Specific Induced Resistance of Mice to Endotoxin. (27447)

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Recently, Schaedler and Dubos(1) provided evidence that mice, raised and maintained free of ordinary bacterial pathogens, became more susceptible to lethal effects of endotoxin after vaccination with certain strains of enterobacteriaceae. Interestingly enough, the induced susceptibility to endotoxin exhibited a certain degree of specificity for the bacterial strain used for vaccination. A recent study of lipopolysaccharides extracted from strains of oral gram negative bacteria belonging to the genus *Veillonella* provided us with serologically specific endotoxin preparations whose specificity could be shown by hemagglutination inhibition technics(2,3). The experiments reported here demonstrate that a strain of mice, harboring very few if any veillonellae in their oral or intestinal contents, can be made resistant to lethal effects of specific lipopolysaccharides derived from veillonellae by prior vaccination with such organisms.

Materials and methods. Vaccines were prepared from several strains of oral anaerobic gram negative cocci conforming to the genus *Veillonella* (a strain of *Veillonella parvula,* unidentified human strains designated as V2, VH-4, VH-11, VH-13, VH-14, VH-15, VH-16, an unidentified rabbit strain, VR-1) and from a human oral strain of *Fusobacterium,* strain F-RP. Cells from 24- to 48-hour broth(4) cultures were collected by centrifugation, washed in 0.85% sodium chloride (saline), and finally resuspended in saline to an optical density 1.5-2.0. Suspensions of bacteria were either used viable in volumes of 0.25 ml or after heating at 80°C for 1 hour in volumes of 0.5 ml. *Bordetella pertussis* vaccine was a commercial preparation obtained from Lederle Laboratories.

Purified lipopolysaccharides (LP) were prepared from 2 strains of veillonella, strain V2 and VH-14, and from *Escherichia coli,* strain BJ-3 by the phenol-water extraction procedure(4). Further purification was accomplished by centrifugation of the water solubilized endotoxin at 100,000 $\times$ g for 2 hours to remove residual contaminating nucleic acids. The acetone-dried endotoxins were dissolved in saline for injection in volumes of 1.0 ml.

Serotonin creatinine sulfate and histamine dihydrochloride (Nutritional Biochemicals Corp.) were dissolved in saline and injected in 1.0 ml volumes.

Female NIH general purpose mice (Swiss-Webster strain), initially weighing 12-16 g were utilized throughout this study. All injections in mice were made by the intraperitoneal route.

Results. Normal mice (saline injected) are quite resistant to the lethal effects of veillonella endotoxin (Table I). Higher dosages of this endotoxin are needed to produce lethality in mice than are ordinarily needed with lipopolysaccharides derived from enterobacteriaceae(4). The vaccination of mice by 2 intraperitoneal injections with heat-killed veillonellae conferred resistance to lethal effects of as much as 2.5 LD_{50} of specific lipopolysaccharide(3). While it appears that there is some overlapping in resistance to endotoxin as seen in Table II, the protection afforded the animals by prior vaccination was most striking with endotoxin prepared from the strain of veillonella used for vaccination. Also indicated in Table II is the finding that viable cells or heat-killed cells are equally effective in induction of a specific state of resistance.

A further experiment was performed by utilizing additional strains of veillonella for vaccination of mice which were then challenged with specific lipopolysaccharide from strain VH-14 (Table III). Most interesting is the finding that the protection was quite uniform among those animals receiving veillonella whose lipopolysaccharides appear identical by hemagglutination inhibition tests(2). One exception, vaccination with cells from

TABLE I. Response of Veillonella Vaccinated Mice to Various Amounts of Endotoxin.

Vaccinations 4 wk and 2 wk before challenge	Deaths after challenge with V2–LP*				
	2.5 mg	2.0 mg	1.5 mg	1.0 mg	.5 mg
Heat-killed veillonella, V2	2/6	0/7	0/4	1/7	0/6
Saline	5/6	6/6	3/6	3/6	0/6

* Deaths over total inj. 48 hr after challenge.

strain VH-13, whose endotoxin was found to be serologically distinct from that of VH-14, conferred significant protection on mice challenged with VH-14 endotoxin. Mice vaccinated with strains *V. parvula*, V2, and VH-4, whose lipopolysaccharides can be shown to be serologically distinct, are as susceptible as control mice to the lethal effects of VH-14 endotoxin (Table III).

Injection of sublethal dosages of endotoxin has profound effects on weight gain by the normal mouse(1). Evidence for protection against this effect of endotoxin after challenge of vaccinated mice with sublethal dosages has also been obtained. Injection of 250 μg of veillonella endotoxin causes significant physiological disturbance in normal mice (saline group) as indicated by the drop in weight which lasted over a period of several days (Fig. 1). In all cases, however, vaccinated mice challenged with endotoxin show less initial weight loss and more rapid recovery. There is an early positive weight gain in the

TABLE II. Effect of Vaccination with Viable and Heat-Killed Veillonella on Susceptibility of Mice to Lethal Effect of Serologically Specific Endotoxins.

Vaccination with heat-killed veillonella*	Deaths after challenge with V2–LP† (1.0 mg)	Vaccination with viable veillonella*	Deaths after challenge with VH-14–LP† (1.5 mg)
V2‡	0/10	V2	6/10
VH-14‡	2/8	VH-14	1/9
VR-1‡	5/8		
Saline	8/10	Saline	5/10

* Mice received 2 intraper. injections of heat-killed or viable bacteria separated by 2 wk and were challenged 2 wk thereafter.

† Deaths over total inj. 48 hr after challenge.

‡ Serologically specific lipopolysaccharides as determined by hemagglutination inhibition tests(2).

TABLE III. Effect of Vaccination with Homologous and Heterologous Veillonella on Susceptibility of Mice to Endotoxin.

Vaccinations 4 wk and 2 wk before challenge	Lethality 48 hr after challenge with 2.0 mg VH-14–LP		
	Deaths/total inj.	%	Degree of protection,* %
V. parvula	4/8	50	+10
V2	5/10	50	+10
VH-4	6/10	60	+ 0
VH-11†	2/9	20	+40
VH-13	1/7	14	+46
VH-14†	2/9	20	+40
VH-15†	0/7	0	+60
VH-16†	1/4	25	+35
Saline	6/10	60	

* % lethality in saline inj. mice − % lethality in vaccinated mice.

† Serologically identical lipopolysaccharides as indicated by hemagglutination inhibition tests(2).

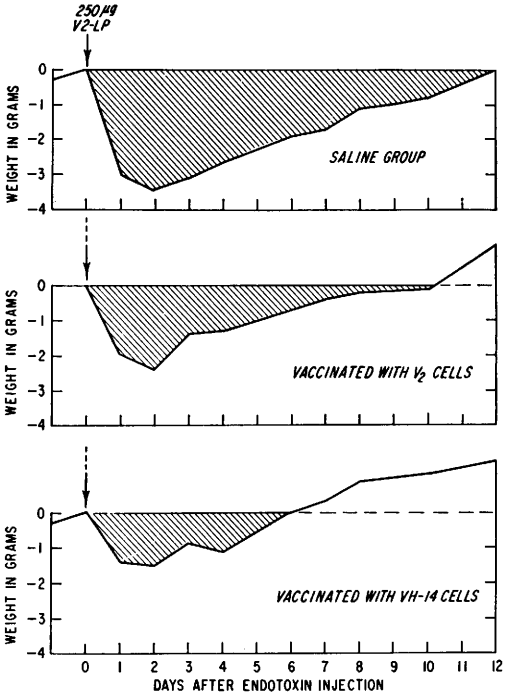

FIG. 1. Wt loss in saline inj. and vaccinated mice (heat-killed cells 4 wk and 2 wk before challenge) after inj. of veillonella endotoxin. Calculated in terms of medium wt in groups of 10-12 mice.

vaccinated groups of mice. In these experiments, there is considerable overlapping in protection against injection of sublethal dosages of homologous and heterologous endotoxin.

Swiss-Webster mice sensitized with *B. pertussis* show an increase in susceptibility to veillonella endotoxin (Table IV), histamine, and serotonin (5-hydroxytryptamine)(5). It seemed desirable to know if vaccination of the Swiss-Webster strain of mice used in these experiments would protect *B. pertussis*-sensitized mice against lethal dosages of serologically related and unrelated endotoxins and against the lethal effects of other agents such as histamine and serotonin. It is clear from Table V that while there is significant protection afforded *B. pertussis*-sensitized mice against veillonella endotoxin by prior vaccination with veillonella cells, the lethal effects of histamine and serotonin are unaltered. Further, prior vaccination with an unrelated strain of gram negative bacteria (*Fusobacterium*), containing a serologically unrelated lipopolysaccharide(3), fails to provide protection against veillonella endotoxin (Table VI). Although there is observed some reduction in mortality in veillonella vaccinated mice after challenge with 500 μg *E. coli* endotoxin, protection is once again most evident in mice challenged with homologous endotoxin (V2-LP).

TABLE IV. Susceptibility of Pertussis-Sensitized Mice to Veillonella Endotoxin.

V2–LP dose, μg	Deaths/total inj. (48 hr) Normal mice	Pertussis sensitized mice*
1000	2/18	10/10
500	0/9	10/10
250	0/9	10/10
125	†	7/10
50	†	0/9

* 0.2 ml *B. pertussis* vaccine 1 wk before challenge with endotoxin.
† Not done.

TABLE V. Effect of Veillonella Vaccination on Susceptibility of Pertussis-Sensitized Mice to Histamine, Serotonin, and Veillonella Endotoxin.

Vaccinations 5 wk and 3 wk before challenge	0.2 ml pertussis vaccine 1 wk before challenge	Deaths after challenge with* V2–LP (250 μg)	Histamine (3 mg)	Serotonin (4 mg)
Veillonella V2	—	0/10	3/10	0/8
Saline	—	0/10	1/10	1/10
Veillonella V2	+	3/10	9/10	5/8
Saline	+	10/10	9/10	6/10

* Deaths over total inj. 48 hr after challenge.

Discussion. Schaedler and Dubos(1) interpreted the increased susceptibility of mice to endotoxin on an immunological basis (allergic-like reactions). Our findings of decreased susceptibility may also have an immunological basis. The apparent specificity of protection indicates that this is not a nonspecific increase in functional capacity of the reticuloendothelial system(6), but rather suggests the presence of specific antibodies which facilitate the uptake of endotoxin by the reticuloendothelial system(7). It may be significant, in view of other workers' reports on prior sensitization of the host as influencing susceptibility to endotoxin(1), that this strain of mice does not normally harbor veillonella in the intestinal tract.

Other investigations have revealed increased susceptibility of mice to endotoxin after infection with virulent strains of gram negative bacilli(8). This may indicate sensitization due to the invasive potential of the infecting organisms. It should be pointed out, however, that viable veillonellae have not been found to be entirely innocuous for the

TABLE VI. Effect of Homologous and Heterologous Vaccination on Susceptibility of Pertussis-Sensitized Mice to Endotoxin.

Inj. 6 wk and 4 wk before challenge	0.2 ml pertussis vaccine 2 wk before challenge	Deaths after challenge with* V2–LP 250 μg	V2–LP 500 μg	*E. coli*–LP 250 μg	*E. coli*–LP 500 μg
Veillonella V2	+	1/14	3/19	9/18	9/19
Fusobacterium	+	11/18	14/18	†	†
Saline	+	12/18	17/19	†	14/15

* Deaths over total inj. 40 hr after challenge. † Not done.

mouse. Certain strains are highly lethal to the mouse and autopsy reveals the presence of viable organisms in such tissues as lung and liver (Mergenhagen, unpublished observations). The mechanism behind this specific induced state of resistance and some of the discrepancies of these results with findings of others require further investigation.

Summary. Vaccination with viable or heat-killed oral anaerobic gram negative cocci (genus *Veillonella*) increased the resistance of a strain of Swiss-Webster mice to the lethal effects of veillonella endotoxin. Protection was best conferred with serologically specific endotoxin prepared from the strain of veillonella used for vaccination. Utilizing weight loss to sublethal dosages as a measure of biological effects of endotoxin, the duration and extent of weight loss in vaccinated mice is also considerably reduced as compared to nonvaccinated animals. Vaccination with heat-killed veillonellae significantly protected *B. pertussis*-sensitized mice against veillonella endotoxin, but not against lethal doses of *E. coli* endotoxin, histamine, or serotonin. Vaccination of mice with another oral gram negative bacterium (*Fusobacterium*) did not protect pertussis-sensitized mice against the lethal effect of veillonella endotoxin.

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"Differential Cleavage Rate" in 2-day Litter Mate Rabbit Embryos.* (27448)

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In multicellular organisms the sizes of cells, other than ova, vary only between certain relatively narrow limits; body size is primarily a reflection of cell number rather than cell size. In most mammalian species, the greatest but not the most rapid growth occurs after the end of fetal period proper, during the later phases of the whole developmental cycle when there is comparatively little change of form (1).

One of the most significant leads in analysis of growth and size in rabbits was reported in connection with breed differences in size between Flemish-giant and small Polish(2). The differences were traced back to early embryonic stages, even though there are no differences in size of ova at the time of ovulation. Similarly, Venge(3) has noted differences in cleavage rate of fertilized ova between large and small sized breeds. At comparable stages, the average number of blastomeres and the blastocyst diameters is greater in large sized breeds than in small breeds(4). At 6½ days of pregnancy, rabbit blastocysts, within the same breed, weigh from 15 to 33 mg(5). Various physio-genetical mechanisms account for size differences in different species. In the rat, the time interval between cleavages from the second to the sixth day of

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