogeneic grafts had a mean survival time of 36 and 42 days, respectively, in thymectomized recipients, while the corresponding values for the sham-operated controls were 15 and 15. The rat grafts had a mean-survival time of 14 days in thymectomized recipients and 10 days in the sham-operated controls. It is evident that an impairment of the response to allogeneic grafts but not to xenogeneic grafts occurs (see *4*). These mice were then again grafted with BALB/c, SWR, rat, and also C3H/HeJ skin 56 days after the primary grafting (86 days after irradiation). The mean survival times for the second-set BALB/c, SWR, and rat grafts in the thymectomized mice were 11, 12, and 5 days, respectively.

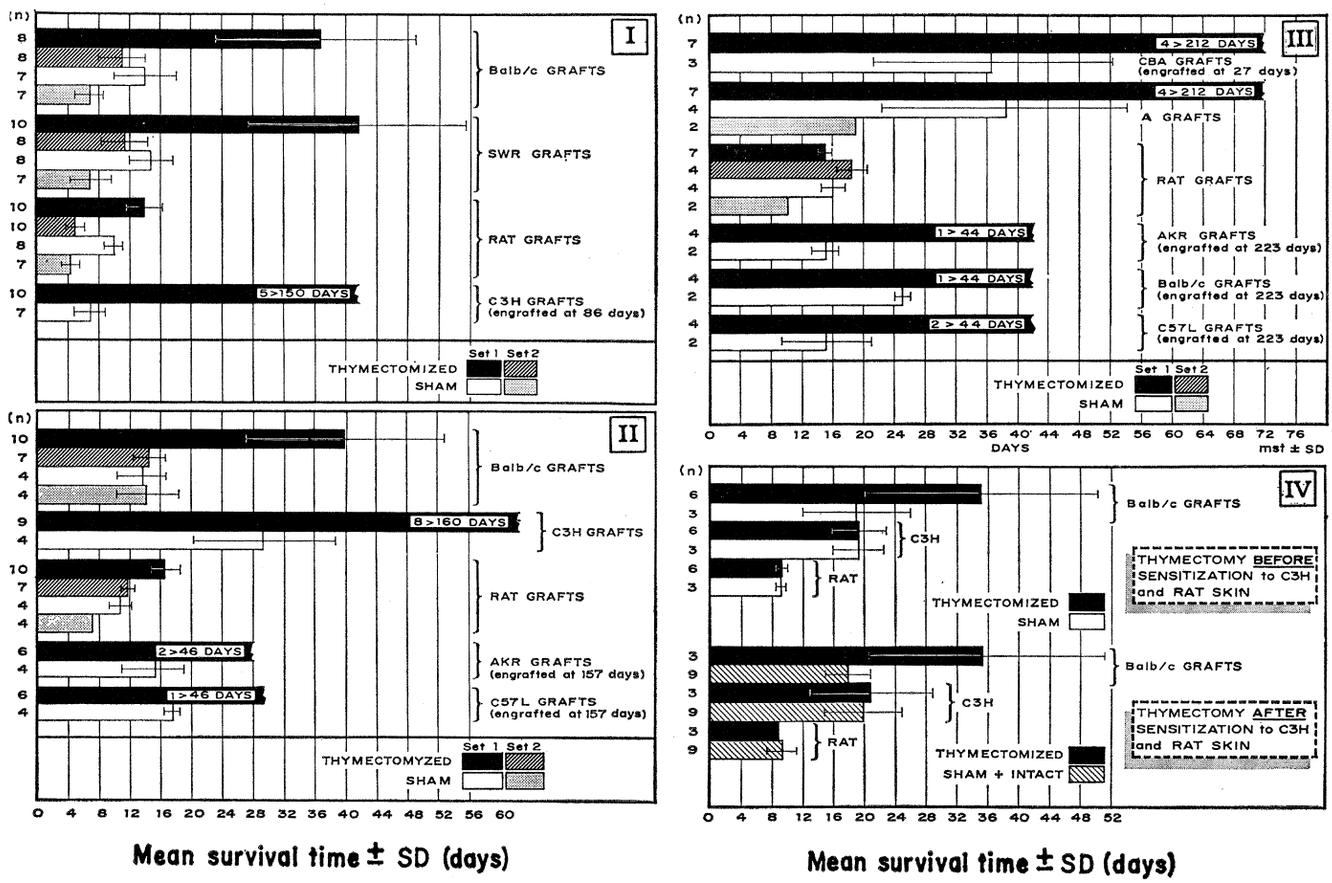

Fig. 1. Survival of first- and second-set homografts in thymectomized-irradiated adult mice. (Group I) LAF_1 mice, grafted at 30 days and 86 days after irradiation. (Group II) CBA/J mice, grafted at 27 days and 157 days after irradiation. (Group III) C3H/HeJ mice, grafted 27 days and 223 days after irradiation. (Group IV) LAF_1 mice, sensitized before irradiation and challenged at 24 days after irradiation (n = number of animals with skin grafts).

In the sham-operated controls the corresponding values were 7, 7, and 4 days. Thus, while the response to the second-set grafts was accelerated, there was an apparent slight delay in this response relative to the controls. The primary C3H skin grafts are still retained at the time of writing (more than 150 days) by five out of ten of the thymectomized recipients; one died (46 days) with an intact graft while four rejected their grafts. In contrast, all the sham-operated controls vigorously rejected their C3H grafts in 7 days. It follows, therefore, that the thymectomized mice were still immunologically impaired 86 days after irradiation.

Similar results were obtained with CBA mice (group II, Fig. 1). It is noteworthy that eight out of nine of the C3H grafts, which share the $H2^k$ histocompatibility locus with the CBA host, were retained indefinitely. Thus, the response to homografts sharing the H2 locus appears to be more markedly suppressed than that towards grafts not sharing the H2 locus, in this case, BALB/c ($H2^d$). Likewise, CBA grafts were accepted by all seven C3H recipients (group III); three of the mice died with intact CBA grafts (at 171 to 203 days) and four have maintained them for over 212 days. The permanency of the impairment is illustrated best by group III (Fig. 1) in which A-strain grafts, in addition to the CBA grafts, have been maintained by several recipients indefinitely; these mice also showed an impaired response to additional primary homografts implanted as late as 223 days after irradiation.

The effect of thymectomy before or after sensitization was then studied. Adult LAF_1 mice were thymectomized and then sensitized by means of two successive grafts of both C3H and rat skin. When these sensitized mice were subjected to x-irradiation (880 rad), injected with syngeneic bone marrow, and challenged with C3H and rat skin grafts 24 days later, there was no difference in the mean survival times between them and the survival times of the sham-operated controls (Fig. 1, group IV). Similarly, when mice were sensitized by two successive grafts of C3H and rat skin and were subsequently thymectomized and irradiated, the mean survival times for the test C3H and rat grafts (engrafted 24 days after irradiation and marrow infusion) were similar to those of the sham-operated controls. Therefore, it appears that thymectomy before or after sensitization of the adult mouse does not alter its responsiveness to skin grafts after irradiation. On the other hand, first-set BALB/c grafts, engrafted on these specifically sensitized thymectomized mice 24 days after irradiation showed a mean survival time of 35, while the value for the controls was 18, indicating that the primary response of these thymectomized mice was impaired.

From our results we can deduce the following. (i) In thymectomized-irradiated adult mice an accelerated response toward second-set allogeneic or xenogeneic skin grafts can occur. (ii) The effect of thymectomy under these conditions is related to the degree of antigenic disparity between the respective skin donors and hosts; that is, the greatest degree of impairment of the homograft response occurs when donor and host are most closely related: non-H2 difference > H2 difference > interspecific difference. A similar differential effect between homografts sharing H2 antigens on the one hand, and differing at the H2 locus, on the other, has been noted by Martinez *et al.* (*1*) in thymectomized neonates. (iii) The unresponsiveness of these mice is long-lasting (probably permanent) and apparently the immunologic functions of the thymus, whatever their nature, cannot be adequately assumed by peripheral cells or tissues in the irradiated mouse. (iv) Thymectomy does not appear to interfere with the induction or maintenance of a state of sensitivity induced in unirradiated adult mice; nor does it interfere markedly with the state of sensitivity in the irradiated mouse once that state has been established.

It is of interest that the thymectomized-irradiated mice in the present study, observed for as long as 267 days after irradiation and bone marrow treatment, have not shown obvious signs of a wasting syndrome. This is in contrast with observations in thymectomized neonates (*2*).

Tyan *et al.* (*4*) showed earlier that thymectomy does not depress xenogeneic graft rejection in irradiated adult mice. We used the same test system and our results are in agreement. However, recent preliminary data indicate that even xenogeneic skin graft rejection can be attenuated in thymectomized-irradiated recipients, when lymph node cells derived from thymectomized-irradiated marrow-restored donors (in contrast with sham-thymectomized controls) were transferred adoptively (*6*). The nonspecific immunologic depression produced by thymectomy in adult x-irradiated mice should not be considered analogous to the state of specific immunological tolerance induced in intact irradiated adult mice or neonatal mice by the injection of allogeneic bone marrow or spleen cells (*7*, *8*).

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References and Notes

1. C. Martinez, J. Kersey, B. W. Papermaster, R. A. Good, *Proc. Soc. Exptl. Biol. Med.* **109**, 193 (1962).
2. J. F. A. P. Miller, *Lancet* **1961-II**, 748 (1961).
3. ———, S. M. A. Doak, A. M. Gross, *Proc. Soc. Exptl. Biol. Med.* **112**, 785 (1963).
4. M. L. Tyan, L. J. Cole, W. E. Davis, Jr., *Science* **142**, 584 (1963).
5. D. W. Bailey and B. Usama, *Transpl. Bull.* **7**, 424 (1960).
6. W. E. Davis, Jr. and L. J. Cole, presented at Bone Marrow Transplantation and Chemical Protection Discussion, Chicago, 13 April 1964; *Blood* (Abstr.), in press.
7. L. J. Cole and W. E. Davis, Jr., *Proc. Natl. Acad. Sci. U. S.* **47**, 594 (1961); C. Martinez, J. M. Smith, R. A. Good, *Transpl. Bull.* **30**, 123 (1962).
8. It is recognized that thymic remnants remaining after surgery could significantly influence these data and their interpretation. Therefore an evaluation of the completeness of thymectomy is being done on each mouse as it dies.
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Inhibition of 19S Antibody Synthesis by 7S Antibody

Abstract. *A brief course of treatment with 6-mercaptopurine prevented conversion of 19S to 7S antibodies during the immune response of rabbits to bovine gamma globulin. Specific reactive homologous 7S antibodies given intravenously to animals with such incompletely developed humoral reactions led to an abrupt fall in 19S antibodies. After a lag period the synthesis of 7S antibodies was apparently normal. These results suggest that the concentration of 7S antibodies controls the synthesis of 19S antibodies.*

The administration of the antimetabolite 6-mercaptopurine (6-MP) can lead to the complete suppression of several types of immune responses in experimental animals and man (*1*). When given in sub-suppressive doses (Fig. 1), this compound blocked the normal maturation of the humoral antibody response (*2*). Six normal rabbits were injected in the hind footpads with alum-precipitated bovine γ-glob-