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Immunogenicity of Deoxycholate-disrupted Endotoxins

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A comparison of the immunogenicity of sodium deoxycholate-disrupted lipopolysaccharides from *Escherichia coli* cell walls revealed that these fragments, which are nonimmunogenic in the rabbit, have some activity in the mouse. This relationship was independent of the route of immunization and sex, but in both species immunogenicity was restored by dilution or dialysis. Adsorption of disrupted lipopolysaccharides onto bentonite particles or administration with methylated bovine serum albumin and Freund's adjuvant did not appreciably augment activity in vivo. It is postulated that in the mouse the requirements for immunogenicity of these lipopolysaccharides are either less stringent with regard to the three-dimensional structure of the antigen, or that a reaggregation to toxic, native lipopolysaccharides may occur in vivo.

The continuing efforts of many investigators to detoxify the endotoxic lipopolysaccharides of the *Enterobacteriaceae* generally has been conceded to yield a less toxic product with either a diminished or altered ability to induce a humoral antibody response (2, 4). Rudbach et al. (7) described sodium desoxycholate (NaD) treatment of endotoxin which will dissociate these high-molecular-weight substances into small homogenous subunits. In addition, it was reported that, in contrast to most other treatments these dissociated molecules will reaggregate into biologically active, toxic elements when the NaD is removed by dilution or dialysis. Tarmina et al. (8) indicated that in rabbits the immunogenicity of the disrupted material is greatly decreased, but that after dialysis, both toxicity and the ability to induce antibody formation were restored. The experiments to be described involve a comparison of the immunogenicity of NaD-disrupted lipopolysaccharides of *Escherichia coli* in the rabbit and in the mouse.

MATERIALS AND METHODS

Animals. New Zealand white rabbits of mixed sex were used throughout. Male and female mice (CD-1) were obtained from Charles River Laboratories.

Antigens. Lipopolysaccharide (LPS) from *E. coli* 0127:B8 was purchased commercially (Difco lot no. 471779). Cultures and endotoxin from *E. coli* 0113 were generously provided by W. P. Weidanz. The 0113 LPS was extracted by the aqueous ether procedure (5), and the 0127 by Boivin's technique (1). The antigens were stored at a concentration of 1 mg/ml in saline and diluted prior to use. NaD (Difco lot no. 469868) was dissolved in 0.15 M phosphate buffer, *p*H 7.3, to prevent gel formation which occurred in acid solutions.

Antigen in NaD was absorbed on bentonite particles (10) and washed; the suspension was then injected intravenously into rabbits and intradermally into mice. Solutions containing NaD and LPS were also mixed with methylated bovine serum albumin (MBSA) in complete Freunds adjuvant (5) and injected subcutaneously into rabbits. Control animals received antigen mixed with bentonite or MBSA alone. Reassociation of NaD-disrupted endotoxin was carried out by dialysis against phosphate buffer, *p*H 7.3, or passage through Sephadex G-25.

Immunization. Animals were injected by a variety of routes with either the parent endotoxin or endotoxin in 2% NaD (w/v) which was preincubated for 30 min at room temperature.

Antibody assay. The animals were bled by cardiac puncture at days 0, 5, 10, and where indicated after immunization; the serum was separated and stored at −20 C prior to assay. Rabbits were assayed individually and mice in groups of three to five. Antibody titers to *E. coli* 0127 were measured by the bactericidal assay (9) and passive hemagglutination of LPS-coated erythrocytes (3). No bactericidal assays were performed on the sera from *E. coli* 0113-injected animals.

RESULTS

The findings presented in Fig. 1 show that treatment with 2% NaD completely suppresses

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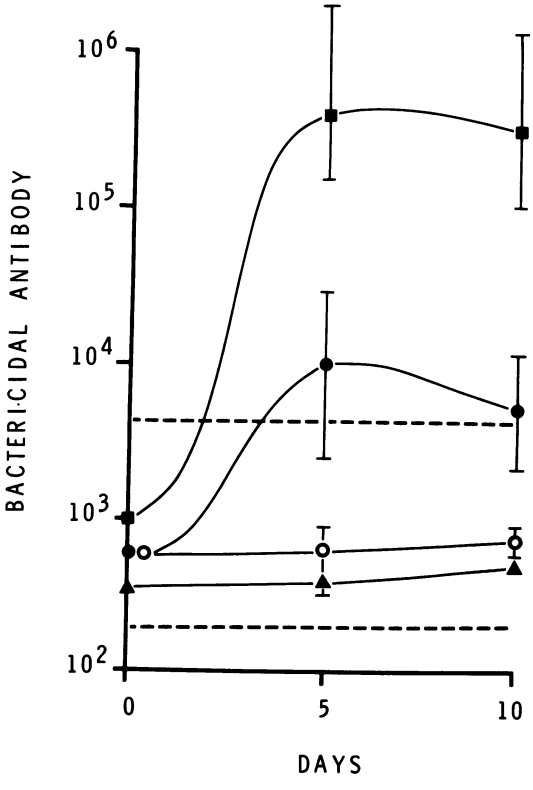

FIG. 1. *Bactericidal antibody titers of rabbit immunized intravenously with: ■, 1 μg of E. coli endotoxin ▲, 1 μg of endotoxin in 2% NaD ○, 10 μg of endotoxin in 2% NaD; ●, 100 μg of endotoxin in 2% NaD. Range marks show variation found in groups of three to five rabbits. Day 0 for each group is shown separately. Dashed lines show range of natural antibody in unimmunized rabbits.*

the antibody response in the rabbit to amounts (1.0 μg) of *E. coli* 0127 endotoxin which are more than sufficient to evoke maximal titers of bactericidal antibody; 100 μg also induced similar high titers in control rabbits. The dose of 100 μg in NaD induced a limited response, but this may be owing either to some low percentage (< 1%) of the parent preparation which is resistant to NaD or merely an excess of endotoxin. Hemagglutination titers did not change or increased only one dilution in the nonresponders. When the greater part of the NaD was removed by dialysis or gel filtration, bactericidal antibody responses were restored to approximately 50% of the average peak titer expected for these antigen doses.

In mice, the same lot of *E. coli* 0127 endotoxin gives rise to lower bactericidal antibody titers for a given dose (10 μg) per animal (Table 1) but, in addition, the effect of treatment with NaD did not suppress antibody production completely. This occurred whether the mice were injected intradermally or intraperitoneally. To ascertain the basis for this difference, *E. coli* 0113 endotoxin extracted by the aqueous ether procedure was subjected to the same treatment with NaD and injected into mice. Since this strain of *E. coli* is not serum-sensitive, antibody measurements were made by hemagglutination (Table 1). These data make it evident that, in contrast to the rabbit system, (i) NaD treatment has less effect on immunogenicity of *E. coli* endotoxin for mice, and (ii) an increase or no difference between control and NaD-treated antigen was seen in passive hemagglutination titers when endotoxins of *E. coli* were prepared by different precedures.

Since the NaD-disrupted LPS was nonimmunogenic in rabbits, the small units were "reaggregated" by association with particulate carriers, i.e., bentonite and MBSA. To assure that adsorption on bentonite had occurred, the particles were incubated with the NaD-disrupted LPS, washed, and exposed to specific anti-*E. coli* antiserum. Strong agglutination of these particles indicated that at least a portion of the original somatic O-determinants were being injected in a particulate form. A comparison of peak antibody titers for both rabbits and mice is shown in Table 2. The results are similar to those seen previously, i.e., no increase or a slight rise in titers when NaD-treated endotoxin was given to rabbits in either "soluble" or particulate form, whereas mice were able to respond to both.

DISCUSSION

Although the capacity to evoke an antibody response in mice was not abolished by NaD treatment, neither was there any significant enhancement by the bentonite particles. In a few cases, an antibody response could be detected in the rabbits injected with bentonite or MBSA as carriers of the treated endotoxin. Thus, it seems apparent that a particulate nature and high-molecular weight are not the only criteria which explain the striking immunogenicity of these LPS antigens. Rather, these findings suggest that, in addition, the three-dimensional micellar arrangement of the molecule must be presented to the immune recognition mechanism in a manner similar to that of the intact parent preparation to induce an antibody response of appreciable magnitude following a single injection. It is noteworthy, however, that the mouse is able to respond to the NaD-treated material, and thus a difference in antigen "processing," a reaggregation in vivo, or an altered immunoglobulin response between the species are also considerations.

TABLE 1. *Bactericidal and hemagglutinating antibody responses of mice to E. coli endotoxins*

Antigen (10 μg)	Antibody titer[a]					
	Bactericidal[b]			Hemagglutination[c]		
	0 Day	5 Days	10 Days	0 Day	5 Days	10 Days
E. coli 0127	<320	26,000	20,000	<2	64	64
E. coli 0127 + 2% NaD	<320	9,000	7,200	<2	64	32
E. coli 0113	—[d]	—	—	<2	4–8	80
E. coli 0113 + 2% NaD	—	—	—	<2	32–64	256

[a] Average of five mice per sample.
[b] Reciprocal dilution of serum which kills 50% of the bacterial inoculum.
[c] Reciprocal dilution of serum giving visible hemagglutination of LPS-coated sheep erythrocytes.
[d] Not done.

TABLE 2. *Bactericidal antibody response to NaD-treated E. coli endotoxin in particulate form*

Antigen (10 μg)	Peak antibody titers	
	Rabbits[a]	Mice[b]
None	0.2×10^3–2×10^3	0.01×10^3–0.1×10^3
E. coli 0127	150–$2{,}000 \times 10^3$	26×10^3
E. coli 0127 + NaD	NC[c]	9×10^3
E. coli 0127 + bentonite	150–300×10^3	26×10^3
E. coli 0127 + NaD + bentonite	NC-3 $\times 10^3$	6×10^3
E. coli 0127 + MBSA	10–270×10^3	—[d]
E. coli 0127 + NaD + MBSA	NC-2 $\times 10^3$	—

[a] Range of values of at least three animals.
[b] Pooled titers from 5 to 10 animals.
[c] No change from background value at day 0.
[d] Not done.

Distribution of antibody activity in the various classes of the immunoglobulins was not investigated; however, all sera were found to be sensitive to treatment with 0.1 M mercaptoethanol, indicating that the majority of the early hemagglutinating antibodies is of the γM and possibly γA class. From studies on mouse spleen cells releasing antibody to the somatic O-antigen of *E. coli* 0127, it is known that by day 10, low-molecular-weight (γG) antibody would be expected to be present (C. S. Walters et al., Federation Proc., p. 735, 1968). Studies are in progress to determine the classes of immunoglobulins which are released from antibody-producing cells in mice injected with NaD-disrupted LPS.

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