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Plasmid-Cured *Salmonella enteritidis* AL1192 as a Candidate for a Live Vaccine

MASAYUKI NAKAMURA,[1]* SHIZUO SATO,[2] TATSUO OHYA,[2] SHOKO SUZUKI,[1] SUMIO IKEDA,[1] AND TETSUO KOEDA[1]

National Veterinary Assay Laboratory, Kokubunji, Tokyo 185,[1] and National Institute of Animal Health, Tsukuba, Ibaraki 302,[2] Japan

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We report the immunizing capacity of *Salmonella enteritidis* AL1192, a strain that has been cured of a 36-megadalton plasmid, to protect ddY mice against subsequent challenge with virulent salmonellas. This strain, which was given subcutaneously at a dose of 10^6 organisms, provided significant protection against oral, subcutaneous, or intraperitoneal challenge by virulent wild-type strains of not only *S. enteritidis*, but also *S. dublin*, *S. naestved*, and *S. typhimurium*.

Recently, three reports (3, 5, 9) showed that virulence was associated with the presence of a plasmid in *Salmonella typhimurium*, *S. dublin*, and *S. enteritidis*, the principal causes of animal and human salmonellosis. Because curing the plasmid of these three serovars resulted in a decrease in virulence for mice, it is reasonable to assume that plasmid-cured derivatives of these strains may be effective live vaccines. Therefore, we tested the potential of a plasmid-cured derivative of *S. enteritidis* as an effective live vaccine.

Virulent *S. enteritidis* AL1190, which carries a 36-megadalton plasmid, was isolated from the spleen of a dairy cow; AL1192 is a plasmid-cured derivative of AL1190 (5). The 50% lethal doses (LD_{50}) of these two strains in 5-week-old ddY mice were $10^{4.51}$ and $10^{7.85}$ bacteria on subcutaneous administration and $10^{5.71}$ and $>10^8$ on oral administration, respectively (5). Figure 1 shows the viable counts of *S. enteritidis* AL1190 and AL1192 organisms in the spleens of C57BL mice (salmonella-susceptible inbred line [6]) up to 14 days after the administration of various doses of bacteria.

We tested the ability of AL1192 to protect against subsequent challenge with AL1190. The mice (ddY 5-week-old males) were subcutaneously given 10^4 to 10^7 AL1192 bacteria and challenged 2 weeks later with graded doses of virulent AL1190. Deaths were recorded daily for 14 days. AL1192, given subcutaneously at a dose of 10^6, gave adequate protection against oral and subcutaneous challenge, whereas almost all nonimmunized control mice were dead by day 14 (Table 1). Thus, subcutaneous injection of 10^6 live AL1192 bacteria protected mice challenged with a lethal dose. Moreover, AL1192, given orally at a dose of 10^6, also gave protection against subcutaneous challenge with 10^2 LD_{50} of AL1190 (data not shown). Hoiseth and Stocker (2) reported that aromatic-dependent *S. typhimurium* completely protected mice against oral or peritoneal challenge of virulent *S. typhimurium*. Our results are similar, although different *Salmonella* serovars were used.

We next examined the ability of AL1192 to protect against subsequent challenge with another wild-type strain of *S. enteritidis* and several wild-type strains of *S. dublin*, *S. naestved*, and *S. typhimurium*. Sources and O-antigenic formula of these challenge strains are shown in Table 2. AL1192 that was given subcutaneously at a dose of 10^6 provided significant protection against oral, subcutaneous, and intraperitoneal challenge by virulent wild-type strains of not only *S. enteritidis*, but also *S. dublin*, *S. naestved*, and *S. typhimurium* (Table 2). Although there were minor differences in level of protection according to the challenge strain or route, in most cases AL1192 protected mice against 10^2 to 10^5 LD_{50} challenge regardless of challenge strain. Immunization of mice with plasmid-cured *S. enteritidis* AL1192 organisms induced not only protection against *S. dublin* and *S. naestved* having the same O-antigenic formula as *S. enteritidis*, but also cross-protection against *S. typhimurium* strains with different antigenic formulas. Smith et al. (7, 8) reported that an aromatic-dependent live vaccine of *S. typhimurium* protected calves against challenge with virulent *S. dublin* and vice versa. According to their reports, the protection given by one serovar against challenge by other serovars might not be adequately explained by O-specific humoral immunity, although the O-antigenic structures of

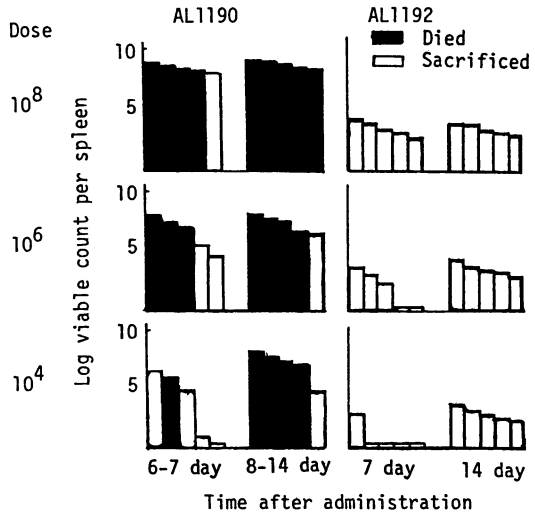

FIG. 1. Log viable count of *S. enteritidis* AL1190 and AL1192 organisms in spleens of C57BL mice.

* Corresponding author.

TABLE 1. Challenge by subcutaneous or oral administration of virulent *S. enteritidis* AL1190 to ddY mice immunized by subcutaneous injection of *S. enteritidis* AL1192

AL1192 immunization dose	Deaths/no. tested at AL1190 challenge dose (oral administration):				AL1192 immunization dose	Deaths/no. tested at AL1190 challenge dose (subcutaneous administration):		
	1.2×10^9	1.2×10^8	1.2×10^7	1.2×10^6		4.8×10^8	4.8×10^7	4.8×10^6
6.1×10^7	0/5	0/5	0/5		3.9×10^6	1/5	0/5	0/5
6.1×10^6	0/5	0/5	0/5		3.9×10^4	4/5	4/5	2/5
6.1×10^5	1/5	0/5	0/5					
6.1×10^4	2/5	1/5	0/5					
Control	5/5	5/5	3/5	2/5	Control	5/5	5/5	5/5

TABLE 2. Challenge of virulent salmonellas to ddY mice immunized by subcutaneous injection of 10^6 *S. enteritidis* AL1192 bacteria[a]

Challenge strain	O-antigen	Source	Route[b]	LD_{50} (log_{10})		
				Immunized mice	Controls	Immunized control
S. enteritidis						
AL1190	1,9,12	Spleen (calf)	i.p.	7.18	1.85	5.33
			s.c.	8.63	4.88	3.75
			p.o.	9.85	6.58	3.27
L-174	1,9,12	Kidney (calf)	s.c.	9.72	6.52	3.20
S. dublin L-107	1,9,12	Liver (calf)	i.p.	8.18	5.18	3.00
S. naestved L-595	1,9,12	Spleen (calf)	s.c.	9.12	6.73	2.39
S. typhimurium						
L-535	4,5,12	Lymph node (calf)	i.p.	4.86	1.86	3.00
			s.c.	7.20	5.51	1.69
L-545	1,4,12	Feces (calf)	i.p.	6.18	2.15	3.67

[a] Five-week-old male mice were challenged 14 days after immunization. Deaths were recorded daily for 14 days after challenge.
[b] i.p., s.c., and p.o. indicate intraperitoneal, subcutaneous, and per os administration, respectively.

these strains share a common component. Recently, Killar and Eisenstein (4) also reported that an aromatic-dependent live vaccine of *S. typhimurium* SL3235 seemed to induce cellular immunity, involving both nonspecific and specific resistance. Moreover, it had already been reported that cellular immunity played an important role in guaranteeing survival of challenged mice (1). Taking these into consideration, we agree with the opinion mentioned above that live vaccines may stimulate specific and nonspecific cellular as well as humoral immune mechanisms.

At present, we consider AL1192 as a candidate for an effective live vaccine against animal and human salmonellosis. However, further investigations of the following two points are necessary. First, this AL1192 is not completely nonvirulent. The LD_{50} of this strain was $10^{7.85}$ by subcutaneous injection into ddY mice, although AL1192 is less virulent than the parent wild type, AL1190 (5). Second, AL1192 apparently differs from wild-type strains by a single characteristic, i.e., it harbored no plasmids, whereas all other *S. enteritidis* strains isolated in Japan harbored at least one plasmid (5). It is recommended that AL1192 be distinguished by more than two markers (independent markers) from field strains of *S. enteritidis*.

Moreover, it is important to investigate the actual basis of strain attenuation. To show this, reintroduction of the 36-megadalton plasmid into cured strain and a detailed study of the virulence factors should be carried out. The paper presented here concentrates, however, on the immunizing capacity of the cured AL1192 as a candidate for an effective live vaccine.

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