

The induction of immunological tolerance by antigen feeding. The effect of thymectomy on orally induced tolerance

G. Filipp, M.D.,* and B. v. Boros, M.D.,**
Saarbrücken, West Germany

The already published work concerning immunological tolerance induced by antigen feeding in the first days of life was extended. Thymectomy or thymectomy plus appendectomy performed in the neonatal period of life deepened the state of immunological tolerance. Antibody synthesis was not influenced in control experiments by thymectomy performed in adult life. The possible practical consequences of these experimental results were discussed.

We have already reported our experiments describing immunological tolerance achieved through oral introduction of antigens in the neonatal period of life.¹⁻⁵ This phenomenon is probably based on the physiologically increased permeability of the mucous membrane of the intestinal tract. It is considerably dependent on the antigen dose given and the time of the neonatal antigen feeding.

In our next experiments we tried to perpetuate and to deepen the orally provoked immunological tolerance by thymectomy or by thymectomy plus appendectomy in the neonatal life period. Claman and Talmage⁶ showed that the loss of acquired immunological tolerance of mice to bovine gamma globulin is dependent on the presence of the thymus. In these experiments mice were repeatedly injected with bovine gamma globulin from birth until the age of 5 to 10 weeks and were then thymectomized. After 130 to 160 days without antigen,

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*Medical faculty of the University of Saarland, West Germany.

**Institute of Public Health of Saarland, West Germany.

an accelerated immune disappearance of I¹²⁵ bovine gamma globulin could be uniformly induced in controls, while thymectomized mice remained tolerant. The role of the thymus and of so-called central lymphatic organs in immunogenesis is a well-known fact.

MATERIALS AND METHODS

Newborn rabbits were fed orally on the second, fourth, sixth, and eighth days of life with 2 ml. of 20 per cent bovine serum albumin (BSA), containing 1 Gm. egg albumin (EA), through a plastic tube, one end of which was put into the mouth and provoked reflex sucking movements. The animals were removed from their mothers ½ hour before feeding. The thymectomy was performed on the third or fifth day of life under ether anesthesia.* Other animals were thymectomized and appendectomized.

Three and 6 months later the homologous antigens (BSA, EA, and, as control antigen, horse serum [HS]) were injected in complete Freund's adjuvant. (In some cases the neonatal feeding and the later immunization were performed only with BSA.)

In the control experiment we studied the influence of thymectomy performed at 3 months of age on the antibody synthesis of animals fed neonatally with antigens. (Last, we wanted to learn the consequence of postnatal thymectomy on the antibody synthesis of adult rabbits without previous antigen feeding.) Animals were bled 6 to 7 days after the last challenge for detection of antibodies. The antibody determination was carried out in all cases by agar gel precipitation and capillary precipitation and in some cases by the passive hemagglutination method of Boyden.† We give our results obtained by capillary precipitation.

EXPERIMENTS AND RESULTS

Seventy-seven rabbits were divided into five groups as indicated in Table I. Groups I, II, III, and IV received BSA and EA orally at birth. Group V was the control group. Group I was thymectomized at birth and Group IV at 3 months. The animals of Group II were thymectomized and appendectomized at birth. All animals were immunized subcutaneously on three alternate days at 3 months and at 6 months with 1, 3, and 5 ml. of BSA, 0.5, 1, and 2 Gm. EA, and 1, 2, and 3 ml. HS in separate complete Freund's adjuvant preparations. Animals were bled 6 to 7 days after the last challenge for detection of antibodies.

In separate experiments only BSA was injected. Group VI and Group VII (Table II) received BSA orally at birth. Group IX represented the control. Group VI was thymectomized at birth. In Group VIII thymectomy was performed in the first 4 days of life. Three months later the animals were immunized on three alternate days with BSA.

As indicated in Table I, thymectomy or thymectomy and appendectomy

*By Professor D-J. Comsa, Department of Experimental Medicine, University Medical Faculty of Saarland, West Germany.

†Performed in part by Professor Dr. F. Scheiffarth, Department of Clinical Immunology, University Medical Faculty of Erlangen, West Germany.

Table I. *Effect of thymectomy or thymectomy plus appendectomy on the synthesis of antibody by rabbits fed BSA and EA neonatally*

Neonatal treatment	Antigen	Mean log ₂ precipitin titer	
		3 months	6 months
<i>Group I</i> Thymectomy, BSA, and EA	BSA	0.4 (3/20)*	1.3 (6/20)
	EA	0.7 (5/20)	1.8 (6/20)
	HS	5.7 (20/20)	7.5 (20/20)
<i>Group II</i> Thymectomy, appendectomy, BSA, and EA	BSA	0.2 (1/16)	0.8 (3/16)
	EA	0.7 (5/16)	1.5 (5/16)
	HS	5.2 (16/16)	7.3 (16/16)
<i>Group III</i> BSA and EA	BSA	1.3 (10/20)	2.9 (12/21)
	EA	1.9 (10/20)	3.4 (12/21)
	HS	8.0 (20/20)	9.4 (21/21)
<i>Group IV</i> BSA, EA, and thymectomy at 3 months	BSA	1.9 (5/10)	2.7 (5/ 9)
	EA	1.0 (4/10)	2.5 (5/ 9)
	HS	7.8 (10/10)	9.5 (9/ 9)
<i>Group V</i> None	BSA	7.2 (9/ 9)	9.2 (10/10)
	EA	6.8 (9/ 9)	8.2 (10/10)
	HS	7.1 (9/ 9)	9.8 (10/10)

Chi square analysis indicates a significant difference ($p < 0.05$) between Groups I and II combined and Groups III and IV combined with both BSA and EA.

*Number responding and number tested.

Table II. *Effect of thymectomy on the synthesis of antibody by rabbits fed BSA neonatally*

Neonatal treatment	Mean log ₂ precipitin titer (3 months)
<i>Group VI</i> Thymectomy and BSA	1.5 (1/ 4)*
<i>Group VII</i>	
<i>Group VIII</i> BSA	0 (0/ 4)
<i>Group IX</i> Thymectomy at birth	7.1 (7/ 7)
Control	8.1 (10/10)

*Number responding and number tested.

(Groups I and II) performed in the neonatal period of life caused a deepening of the immunological tolerance produced by antigen feeding. There were no differences between the thymectomized and the thymectomized plus appendectomized rabbits concerning the depth and breadth of the tolerance. A slightly reduced production of antibody was found after the thymectomy or thymectomy plus appendectomy with the control antigen (horse serum), which was not given in the neonatal period of life. Thymectomy performed at 3 months did not deepen the orally induced immunological tolerance. Thymectomy in the neonatal period of life without antigen feeding did not suppress greatly the synthesis of antibodies by adult rabbits (Table II).

DISCUSSION

The induction of the state of immunological tolerance by introduction of antigens orally in the first days of life seems to be well established on the basis of our own experiments and those of others. We have been able in this manner to suppress specifically and for a long time the production of antibodies. In our experiments thymectomy performed in the neonatal period of life deepened the orally induced tolerance. This result underlines the central role of the thymus in immunological processes.

The practical purpose of our experiments was to study the possibility of the induction of the tolerant state by oral feeding in human beings. We consider here the oral induction of immunological tolerance in atopic children or the neonatal feeding of atopic children with a combination of various antigens. As a first step some experience with atopic dogs would be important. The prophylactic neonatal induction of immunological tolerance by oral feeding in newborn animals, which have a high probability of becoming allergic after a natural exposure, would be the basis for a prophylactic treatment in babies with a bilateral or unilateral allergic diathesis.

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