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Translocation of *Escherichia coli* from the Gastrointestinal Tract to the Mesenteric Lymph Nodes in Gnotobiotic Mice Receiving *Escherichia coli* Vaccines Before Colonization

RODNEY D. BERG* AND ALVA W. GARLINGTON†

Department of Microbiology and Immunology, Louisiana State University Medical Center, School of Medicine in Shreveport, Shreveport, Louisiana 71130

Germfree mice were immunized orally or intraperitoneally for 6 weeks with heat-killed vaccines of indigenous *Escherichia coli* or nonindigenous *E. coli* O127:B8 before colonization with these strains. The mice exhibited increases in specific serum antibodies and intestinal immunoglobulin A reacting with the *E. coli* antigens. Prior immunization did not reduce the gastrointestinal population levels of the *E. coli* strains attained 3 and 7 days after colonization. Neither oral nor intraperitoneal immunization with the *E. coli* strains before colonization decreased the incidence of bacterial translocation to the mesenteric lymph nodes or reduced the number of viable *E. coli* cells per mesenteric lymph node. There also was no relation in individual mice between serum antibody titers and the numbers of viable *E. coli* cells translocating to the mesenteric lymph nodes. Thus, prior vaccination with *E. coli* in this study did not decrease the incidence or reduce the numbers of viable *E. coli* translocating to the mesenteric lymph nodes in gnotobiotic mice monoassociated with *E. coli*.

Bacteria of the indigenous gastrointestinal flora are not cultured from the mesenteric lymph nodes, spleens, or livers of specific pathogen-free (SPF) mice (3). Certain types of indigenous bacteria are cultured, however, from the mesenteric lymph nodes of ex-germfree mice inoculated intragastrically with the whole cecal microflora from SPF mice (3). For example, viable indigenous *Escherichia coli* are present in the mesenteric lymph nodes of 96% of gnotobiotic mice monoassociated with this organism, but in none of the mesenteric lymph nodes of control SPF mice also inoculated with this indigenous *E. coli* (3). Thus, there are mechanisms active in adult SPF mice which inhibit certain viable indigenous bacteria from passing from the gastrointestinal tract to the mesenteric lymph nodes, spleens, or livers. This passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes and possibly other organs is called bacterial translocation (3, 4). The inhibitory mechanisms preventing bacterial translocation in SPF mice are either absent or not as efficient in gnotobiotic mice colonized with these bacteria. One mechanism that inhibits certain viable bacteria from translocating from the gastrointestinal tract to other organs in adult SPF mice is the reduction in the population levels of these bacteria in the gastrointestinal tract due to antagonism by other members of the indigenous microflora (2, 5).

The host immune system also appears to inhibit the translocation of certain viable indigenous bacteria from the gastrointestinal tract to other organs. We cultured viable aerobic and strictly anaerobic bacteria from 50% of the mesenteric lymph nodes, spleens, livers, and kidneys of athymic (nu/nu) mice, whereas viable bacteria were detected in only 5.2% of these organs from heterozygous (nu/+) mice (12). Grafting thymuses from nu/+ to nu/nu mice decreases the incidence of bacterial translocation from the gastrointestinal tract from 50% in the nu/nu mice to 7.8% in the thymus-grafted (nu/nu) mice (12). Thymectomy also increases the incidence of bacterial translocation from the gastrointestinal tract in SPF, CD-1 mice (R. D. Berg, Am. J. Clin. Nutr., in press). Furthermore, intraperitoneal injections of SPF mice with immunosuppressive agents such as cyclophosphamide, prednisone, methotrexate, 5-fluorouracil, and cytosine arabinoside increases the incidence of bacterial translocation from the gastrointestinal tract (Berg, in press). Germfree animals do not possess lymphoid tissues as well developed or defined as those of conventional animals (7, 11). Apparently, the lymphoid tissues of germfree animals have not been activated or "primed" by bacterial antigens; consequently, their immunological responses develop more slowly than those of conventional animals (5, 6). Thus, bacterial

† Present address: Willis-Knighton Medical Center, Shreveport, LA 71103.

translocation from the gastrointestinal tract may occur in gnotobiotic mice but not in SPF mice because of an inadequately primed immune response in the gnotobiotic animals. We immunized germfree mice orally or intraperitoneally with heat-killed indigenous *E. coli* or nonindigenous *E. coli* O127:B8 before colonization with these organisms in an attempt to inhibit their translocation from the gastrointestinal tract to the mesenteric lymph nodes.

Germfree and gnotobiotic mice (CD-1 strain; Charles River Breeding Laboratories, Wilmington, Mass.) were housed in autoclaved polypropylene cages with stainless steel wire lids inside Trexler-type, vinyl isolators (Germfree Supply Division, Standard Safety Equipment Co., Palatine, Ill.) sterilized with 2.0% peracetic acid (FMC Corp., Buffalo, N.Y.) as previously described (1, 6). The mice were fed autoclavable Purina Laboratory Chow 5010 (Ralston Purina Co., St. Louis, Mo.) and supplied with San-I-Cel bedding (Paxton Processing Co., Inc., White House Station, N.J.). Two groups of germfree mice were vaccinated intraperitoneally twice weekly for 6 weeks with 10^8 heat-killed (65°C for 1 h) indigenous *E. coli* or nonindigenous *E. coli* O127:B8. Control germfree mice received injections of normal saline twice a week. Two other groups of germfree mice were given 10^8 heat-killed indigenous *E. coli* or *E. coli* O127:B8 per ml in their drinking water for 6 weeks. The indigenous *E. coli* strain was isolated from the mesenteric lymph nodes of gnotobiotic mice inoculated intragastrically with a suspension of cecal contents from SPF (CD-1) mice as previously described (1). Nonindigenous *E. coli* O127:B8 (ATCC 12740) was obtained from the American Type Culture Collection, Rockville, Md. After 6 weeks of immunization, the mice were allowed to ingest food pellets inoculated with viable indigenous *E. coli* or nonindigenous *E. coli* O127:B8. Mesenteric lymph nodes and ceca from the mice were cultured on Tergitol-7 agar 3 and 7 days after colonization with the *E. coli* by using procedures described previously (3). Sera from the mice also were tested by passive hemagglutination with sheep erythrocytes sensitized with cell wall lipopolysaccharide prepared from the *E. coli* strains (6, 8, 17). Intestinal washings from these mice also were tested by indirect immunofluorescence to demonstrate an increase in intestinal immunoglobulin A (IgA) to antigens of the indigenous *E. coli* or *E. coli* O127:B8. Ascites fluid from BALB/c AnNCrlBR mice (Charles River Breeding Laboratories) injected intraperitoneally with 5.0×10^5 MOPC 315 plasmacytoma cells was purified according to the method of Goetzl and Metzger (10) on a dinitrophenyl-lysine Sepharose adsorbent column. The purity of the IgA protein was tested by sodium dodecyl sulphate-gel electrophoresis (16) and by immunoelectrophoresis against goat anti-mouse IgA, IgM, and IgG (Litton Bionetics, Inc., Kensington, Md.). A goat was immunized with the purified mouse IgA, and the serum was harvested and purified on Sepharose 4B-200 (Sigma Chemical Co., St. Louis, Mo.) immunoadsorbent with purified mouse IgA by a batchwise procedure (9). The eluted goat anti-mouse IgA was absorbed with mouse IgG (Litton Bionetics) to remove any reactivity to immunoglobulin light chains. The goat anti-mouse IgA was conjugated with fluorescein (0.025 ml of fluorescein per ml of protein) (15). The fluorescein-conjugated goat anti-mouse IgA was utilized to detect mouse IgA reacting specifically with antigens of the *E. coli* strains in mouse intestinal washings by indirect immunofluorescence (9).

Mice immunized either orally or intraperitoneally with indigenous *E. coli* or nonindigenous *E. coli* O127:B8 exhibited specific, systemic immune responses (Fig. 1). As expected, the serum

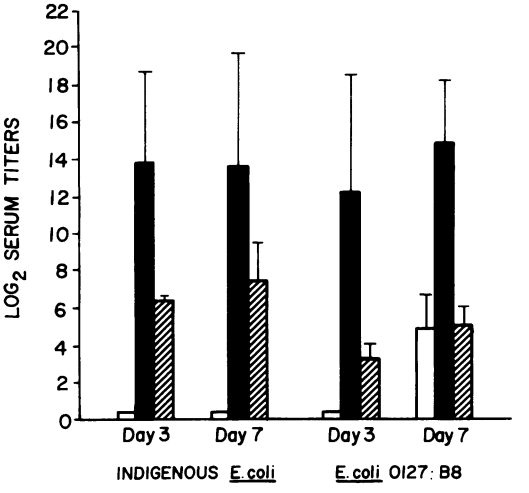

FIG. 1. *Serum immune responses in gnotobiotic mice vaccinated with indigenous E. coli or nonindigenous E. coli O127:B8. Germfree mice were injected intraperitoneally with 10^8 heat-killed indigenous E. coli or nonidigenous E. coli O127:B8 twice weekly for 6 weeks (■). Another group of germfree mice was inoculated orally with 10^8 heat-killed indigenous E. coli or nonindigenous E. coli O127:B8 per ml in drinking water for 6 weeks (▨). Control germfree mice received intraperitoneal injections of normal saline twice weekly for 6 weeks (□). Two days after the last inoculation, the germfree mice were colonized with indigenous E. coli or nonindigenous E. coli O127:B8. Passive hemagglutination titers of serum antibodies to E. coli antigens were determined at 3 and 7 days after colonization with viable E. coli. Each bar represents the mean of the titers from five mice, and the brackets represent the standard errors.*

TABLE 1. *Comparison of the relationship of serum antibody titers and cecal population levels to translocation of indigenous E. coli and nonindigenous E. coli O127:B8 from the intestinal lumen to the mesenteric lymph nodes in individual gnotobiotic mice*

immunization	INDIGENOUS *E. COLI*[a]								NONINDIGENOUS *E. COLI* 0127:B8							
	3 DAYS				7 DAYS				3 DAYS				7 DAYS			
	mouse no.	serum titer[b]	cecal population level[c]	no. bacteria per MLN	mouse no.	serum titer	cecal population level	no. bacteria per MLN	mouse no.	serum titer	cecal population level	no. bacteria per MLN	mouse no.	serum titer	cecal population level	no. bacteria per MLN
Saline (I.P.)	1	0	9.5	500	6	0	10.3	<100	31	1	9.1	<100	36	7	9.6	100
	2	0	10.0	<100	7	1	9.2	1000	32	0	9.0	<100	37	3	9.6	200
	3	0	9.3	200	8	0	9.6	<100	33	0	8.1	100	38	6	9.4	0
	4	0	9.4	200	9	0	10.1	100	34	0	10.0	<100	39	3	9.8	400
	5	0	9.8	<100	10	0	9.9	400	35	1	10.0	<100	40	5	9.6	100
I.P.[d]	11	17	9.7	100	16	5	9.9	100	41	12	4.8	<100	46	15	8.1	100
	12	6	9.9	<100	17	22	9.8	200	42	22	7.2	200	47	12	9.9	<100
	13	14	9.9	0	18	14	10.2	<100	43	9	9.6	<100	48	12	9.9	100
	14	13	10.0	<100	19	12	9.4	<100	44	13	6.8	200	49	20	9.8	300
	15	19	10.3	<100	20	15	9.9	300	45	5	9.6	<100	50	15	10.0	<100
Oral[e]	21	6	9.5	<100	26	10	9.8	0	51	3	10.2	<100	56	7	9.6	<100
	22	7	10.0	500	27	9	9.9	<100	52	4	9.7	<100	57	3	9.9	300
	23	5	9.8	100	28	7	9.3	<100	53	3	10.2	200	58	6	9.8	<100
	24	7	9.4	<100	29	5	10.1	<100	54	4	9.8	100	59	3	9.5	<100
	25	7	9.8	<100	30	6	9.5	100	55	2	ND	<100	60	5	9.8	<100

[a] 1×10^{10} viable *E. coli* placed on food 2 days following the last immunization.

[b] Log_2 reciprocal of highest serum dilution causing hemagglutination of sheep RBC adsorbed with *E. coli* lipopolysaccharide in Microtiter U-plates

[c] Log_{10} number of viable *E. coli* per gram of cecum cultured at 3 or 7 days following inoculation of food with viable *E. coli*.

[d] 1×10^8 heat-killed *E. coli* cells injected intraperitoneally twice weekly for 6 weeks.

[e] 1×10^8 heat-killed *E. coli* cells per ml suspended in drinking water for 6 weeks.

immune responses were greater in the mice vaccinated intraperitoneally than in the mice vaccinated orally. Control mice colonized but not immunized with nonindigenous *E. coli* O127:B8 showed no immune response after 3 days of colonization, but demonstrated a primary immune response after 7 days of ingesting viable *E. coli* O127:B8. Interestingly, no immune response was detected at either 3 or 7 days in the nonvaccinated, control mice monoassociated with the indigenous *E. coli*. Thus, 7 days of colonization with viable nonindigenous *E. coli* O127:B8 was sufficient to stimulate a specific serum immune response in nonimmunized gnotobiotic mice, but 7 days of colonization with indigenous *E. coli* was not sufficient, reaffirming that these mice respond immunologically to a greater degree to antigens of *E. coli* O127:B8 than to antigens of the indigenous *E. coli* strain (5, 6). Nevertheless, the gnotobiotic mice immunized orally or intraperitoneally with indigenous *E. coli* exhibited levels of specific serum antibodies as great as those of gnotobiotic mice immunized with nonindigenous *E. coli* O127:B8.

These immunized and control mice also were tested for intestinal IgA reacting specifically with indigenous *E. coli* or nonindigenous *E. coli* O127:B8. Intestinal washings from the immunized gnotobiotic mice contained IgA reacting strongly with antigens of the *E. coli* strains as detected by indirect immunofluorescence, whereas control gnotobiotic mice did not exhibit any intestinal IgA reacting with these *E. coli* strains. Thus, 6 weeks of either oral or intraperitoneal immunization was sufficient to stimulate intestinal IgA as well as serum antibodies reacting with *E. coli* antigens.

Intraperitoneal or oral immunization for 6 weeks with vaccines of these *E. coli* strains did not decrease the incidence of bacterial translocation of either indigenous *E. coli* or nonindigenous *E. coli* O127:B8 to the mesenteric lymph nodes (Table 1). Neither specific intraperitoneal nor oral immunization reduced the numbers of indigenous *E. coli* or *E. coli* O127:B8 colonizing the ceca of these mice. There was no obvious relationship in individual mice between their serum antibody titers and the numbers of viable *E. coli* translocating to their mesenteric lymph nodes, or between their serum antibody titers and the numbers of *E. coli* populating their ceca.

The inhibition of bacterial translocation from the gastrointestinal tracts in thymus-grafted (nu/nu) mice (4), the promotion of bacterial translocation in thymectomized CD-1 mice (Berg, in press), and the increase in bacterial translocation in CD-1 mice injected with immunosuppressive agents (Berg, in press) suggest that the host immune system operates to confine certain bacteria to the gastrointestinal tract. However, neither oral nor intraperitoneal immunization of germfree mice with heat-killed *E. coli* before colonization with *E. coli* decreased the numbers of *E. coli* translocating to the mesenteric lymph nodes in our study. It may be worthwhile to attempt other immunization procedures in efforts to produce an immunity effective in reducing or inhibiting bacterial translocation from the gastrointestinal tract. Combining oral and intraperitoneal vaccinations possibly could stimulate a more effective immune response. Pierce et al. (13) suggest that, in dogs at least, subcutaneous priming with cholera toxoid followed by oral boosting is a more effective means of providing protection against challenge with *Vibrio cholerae* than either oral or parenteral vaccination alone. Perhaps the 1,000-fold-higher numbers of *E. coli* populating the gastrointestinal tracts of monoassociated gnotobiotic mice compared with the numbers of *E. coli* in the gastrointestinal tracts of conventional mice overwhelm the immune system of the gnotobiotes. Shedlofsky and Freter (14) observed that immunization had no reducing effect on the population levels of *V. cholerae* in the gastrointestinal tracts of monoassociated gnotobiotic mice unless the vibrio numbers first were reduced by colonization of the mice with an antagonistic bacterial flora. Thus, prior immunization might reduce the translocation of viable *E. coli* to the mesenteric lymph nodes or other organs if the *E. coli* population levels in the gastrointestinal tract first could be reduced by a select anatagonistic flora to levels closer to that found in conventional mice, but still at population levels able to promote translocation from the gastrointestinal tract.

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