Survival of Skin Homografts in Adult Mice After Combined Thymectomy-Splenectomy and Steroid Therapy.* (29864)

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The postulated role of the thymus in the development of immune functions(1,2) may not be strictly limited to the neonatal period since skin homotransplantation across weak histocompatibility barriers was observed in mice thymectomized at 30 days of age(2). Furthermore, mice thymectomized at 2 months of age and given lethal doses of irradiation and syngeneic (isologous) bone marrow therapy failed to reject skin homografts and to produce a primary or secondary response to sheep erythrocytes(3). Splenectomy(4) and regional lymphadenectomy(5) appear to have little or no effect on the behavior of skin homografts. The effect of corticosteroids and other steroid hormones on various immune functions, including transplantation immunity, has been extensively studied(6,7,8).

In 3-month-old C_3H mice combined thymectomy and splenectomy resulted in an appreciable impairment of the expected rise of serum γ-globulin concentration following intraperitoneal administration of horse serum (9). This led us to investigate the effect of combined thymectomy and splenectomy on the behavior of skin homografts. Hydrocortisone or testosterone were also used in this work to supplement the effect of the combined operation and because of the reported thymus-suppressive action of these two components and of the adrenal cortex and testis(10,11).

Materials and methods. Animals were 3-month-old C_3H/HeJ mice of both sexes.† Half of the animals were sham-operated; the remainder underwent combined thymectomy and splenectomy. Both sham-operated and thymectomized-splenectomized animals were sub-divided into untreated, hydrocortisone and testosterone-treated groups. Animals in each group were either subjected to skin grafting or were injected intraperitoneally with standard antigens.

Thymectory and splenectomy were performed on the same mice under intraperitoneal Nembutal anesthesia and in immediate succession. The overall operative mortality rate was about 15%. Sham-operations consisted in exploring the thymus and spleen regions through upper sternal and left flank incisions.

Steroid therapy. Hydrocortisone, 1 mg or testosterone, 2 mg, suspended in Tween 80, were administered subcutaneously daily from the 3rd post-operative day for 10 days after which the mice were either grafted or immunized. The injections were continued at intervals of 2 days and until the grafts were rejected or immune blood samples were withdrawn. After rejection of their first graft, the animals were left untreated for 3 months and then subjected to another course of hydrocortisone or testosterone followed by a second grafting procedure. Mice left untreated with steroids received 0.1 ml injections of the Tween 80 vehicle. All animals were given oxytetracycline-HCl (Tetracycline) in drinking water.

Skin grafting. Full-thickness "pinch" skin grafts were performed according to the method of Billingham. The graft was first examined at 7 days and its survival estimated from gross appearance. Two separate procedures were carried out: (1) 1st grafting following initial course of steroids—donors were CE/J *or* SWR/J mice; (2) 2nd grafting of same recipients after 2nd course of steroids with *both* CE/J and SWR/J homotransplants. Thus, during the 2nd grafting procedure animals were subjected simultaneously to a new graft as well as a graft against which they had been previously sensitized.

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† These and donor animals were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

TABLE I.

Treatment C_3H/HeJ mice	1st grafting* No. survivors	1st grafting* Donor strain	1st grafting* Skin homograft survival avg days (range)	2nd grafting† No. survivors	2nd grafting† Donor strain	2nd grafting† Skin homograft survival avg days (range)	2nd grafting† Donor strain	2nd grafting† Skin homograft survival avg days (range)
1. Control	5	CE/J	11(9-12)	5	CE/J	7(7- 8)	SWR/J	9(8-10)
(sham operated)	4	SWR/J	10(8-11)	3	*Idem*	died	*Idem*	died
2. Hydrocortisone	6	CE/J	14(13-15)	4	"	10(9-10)	"	12(10-12)
	5	SWR/J	12(10-13)	5	"	9(8-10)	"	11(11-13)
3. Testosterone	5	CE/J	11(10-13)	2	"	7(7& 7)	"	8(7& 8)
	4	SWR/J	11(10-12)	3	"	9(9-10)	"	6(6- 7)
4. Thymectomy—	7	CE/J	12(11-12)	5	"	10(9-12)	"	12(11-14)
Splenectomy	6	SWR/J	11(9-12)	4	"	13(13-15)	"	10(9-11)
5. Thymectomy—	9	CE/J	11(11-12)	4	"	18(16-18)	"	18(17-18)
Splenectomy with hydrocortisone	8	SWR/J	12(10-13)	5	"	20(19-22)	"	16(15-17)
6. Thymectomy—	7	CE/J	11(11-12)	6	"	10(8-12)	"	10(9-12)
Splenectomy with testosterone	6	SWR/J	10(9-11)	3	"	died	"	died

* Animals received only one type of skin homograft (CE/J or SWR/J).
† Each animal received both types of skin homografts (CE/J and SWR/J).

C_3H and SWR mice have different H_2 (strong) histocompatibility genetic loci whereas C_3H and CE strains share the same H_2 locus but differ at other loci. It has therefore been possible to study homograft survival across both strong (H_2) and weak histocompatibility barriers(12).

Blood studies. Absolute and differential white cell counts were performed on tail blood on mice immediately after the complete rejection of their first skin transplants.

Immunization. Animals which were not submitted to grafting received 3 successive 0.5 cc intraperitoneal injections of a 10% saline mixture of horse serum, bovine serum, albumin, human immune globulin and human serum albumin given at 3-day intervals. Animals were sacrificed 3 weeks from initial injection of antigens. Blood samples were obtained before immunization and at autopsy. Pooled sera were used for total protein determination, paper electrophoretic and immunoelectrophoretic analysis, and to test for precipitin antibody reaction on Ouchterlony agar plates. Precipitin titers were obtained only against serial dilutions of human serum albumin.

Results. The majority of C_3H mice appeared healthy and gained weight at the conclusion of experiment. Thymectomized-splenectomized male mice which were treated with testosterone became slightly wasted and lost their vigor. Female mice withstood testosterone therapy without any apparent effect on weight. Hydrocortisone was well tolerated by both sexes.

Homograft survival. The survival time of skin transplants from SWR and CE donors to C_3H/He recipients is recorded in Table I. After the first grafting, average homograft survival time in all groups ranged from 10 to 14 days. There were no significant differences in graft survival among the 6 treated groups. As expected, CE transplants survived slightly longer than groups from SWR donors. After the second grafting, average graft survival time was approximately the same as or shorter than after the first grafting with the notable exception of grafts on thymectomized-splenectomized mice treated with hydrocortisone. In this group, graft survival time was significantly prolonged (16 to 20 days) even in animals that had been previously sensitized to CE or SWR skin.

Lymphocyte count. As shown in Table II, on sham-operated mice, hydrocortisone therapy was associated with a slight drop in number of circulating lymphocytes whereas testosterone-treated mice showed a slightly elevated lymphocyte count. A significant in-

TABLE II.

Treatment	No. mice*	Lymphocytes/ cu mm ± s.e.	Serum γ-globulin g% † Pre-immunization	Post-immunization	
1. Control (sham operated)	8	7,862 ± 711	.58	.93	1.
2. Hydrocortisone	10	6,815 ± 736	.49	.59	2.
3. Testosterone	9	10,905 ± 1,708	.37	.64	3.
4. Thymectomy—splenectomy	11	12,650 ± 1,279	.54	.68	4.
5. Thymectomy—splenectomy with hydrocortisone	13	12,038 ± 3,552	.55	.37	5.
6. Thymectomy—splenectomy with testosterone	8	12,456 ± 666	.28	.38	6.

* No. of mice on which lymphocyte count was done after complete rejection of 1st skin homograft.

† Values obtained on pooled sera of non-grafted mice immunized with antigen mixture.

crease in number of circulating lymphocytes above that of sham-operated animals was observed in all thymectomized-splenectomized mice.

Serum electrophoretic analyses are also recorded in Table II. Pre-immunization levels of serum γ-globulin ranged between 0.28 to 0.58 g%, however, the lowest levels were in testosterone-treated groups which included a high proportion of weak and slightly wasted male animals. Combined thymectomy-splenectomy or hydrocortisone did not appear to affect significantly pre-immunization γ-globulin values. The anticipated post-immunization rise of serum γ-globulin was considerably impaired in animals which underwent combined thymectomy-splenectomy or which were treated with hydrocortisone. In the group of thymectomized-splenectomized mice treated with hydrocortisone, post-immunization level of serum γ-globulin was 0.37 g% as compared to a pre-immunization level of 0.55 g%. This marked depression of γ-2 component of the immunoglobulins in thymectomized-splenectomized and in hydrocortisone-treated mice was likewise shown on immunoelectrophoresis.

Precipitin antibody reactions. Sera of immunized mice of all groups when reacted against each one of the 4 antigens used in the experiment showed a distinct and strong precipitin line on double-diffusion Ouchterlony agar plates. Immune mice sera reacted against human albumin to dilutions ranging from 1:100 to 1:400, however, the highest titers were observed among sham-operated, thymectomized-splenectomized as well as hydrocortisone-treated mice.

Discussion. In adult subjects, alterations of host factors in an effort to prolong survival or achieve lasting tolerance of homografts have in the past either met with failure or have seriously endangered the host (13). Recent experiments in neonatally thymectomized rodents(1,2) and in thymectomized young adults subject to lethal total body irradiation and protected with isologous bone-marrow(3) have offered a new approach in transplantation immunity. Presently prevailing concepts of the primary role of the thymus in immunity need to perhaps be re-evaluated in view of newer data suggesting that the apparent immunological deficiency of neonatally thymectomized rodents may be at least partly an expression of immunological preoccupation with intercurrent infections (14). Wilson *et al*(15) have also reported that the incidence of the wasting syndrome, one of the hallmarks of the neonatally thymectomized state in certain strains of mice, is markedly decreased in germ-free animals and increases after bacterial contamination. Past the neonatal period, thymectomy alone fails to achieve any striking delay in homograft rejection with the possible exception of Miller's experiments in which lethal total body irradiation had to be resorted to(3). Children who were subjected to full-thickness skin homografts on the day of thymectomy done during the course of corrective heart surgery failed to show any appreciable delay in graft rejection(16).

It is clear from our data that combined thymectomy-splenectomy or steroid therapy did not appreciably affect the fate of skin homotransplants. Combined thymectomy-

splenectomy supplemented with hydrocortisone injections resulted in only a modest but significant delay in homograft rejection after the second transplantation. Combined thymectomy-splenectomy, steroid therapy or both were effective in reducing the anticipated rise of γ-globulin, particularly γ-2, in response to injection of antigens. This reduction of γ-globulin was not, however, corroborated with an effective suppression of precipitin antibody formation or homograft rejection.

The paradoxical rise in number of circulating lymphocytes in animals subjected to combined thymectomy-splenectomy needs to be explained. In our experience, thymectomy in young adult C_3H mice leads to a reduction of about ⅓ of the total number of circulating lymphocytes whereas splenectomy is associated with both total leucocytosis and lymphocytosis(17). It is assumed that in the combined thymectomized-splenectomized state, the effects of splenectomy take precedence over those of thymectomy.

Summary. In young adult C_3H mice combined thymectomy-splenectomy with or without hydrocortisone and testosterone therapy failed in general to affect the fate of skin homografts from CE/J and SWR/J donors. A significant but modest delay in "second-set" graft rejection was achieved by combined thymectomy-splenectomy supplemented with hydrocortisone injections. Likewise, following the intraperitoneal injections of several antigens, animals which had undergone combined thymectomy and splenectomy and/or had been treated with hydrocortisone or testosterone showed a significant impairment in the anticipated rise of serum γ2-globulin without, however, showing any appreciable suppression of precipitin antibody response. These findings underline the difficulty of effectively suppressing homograft rejection and humoral antibody formation in adult mice in spite of ablation of 2 major lymphoid organs and intensive steroid therapy.

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1. Miller, J. F. A. P., Proc. Royal Soc., (Biology), 1962, v156, 415.

2. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., Papermaster, B. W., J. Exp. Med., 1962, v116, 773.

3. Miller, J. F. A. P., Doak, S. M. A., Cross, A. M., Proc. Soc. Exp. Biol. and Med., 1963, v112, 785.

4. Krohn, P., Transpl. Bull., 1953, v1, 21.

5. Billingham, R. E., Brent, L., Medawar, P. P., Proc. Royal Soc. (Biology), 1954, v143, 58.

6. Billingham, R. E., Krohn, P. L., Medawar, P. B., Brit. Med. J., 1951, v1, 1157.

7. Stoerk, H. C., The Effect of ACTH and Cortisone upon Infection and Resistance, 1953, Ch.7.

8. Krohn, P. L., J. Endocrinol., 1954, v11, 78.

9. Azar, H. A., Arch. Path., 1963, v76, 653.

10. Dorfman, R. I., Dorfman, A. S., Endocrinology, 1961, v69, 283.

11. Scherzer, A. L., Azar, H. A., Naujoks, G., Williams, J., Arch. Path., 1963, v76, 647.

12. Counce, S., Smith, P., Barth, R., Snell, G. D., Ann. Surg., 1956, v144, 198.

13. Woodruff, M. F. A., The Transplantation of Tissues and Organs, C. C Thomas, Springfield, Ill., 1960.

14. Azar, H. A., Proc. Soc. Exp. Biol. and Med., 1964, v116, 817.

15. Wilson, R., Sjodin, K., Bealmear, M., ibid., 1964, v117, 237.

16. Zollinger, R. M., Jr., Lindem, M. C., Jr., Filler, R. M., Corson, J. M., Wilson, R. E., New Eng. J. Med., 1964, v270, 707.

17. Azar, H. A., Naujoks, G., Williams, J., Am. J. Path., 1963, v43, 213.

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