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Bacterial Translocation from the Gastrointestinal Tracts of Thymectomized Mice

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Abstract. The incidence of translocation of viable indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node, spleen, liver, and kidney was compared in neonatally thymectomized mice and sham-thymectomized specific pathogen-free mice. The immunologic responses of the thymectomized mice to sheep erythrocytes were decreased compared to the responses of sham-thymectomized mice. Strictly anaerobic bacteria were isolated from only 1.8% of the organs from thymectomized mice and from none of the organs of sham-thymectomized mice. Aerobic or facultatively anaerobic bacteria were cultured from 27.4% of the organs of thymectomized mice. Of the thymectomized mice, 70.7% contained viable aerobic or facultatively anaerobic bacteria in one or more of their organs tested, compared with only 10% of the sham-thymectomized mice. *Escherichia coli* was the predominant bacterial species isolated from these organs, although *Staphylococcus aureus*, *Streptococcus*, and *Corynebacterium* also were present. *Bacteroides* were the only strictly anaerobic bacteria cultured. Neonatal thymectomy promotes the translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node, spleen, liver, and kidney.

Bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal tract to the mesenteric lymph nodes and possibly other organs [7]. Certain viable bacteria translocate from the gastrointestinal tract through the lamina propria to the mesenteric lymph nodes of gnotobiotic mice colonized with the whole cecal flora from specific pathogen-free, (SPF) mice [7]. Gnotobiotic mice monoassociated with a particular bacterial species, such as indigenous *Escherichia coli,* exhibit increased cecal population levels of *E. coli* compared to the *E. coli* levels in conventional mice [5,8]. There also is an increase in the incidence of translocation of *E. coli* from the gastrointestinal tracts of these gnotobiotic mice compared to its translocation in conventional mice [5,8]. The translocation of *E. coli* is inhibited, however, when gnotobiotes monoassociated with *E. coli* are inoculated intragastrically with the whole cecal flora from SPF mice [5,8]. The normal flora bacteria antagonize *E. coli* to a population level at which translocation of *E. coli* no longer occurs. Enteric bacteria, such as *E. coli,* also translocate from the gastrointestinal tract to the mesenteric lymph nodes of SPF mice treated orally with either penicillin, clindamycin, or metronidazole [6]. These antibiotics disrupt the normal flora ecology allowing Gram-negative enteric bacilli to overpopulate the ceca, thereby promoting translocation of these bacteria to the mesenteric lymph nodes. Thus, bacterial antagonism of the gastrointestinal population levels of particular indigenous bacteria by other members of the normal bacterial flora is one mechanism inhibiting translocation and confining these bacteria to the gastrointestinal tract.

The host's immune system also appears to be a defense mechanism preventing the translocation of certain indigenous bacteria from the gastrointestinal tract. It has been reported that viable aerobic and anaerobic bacteria are cultured from 50% of the mesenteric lymph nodes, spleens, livers, and kidneys of congenitally athymic (nu/nu) mice, whereas viable bacteria are cultured from only 5.2% of these organs from heterozygous (nu/+) mice [16]. Grafting thymuses to athymic (nu/nu) mice restores their immunological responses to sheep erythrocytes and decreases the incidence of bacterial translocation from 50% in athymic mice to 7.8% in thymus-grafted (nu/nu) mice [16]. Therefore, T-cell depen-

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dent immune mechanisms restored by thymic grafts appear to inhibit the translocation of certain indigenous bacteria from the gastrointestinal tract.

Congenitally athymic mice possibly possess abnormalities, other than the absence of a thymus, that may promote bacterial translocation. Thymectomy of SPF mice should increase the incidence of bacterial translocation if thymus-dependent immunity is a mechanism confining certain bacteria to the gastrointestinal tract. This paper presents evidence that neonatal thymectomy of SPF mice promotes the translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node, spleen, liver, and kidney.

Materials and Methods

Mice. Specific pathogen-free (SPF) mice of the CD-1 strain were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Massachusetts). These animals were used to establish an SPF mouse-breeding colony maintained by our laboratory. Mice were housed in individual polycarbonate cages with stainless steel wire tops (Maryland Plastics, Inc., New York, New York) fitted with autoclavable, polyester tops (Econo-Filter, Maryland Plastics). Ground corn cobs (San-I-Cel, Paxton Processing Co., Paxton, Illinois) were used for bedding. The animals were fed an autoclaved commercial diet preparation (Laboratory Chow No. 5001, Ralston Purina Co., St. Louis, Missouri) and given acid-water (0.001 N HCl) [17] ad libitum. The SPF mice were maintained under barrier-sustained conditions in a special housing facility with automatically-controlled temperature, humidity, and light conditions.

Thymectomy procedure. Neonatal mice, 12- to 24-h old, were anesthetized by placing them on ice for 2 to 3 min [10]. The mouse was taped to a metal canister lid and placed over ice to maintain the mouse in a hypothermic state throughout the procedure. All further manipulations were carried out while viewing through a dissecting microscope (10×). A 3-mm incision was made through the skin and sternum of the chest wall between the second and third ribs using a number six stainless steel scalpel. The incision was enlarged slightly by blunt dissection so that the thymus was visible adhering to the underside of the sternum. The thymus was removed by vacuum aspiration using a Pasteur pipet attached to a vacuum system. The incision was closed with one 7-0 dexon absorbable suture (Davis and Geck, American Cyanamid Co., Pearl River, New York). The incision then was painted with a thin film of collodion (Matheson Coleman and Bell Co., Cincinnatti, Ohio). Sham-thymectomized control mice received the same operative procedures except for actual removal of the thymus

Evaluation of thymectomy procedure. Reduction in serum antibody response to sheep erythrocytes and gross morphological examination for the thymus at autopsy were used to evaluate the success of the thymectomy. Six weeks after the operations, the thymectomized and sham-thymectomized mice received one intraperitoneal injection of 0.1 ml of a 10% suspension of sheep erythrocytes. The mice were bled from the retro-orbital plexus 7 days later. The sera were collected and heat-inactivated at 56°C for 30 min. Hemagglutination tests were performed in microtiter plates (Dynatech Laboratories, Inc., Alexandria, Virginia) following the method of Sever [18]. All mice received a second intraperitoneal injection of 0.1 ml of 10% sheep erythrocytes 14 days after the first injection. The mice were bled again 7 days after this second vaccination and the sera tested by hemagglutination. The thoracic cavity also was examined for thymic remnants when the 9-week-old mice were sacrificed to test the various organs for translocating bacteria.

Assay for translocation of aerobic, facultatively anaerobic, and obligately anaerobic bacteria. Mice were killed by cervical dislocation and placed in an anaerobic glove box (Coy Manufacturing Co., Ann Arbor, Michigan) [1] maintained at less than 10 parts of oxygen per 10^6 parts of an atmosphere consisting of 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. The oxygen level inside the anaerobic glove box was monitored daily with a Trace Oxygen Analyzer (Lockwood and McLorie, Inc., Horsham, Pennsylvania). The abdomens of the mice were soaked with 70% ethanol. An incision was made through the skin with sterile scissors. A second incision then was made through the peritoneum with another pair of sterile scissors. The abdominal wall was reflected with sterile forceps and the exposed viscera swabbed with two sterile, cotton-tipped applicator sticks. The sticks were placed in tubes of brain heart infusion (Difco Laboratories, Detroit, Michigan) and one tube incubated anaerobically at 37°C for 48 h and the other tube aerobically at 37°C for 24 h to test for any bacterial contamination of the viscera: None was detected in these experiments. The middle mesenteric lymph node draining the jejunum, ileum, and cecum was located in the mesentery of the ascending colon and excised with another set of sterile instruments. The spleen, kidney, and liver also were excised with a sterile set of instruments for each organ.

The mesenteric lymph node was transferred under anaerobic conditions in the glove box to a grinding tube (Tri-R Instruments, Rockville Center, New York) containing 0.3 ml of prereduced, enriched Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Maryland) [1,4]. The mesenteric lymph node was homogenized with a sterile Teflon grinder (Tri-R Instruments) and divided into equal portions. Half of the entire mesenteric lymph node homogenate was spread with glass L-rods onto plates of enriched Trypticase soy agar [1] and incubated at 37°C in the anaerobic glove box for 72 h. The remaining half of the mesenteric lymph node homogenate was removed from the anaerobic glove box and the entire amount cultured on blood agar plates. The blood agar plates were incubated aerobically at 37°C for 24 h. The entire spleen and kidney and a portion of the liver also were homogenized in grinding tubes and each divided into two equal portions. Half of each organ homogenate was cultured anaerobically at 37°C on enriched Trypticase soy agar in the anaerobic glove box for 72 h and the other half was cultured aerobically on blood agar at 37°C for 24 h.

Identification of bacteria. All aerobic and facultatively anaerobic organisms were identified by using information and procedures in *Bergey's Manual of Determinative Microbiology*, eighth ed., and by using the API 20 E System (Analytab Products, Plainview, New York). Obligate anaerobes were identified by gas liquid chromatography of volatile and nonvolatile fatty acids as outlined in the *CDC Laboratory Manual of Laboratory Methods in Anaerobic Bacteriology* using a Capco model 700 gas chromatography (Capco Clinical Analysis Products, Co., Sunnyvale, California), by biochemical tests utilizing the API 20 A system and by their morphology in Gram-stained preparations.

Results

Thymectomized and sham-thymectomized mice were vaccinated intraperitoneally at 6 weeks of age

with sheep erythrocytes (Fig. 1). The sham-thymectomized mice produced a greater hemagglutinin response after the first vaccination than the thymectomized mice ($P < .0001$). A second intraperitoneal vaccination with sheep erythrocytes increased the hemagglutinin titers in the thymectomized mice to $\log_2$ 7.4, but this response was still significantly reduced when compared to the mean $\log_2$ titer of 12.7 in the sham-thymectomized mice after a second vaccination ($P < .0001$). No thymic remnants were seen in the thoracic cavities of the thymectomized mice upon autopsy; thus, the thymectomized mice were immunologically impaired.

The incidence of bacterial translocation from the gastrointestinal tract to the mesenteric lymph node, spleen, liver, and kidney of thymectomized and sham-thymectomized mice was compared to determine whether thymectomy would promote bacterial translocation (Table 1). Only 3 of 164 organs from the thymectomized mice yielded positive cultures of strictly anaerobic bacteria. Strictly anaerobic bacteria were not cultured from any of the organs of sham-thymectomized mice. On the other hand, of the organs from the thymectomized mice, 27.4% contained viable aerobic or facultatively anaerobic bacteria, compared with only 2.5% of the organs from sham-thymectomized mice. Of the thymectomized mice, 70.7% contained viable aerobic or facultatively anaerobic bacteria in one or more of their organs tested, compared with only 10% of the sham-thymectomized mice. *Escherichia coli* was the predominant bacterial species isolated from these organs, although *Staphylococcus aureus, Streptococcus* sp., and *Corynebacterium* sp. also frequently were present (Table 2). Thymectomy did not increase the cecal population levels of the Gram-negative enteric bacilli. *Escherichia coli,* for example, was present at 10^4 to 10^5/g cecum. These results demonstrate that neonatal thymectomy of specific pathogen-free mice promotes the translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node, spleen, liver, and kidney. Consequently, thymus-dependent immunity is a defense mechanism inhibiting bacterial translocation and confining certain indigenous bacteria to the gastrointestinal tract.

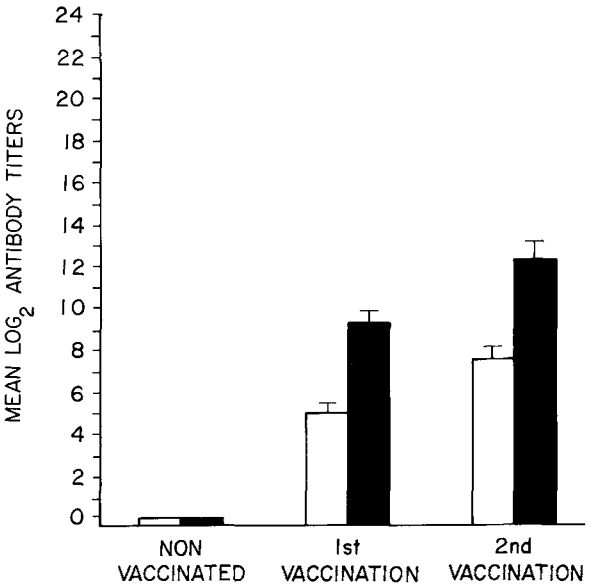

Fig. 1. Comparison of serum hemagglutinin responses of thymectomized and sham-thymectomized mice to sheep erythrocytes. Data are expressed as mean $\log_2$ of serum antibody titers, with brackets denoting standard errors. Open bars represent thymectomized mice and solid bars sham-thymectomized mice.

Discussion

Viable indigenous bacteria were cultured from 50% of the mesenteric lymph nodes, spleens, livers, and kidneys of congenitally athymic (nu/nu) mice, but from only 5.2% of these organs from heterozygous (nu/+) mice [16]. Grafting thymuses to athymic (nu/nu) mice reduced the incidence of bacterial translocation to these organs from 50% to 7.8% [16]. The results presented here demonstrate that neonatal thymectomy of specific pathogen-free (SPF) mice increases the incidence of translocation of aerobic and facultatively anaerobic bacteria from 2.5% in sham-thymectomized mice to 27.4% in thymectomized mice. Thus, T-cell-dependent immunity appears to inhibit the translocation of certain indigenous bacteria from the gastrointestinal tract.

The serum-hemagglutinin responses to sheep erythrocytes were reduced in thymectomized mice compared with the response of sham-thymectomized mice. Immune responsiveness to T-cell-dependent antigens, such as sheep erythrocytes, commonly is used to test for the efficiency of thymectomy [2,14]. The maturation of T-cell functions depends upon the presence of the thymus during ontogenetic development. Thus, neonatal thymectomy impairs cell-mediated as well as humoral immunity [3]. Neonatal thymectomy also decreases both secretory and serum IgA levels [15]. A lack of helper T-cells could be responsible for the decreased IgA levels in thymectomized mice.

It seems likely that indigenous bacteria must associate with or attach to epithelial cells of the gastrointestinal mucosa prior to translocating through the gastrointestinal epithelium to the mes-

Table 1. Bacterial translocation from the gastrointestinal tract to various organs in thymectomized (T) mice and sham-thymectomized (ST) mice.

	Aerobic and facultatively anaerobic bacteria			Strictly anaerobic bacteria		
Tested	T	ST	P^a	T	ST	P
Mesenteric lymph node	18/41[b]	1/20	<.0001	1/41	0/20	NS
Spleen	14/41	0/20	.02	0/41	0/20	NS
Liver	8/41	1/20	.03	2/41	0/20	NS
Kidney	5/41	0/20	.008	0/41	0/20	NS
All organs	45/164	2/80	<.0001	3/164	0/80	.04
Percent positive	27.4	2.5		1.8	0	
Mice positive	29/41[c]	2/20	<.0001	3/41	0/20	.04
Percent mice positive	70.7	10.0		7.3	0	

[a] Incidence of bacterial translocation to organs of thymectomized mice compared with the incidence of bacterial translocation to organs of sham-thymectomized mice by the normal difference test (Z test). NS denotes nonsignificant.
[b] Number of organs with positive bacterial cultures over the number of organs tested.
[c] Number of mice with one or more organs positive for viable bacteria.

Table 2. Bacterial species isolated from the mesenteric lymph nodes, spleens, livers, or kidneys of thymectomized or sham-thymectomized mice.

	Aerobic and facultatively anaerobic bacteria				Strictly anaerobic bacteria[a]	
	Thymectomized mice		Sham-thymectomized mice		Thymectomized mice	
Organs	Bacterial species[b]	Pos. organs/ organs tested[c]	Bacterial species	Pos. organs/ organs tested	Bacterial species	Pos. organs/ organs tested
Mesenteric lymph node	*Escherichia coli*	7/41	*Escherichia coli*	1/20	*Bacteroides* sp.	1/41
	Staphylococcus aureus	4/41				
	Streptococcus faecalis	1/41				
	Streptococcus (viridans)	1/41				
	Corynebacterium sp.	3/41				
	Pseudomonas sp.	1/41				
	Klebsiella pneumoniae	1/41				
Spleen	*Escherichia coli*	5/41		0/20		0/41
	Staphylococcus aureus	4/41				
	Streptococcus (viridans)	3/41				
	Klebsiella pneumoniae	1/41				
	Corynebacterium sp.	1/41				
Liver	*Escherichia coli*	3/41	*Staphylococcus aureus*	1/20	*Bacteroides* sp.	2/41
	Staphylococcus aureus	1/41				
	Streptococcus (viridans)	2/41				
	Corynebacterium sp.	2/41				
Kidney	*Escherichia coli*	2/41		0/20		0/41
	Corynebacterium sp.	2/41				
	Lactobacillus sp.	1/41				

[a] The organs of sham-thymectomized mice did not contain viable strictly anaerobic bacteria.
[b] The Gram-negative enteric bacilli were present in the ceca at levels of 10^4 to 10^5/g while the strictly anaerobic bacteria were present at 10^{10} to 10^{11}/g cecum.
[c] Numbers or organs with positive bacterial cultures over the number of organs tested.

enteric lymph nodes and other organs. Freter [12] found that specific secretory IgA prevents the attachment of *Vibrio cholerae* to intestinal epithelia. Therefore, decreased levels of secretory IgA in thymectomized mice or congenitally athymic mice might allow certain indigenous bacteria to attach to, or associate more easily with, epithelial surfaces prior to translocating from the gastrointestinal tract.

Doerhoff et al. [11] suggest that thymectomy decreases the numbers of T-cells present in peripheral lymphoid tissue, such as the mesenteric lymph nodes. The mesenteric lymph nodes and spleens of

thymectomized mice, with decreased numbers of T-cells, may have greater difficulty in clearing viable translocating bacteria than these organs in sham-thymectomized mice.

In our earlier studies [16], incidence of translocation of indigenous bacteria from the gastrointestinal tract to the mesenteric lymph node, spleen, liver, and kidney in athymic (nu/nu) was 50% compared with 27.4% in the thymectomized SPF mice in this study. Also, the incidence of translocation of these organs in heterozygous (nu/+) mice was 7.8% [16] compared to 2.5% in these sham-thymectomized mice. The incidence of bacterial translocation from the gastrointestinal tract may be greater in congenitally athymic mice than in thymectomized mice, and greater in heterozygous (nu/+) mice than in sham-thymectomized mice, because both the athymic and heterozygous mice in the previous study were the inbred BALB/c strain whereas the thymectomized and sham-thymectomized mice in this study are the outbred CD-1 strain. The incidence of translocation in congenitally athymic mice also might be greater than in thymectomized mice because of incomplete immunosuppression of the thymectomized mice. The peripheral lymphoid organs of thymectomized mice could have been partially seeded with T-cells during embryonic or postnatal life prior to thymectomy [13], whereas the peripheral lymphoid organs of congenitally athymic mice would not have been seeded. In support of this hypothesis, the thymectomized mice produced a greater serum-immune response to sheep erythrocytes after vaccination than did the congenitally athymic mice [16], indicating incomplete immunosuppression.

Cantrell and Jutila [9] reported wasting disease in 21% of thymectomized BALB/c mice. Injections of rabbit anti-mouse thymocyte sera increased the incidence and severity of the wasting disease of these thymectomized mice, indicating that thymectomy may have been incomplete. The livers, spleens, and blood of the thymectomized mice receiving the anti-thymocyte sera contained bacteria presumed to originate from the normal flora of the gastrointestinal tract. We did not observe wasting disease in the neonatally thymectomized CD-1 mice. However, we did culture viable indigenous bacteria from the mesenteric lymph nodes, spleens, livers, and kidneys of these thymectomized mice.

The incidence of bacterial translocation from the gastrointestinal tract in congenitally athymic (nu/nu) mice is 50% and only 7.8% in thymus-grafted (nu/nu) mice [16]. The results presented here demonstrate that neonatal thymectomy of SPF mice also increases the incidence of bacterial translocation from the gastrointestinal tract from 2.5% in sham-thymectomized mice to 23.8% in thymectomized mice. These animal models demonstrate that T-cell-dependent immunity is a host defense mechanism inhibiting bacterial translocation and confining certain indigenous bacteria to the gastrointestinal tract.

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