Restoration of Antibody Response to Sheep Erythrocytes in Thymectomized Mice Following Grafting of Rabbit Appendix[1] (35517)

DIETER H. SUSSDORF
(Introduced by A. A. Hirata)
Department of Microbiology, Cornell University Medical College, New York, New York 10021

The discovery of the dissociation of immunologic responsiveness, exemplified in birds by the influence of the thymus on cellular immunity and the bursa of Fabricius on humoral immunity (1, 2), has led to a search for the functional equivalent in mammals of the avian bursa. As a result, lymphoepithelial structures of the intestinal tract, such as the appendix, sacculus rotundus, and Peyer's patches, have been implicated as tissues influencing humoral immunocompetence. This designation has been based in part on morphological and developmental similarities between the appendix of the rabbit and the avian bursa (3), and partly on the effects of extirpation of gut-associated lymphoid tissues on immunocompetence. While appendectomy in the adult rabbit does not affect antibody formation (4, 5), removal of this organ together with Peyer's patches and the sacculus rotundus, especially at a very early age or in conjunction with total-body X-irradiation, is followed by depressed antibody formation against a variety of antigens (6–8). The IgM antibody response appears to be particularly sensitive to these procedures, while cellular immunity remains unaffected (6, 7, 9, 10).

It is well established that in the mouse humoral responsiveness to certain antigens, particularly sheep erythrocytes (SRBC), as well as cellular immunity, are influenced by the thymus (11). Law (12) reported that the response to SRBC could be restored in thymectomized mice by xenogeneic (rat or hamster) thymus grafts. This observation prompted the present study on the effects of implanted rabbit appendix, as well as thymus, on the-antibody response in thymectomized mice. CBA mice, after thymectomy at birth, received several weeks later, appendix or thymus tissue from neonatal New Zealand white rabbits. The hemolysin responses to SRBC were followed in the recipients for 10 days and compared with responses in intact and thymectomized mice.

Materials and Methods. Surgery and grafting. CBA/J mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, and were bred in our animal facility. Within 18 hr after birth, their litters were thymectomized according to the method of Sjodin and associates (13). Control surgery consisted of the same procedure except that the thymus was left intact.

At an average age of 3 weeks, the thymectomized mice received thymus or appendix grafts from neonatal New Zealand white rabbits. These donors were obtained from does which were bred at Camm Research Institute, Wayne, New Jersey, and which littered in our animal facility. Healthy appearing members of each litter were chosen as donors within 24 hr after birth. Under ether anesthesia, the thymus and appendix were removed and rinsed in cold saline. Each organ was finely minced in 0.2 ml of saline, and the tissue suspension was injected immediately into the recipient mice. Each mouse received, either subcutaneously into the nuchal region or intraperitoneally, the tissue suspension prepared from one whole thymus or appendix (av wt, 87 and 31 mg, respectively). An effort was made to have approximately equal numbers of thymus-grafted, appendix-grafted, and control mice in each litter.

[1] This work was supported by grants GB-7351 and GB-23957 from the National Science Foundation.

TABLE I. Peak Serum Titers of Antisheep Erythrocyte Hemolysin in Thymectomized Mice Bearing Implants of Thymus or Appendix from Neonatal Rabbits.

Group	Treatment	Mean peak titer[a]		No. of mice
		Log_{10}	Arithmetic	
I	Control (sham surgery)	2.862 ± 0.363[b]	1000	10
II	Thymectomy	1.918 ± 0.112	69	6
III	+ thymus implant	1.745 ± 0.193	96	4
IV	+ appendix implant	2.498 ± 0.117	379	5

[a] 50% hemolysin units/ml of serum.

[b] These means ± standard errors were used for statistical comparisons shown in Fig. 1.

Antigen injection and serum titration. When the recipients were 4 to 6 weeks of age, they were injected with a 20% saline suspension of washed SRBC, standardized as described in Campbell *et al.* (14). Each mouse received intraperitoneally 0.2 ml of the suspension, equivalent to a dose of 6.4×10^8 cells. (During subsequent statistical analyses of serum titers, the 4- and 6-week-old animals were pooled, since their numbers were too small for separate statistical evaluation).

Blood samples were obtained by orbital puncture just prior to antigen injection; and afterwards, every second day for 10 days. Individual serums were titrated for antisheep isophile hemolysins according to the method of Taliaferro and Taliaferro (15), modified by Chapman and Sussdorf (16). In this assay, a hemolysin unit is defined as the reciprocal of that milliliter fraction of serum which lyses 50% of 1.7×10^7 SRBC in the presence of an excess of guinea pig complement (four 50% units), at 37°, in 30 min, in a reaction volume of 2.5 ml.

Serum titers were plotted semilogarithmically against time for individual mice and peak titers were obtained from visually fitted response curves. Mean arithmetic and log peak titers were calculated for each of the experimental groups of animals listed in Table I. Mean log peak titers were compared by Student's t test. The curves in Fig. 1 were obtained by plotting means of individual titers in each experimental group.

Histological evaluations. All mice were killed and autopsied at the end of the experimental period. Spleens and mesenteric lymph nodes were prepared for histological examination by hematoxylin–eosin staining. In thymectomized mice, the retrosternal area was examined grossly and microscopically by serial sectioning. Animals bearing detectable thymus remnants were excluded from statistical evaluations.

Results and Discussion. As shown in Table I and Fig. 1, thymectomy clearly had a depressing effect on the hemolysin response. The mean peak titer in the thymectomized animals (group II) was approximately one-fifteenth of that in the surgical controls (group I). This titer reduction of about 1 log unit is within the range reported by others for CBA mice (17). Depression of peak titer remained essentially unchanged when thymectomy was followed by the implantation of rabbit thymus (group III). On the other hand, grafting of the appendix (group IV) resulted in antibody responses which were significantly higher than those of groups II and III ($p = .01$ in both cases). A comparison of mean log peak titers in groups I, II, and IV indicates that implantation of the appendix restored 61% of the titer depression caused by thymectomy. Although the peak titers in groups I and IV did not differ at the 5% level, the difference approached significance ($p = .07$).

Grafting of thymus and especially of appendix tissue into thymectomized mice reduced the percentage of animals showing symptoms of wasting disease. Thus, wasting was observed in 6/9[1] (67%) of the animals following thymectomy alone and in 2/6

[1] Several of the wasting animals died before the end of the experimental period. Since such animals were not used for statistical analyses, the group sizes given in connection with the incidence of wasting disease are larger than those listed in Table I.

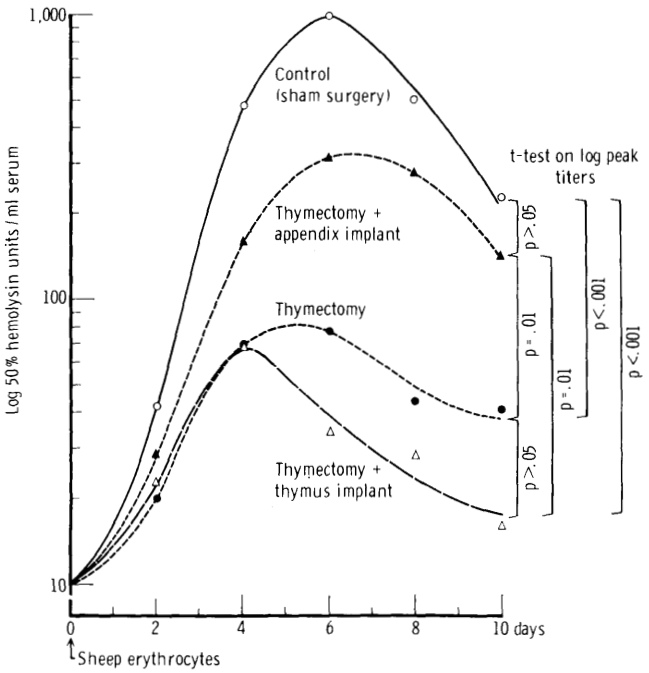

FIG. 1. Hemolysin titers against sheep erythrocytes in thymectomized CBA mice bearing implants of neonatal rabbit thymus or appendix, and in sham-operated and thymectomized controls. Each curve represents 4–10 animals. Brackets on the right indicate groups which were compared statistically, with probabilities obtained by *t* tests on log peak titers.

(33%) of the thymectomized, thymus-grafted mice. None of the 5 animals in the thymectomized, appendix-grafted group appeared to suffer from wasting symptoms.

A relationship was also observed between grafting, serum antibody response, and cellular composition of the splenic white pulp in the thymectomized mice. Figure 2 illustrates differences in splenic cellularity of the periarteriolar zone in the white pulp of the spleen 2 weeks after the injection of SRBC into a thymectomized mouse grafted with thymus (A), and into a thymectomized mouse grafted with appendix (B).

The animal represented in Fig. 2A had a greatly depressed peak titer of 15 hemolysin units/ml. The region immediately surrounding the central arteriole is depleted of small lymphocytes to the extent that reticular cells and scattered macrophages have become the prominent cell types. This characteristic depletion of the splenic thymus-dependent areas by thymectomy (18, 19) apparently was not counteracted by implantation of rabbit thymus tissue.

In contrast, as shown in Fig. 2B, periarterial cellularity approaching normal density was observed after thymectomy and appendix grafting. (The animal represented in Fig. 2B had a peak serum titer of 350 hemolysin units/ml). The area adjacent to the central arteriole is populated with small and medium-sized lymphocytes, and blast cells are scattered throughout the remainder of the follicle. A small, active germinal center is located to the left of the arteriole, and clusters of immature plasma cells can be seen in the transitional zone to the right. Recently Harrison (20) described a similar repopulation of the splenic thymus-dependent area in thymectomized mice which had received intraperitoneal implants of palatine tonsils from perinatal rabbits.

The data and observations cited above lead to the conclusion that the implanted appendiceal tissue influenced, across a xenogeneic barrier, the cellular composition of the host peripheral lymphoid organs and their responsiveness to SRBC antigen. Restoration probably was not due to nonspecific stimulation by

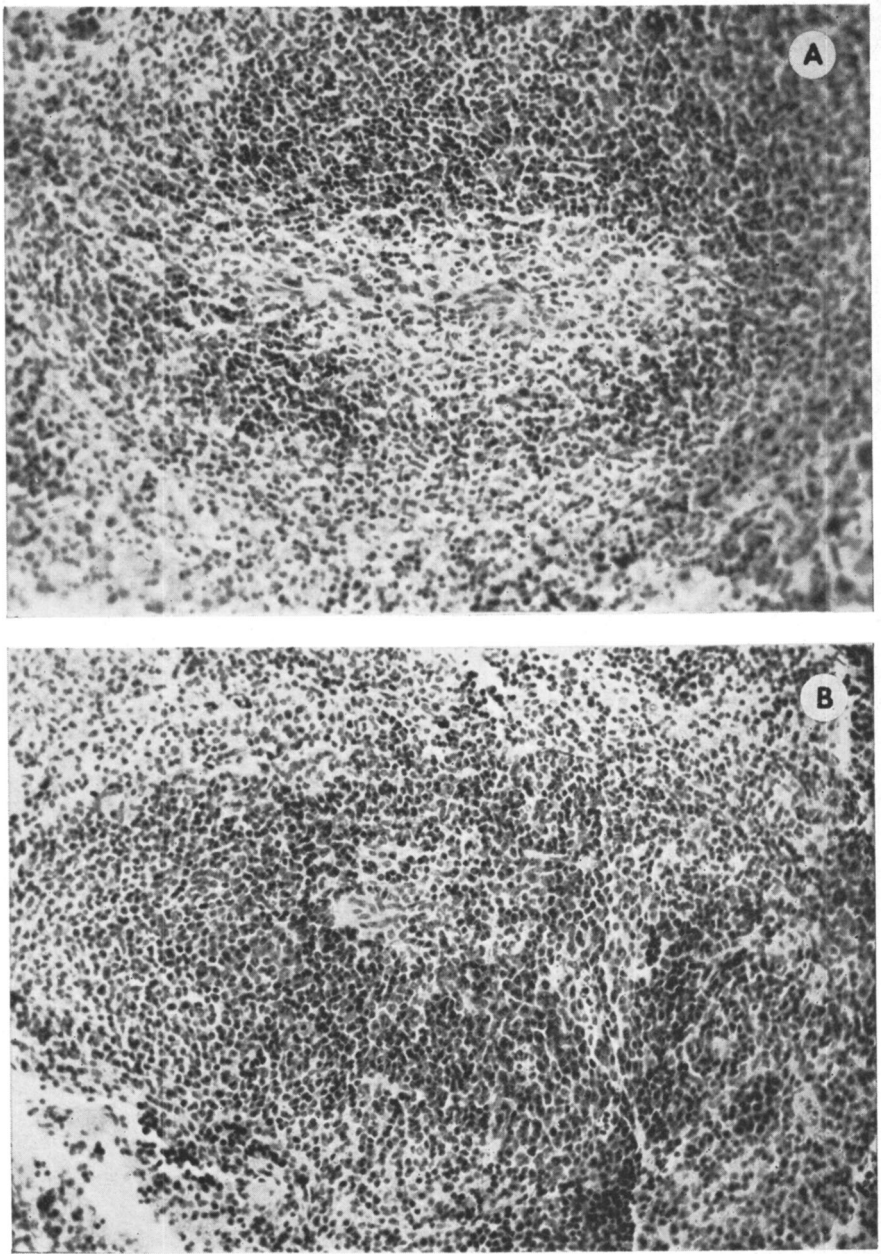

FIG. 2. Periarteriolar region of splenic white pulp in neonatally thymectomized mice following implantation of rabbit thymus (A); or appendix (B); and injection of sheep erythrocytes. See text for details and discussion; 187×.

a foreign, eventually necrotizing tissue, because transplantation of the rabbit thymus, done under identical conditions and involving a tissue mass larger than the appendix, failed to stimulate the antibody response.

Although the rabbit thymus is well populated with lymphocytes at birth, the neonatal appendix is devoid of lymphoid elements and contains epithelial–reticular elements only (3, 21). Therefore, the transplanted appendix may have produced its restorative effect via a humoral factor elaborated by its epithelial cell constitutents, rather than by populating the recipient animal with compe-

tent cells. None of the subcutaneously implanted tissue was microscopically detectable at the end of the experimental period. Other investigators (*12, 22*) have reported immunologic restoration despite eventual resorption of transplants after grafting of xenogeneic (rat or hamster) thymus into neonatally thymectomized mice and of allogeneic thymus into mice thymectomized as adults.

Even though the thymus appears to play a role in the antibody-forming capacity of the rabbit (*23–25*), and evidence for the presence of a thymic humoral factor in rabbits has been reported, implantation of this organ into thymectomized mice did not restore antibody formation. No satisfactory explanation can be offered for this failure. The advanced state of development of the rabbit thymus at birth (*3*), and presumably very early onset of thymic immunologic function, may indicate that humoral activity in this organ peaks during the prenatal period and was too low to be restorative at the time the thymus transfer was done.

Summary. Since the isophile hemolysin response to sheep red blood cells (SRBC) is depressed in thymectomized mice, the effect on the response was studied by implanting the appendix of neonatal, New Zealand white rabbits into young, neonatally thymectomized CBA mice prior to the injection of SRBC. The neonatal appendix, containing only epithelial–reticular elements and no lymphoid cells, restored 61% of the reduction of the peak hemolysin titer caused by thymectomy, whereas the rabbit thymus was ineffective under similar conditions. The appendix, but not the thymus, also led to a repopulation of the periarteriolar region of the white pulp of the spleen of the experimental mice. These data indicate that the neonatal rabbit appendix is capable of partially restoring hemolysin-forming capacity across xenogeneic barriers, possibly by the elaboration of a humoral factor, and support the suggestion that the appendix may represent, in part, the mammalian immunologic equivalent of the avian bursa of Fabricius.

My thanks to Mrs. B. S. Joh for her excellent technical assistance.

1. Szenberg, A., and Warner, N. L., Nature (London) **194**, 146 (1962).

2. Warner, N. L., Szenberg, A., and Burnet, F. M., Aust. J. Exp. Biol. Med. Sci. **40**, 373 (1962).

3. Archer, O. K., Sutherland, D. E. R., and Good, R. A., Lab. Invest. **13**, 259 (1964).

4. Sussdorf, D. H., and Draper, L. R., J. Infect. Dis. **99**, 129 (1956).

5. Draper, L. R., and Sussdorf, D. H., J. Immunol. **95**, 306 (1965).

6. Cooper, M. D., Perey, D. Y., McKneally, M. F., Gabrielsen, A. E., Sutherland, D. E. R., and Good, R. A., Lancet **1966**, 1389 (1966).

7. Cooper, M. D., Perey, D. Y., Gabrielsen, A. E., Sutherland, D. E. R., McKneally, M. F., and Good, R. A., Int. Arch. Allergy Appl. Immunol. **33**, 65 (1968).

8. Perey, D. Y., Cooper, M. D., and Good, R. A., Science **161**, 266 (1968).

9. Konda, S., and Harris, T. N., J. Immunol. **97**, 805 (1966).

10. Konda, S., and Takiguchi, T., Acta Haematol. Japon. **30**, 85 (1967).

11. Miller, J. F. A. P., and Osoba, D., Physiol. Rev. **47**, 437 (1967).

12. Law, L. W., Nature (London) **210**, 1118 (1966).

13. Sjodin, K., Dalmasso, A. P., Smith, J. M., and Martinez, C., Transplantation **1**, 521 (1963).

14. Campbell, D. H., Garvey, J. S., Cremer, N. E., and Sussdorf, D. H., "Methods in Immunology," 2nd ed., p. 121. Benjamin, New York (1970).

15. Taliaferro, W. H., and Taliaferro, L. G., J. Infect. Dis. **87**, 37 (1950).

16. Chapman, S. E., and Sussdorf, D. H., J. Immunol. **98**, 860 (1967).

17. Miller, J. F. A. P., Mitchell, G. F., and Weiss, N. S., Nature (London) **214**, 992 (1967).

18. Metcalf, D., Brit. J. Haematol. **6**, 324 (1960).

19. Parrott, D. M. V., De Sousa, M. A. B., and East, J., J. Exp. Med. **123**, 191 (1966).

20. Harrison, B. H., J. Immunol. **105**, 38 (1970).

21. Thorbecke, G. J., J. Exp. Med. **112**, 279 (1960).

22. Leuchars, E., Cross, A. M., and Dukor, P., Transplantation **3**, 28 (1965).

23. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., and Papermaster, B. W., J. Exp. Med. **116**, 773 (1962).

24. Archer, O. K., Pierce, J. C., Papermaster, B. W., and Good, R. A., Nature (London) **195**, 191 (1962).

25. Trench, C. A. H., Watson, S. W., Walker, F. C., Gardner, P. S., and Green, C. A., Immunology **10**, 187 (1966).

Received Dec. 14, 1970. P.S.E.B.M., 1971, Vol. 137.