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Intestinal Immunoglobulin A Responses in Rabbits to a *Salmonella typhi* Strain Harboring a *Shigella sonnei* Plasmid

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Salmonella typhi 5076-IC, which contains a plasmid that encodes the form I antigen of *Shigella sonnei* and which expresses *S. typhi* 9 and 12 and *S. sonnei* form I antigens, was used to immunize rabbits via chronically isolated ileal loops. Intestinal immunoglobulin A activity was detected against *S. typhi*, *S. sonnei* form I, and *S. typhi* strain 5076-IC. Thus *S. typhi* 5076-IC can effectively elicit mucosal immunoglobulin A to both *S. typhi* and *S. sonnei*.

Living oral vaccines against shigella strains can prevent dysentery in both laboratory animals and humans (1, 3–5, 7, 15–18). This immunity most likely results from the stimulation of the mucosal immune system for studies using chronically isolated ileal loops in rabbits have shown that direct mucosal immunization with attenuated shigella strains results in a secretory immunoglobulin A (IgA) response to the immunizing bacteria (11, 12). Furthermore, when multiple doses of live shigella are given orally, a significant local IgA memory response can be elicited (9, 13). Unlike most attenuated shigella vaccine strains, a galactose epimeraseless (galE) attenuated mutant of *Salmonella typhi* Ty21a has been shown to be both safe and effective as an oral vaccine (6, 19).

Previously, Formal et al. (2) demonstrated that the *Shigella sonnei* plasmid which encodes the form I cell surface antigen could be conjugally transferred to the galE oral vaccine strain *S. typhi* Ty21a. By immunizing mice parenterally with this resultant strain (*S. typhi* 5076-IC), Formal and co-workers found that these animals were protected from subsequent challenge with either *S. sonnei* or *S. typhi* (2). The present study was undertaken to determine whether this *S. typhi* 5076-IC vaccine strain elicits an intestinal IgA response to both *S. typhi* and *S. sonnei* antigens.

Our previously described method was used to construct chronically isolated segments of ileum, 20 cm long, in New Zealand white rabbits weighing 2 to 3 kg (10). Secretions from the isolated loops were collected daily, centrifuged at 3,500 rpm for 10 min in a Sorvall GLC-3 centrifuge to separate mucus and cell debris, and stored at −20°C.

The bacterial strains used here have been characterized previously (2). Briefly, *S. typhi* 643W expresses the typical 9 and 12 somatic antigens. *S. typhi* 5076-IC is a transconjugant strain which carries a *S. sonnei* form I plasmid and expresses the form I antigen in addition to the 9 and 12 somatic antigens. *S. sonnei* 9774 expresses the form I antigen.

Rabbits were given 4 ml of a 1:10 dilution, in saline, of an overnight Trypticase (BBL Microbiology Systems) broth culture of *S. typhi* 5076-IC in their isolated ileal loops on days 1 and 12 after surgery.

Secretions from the isolated ileal loops shed *S. typhi* 5076-IC cells for a minimum of 2 days and a maximum of 10 days (mean of 6 days) after the first mucosal immunization. After the second intramucosal immunization, administered on day 10, bacteria were shed for a minimum of 2 days and a maximum of 8 days (mean of 5 days). No relationship was found between duration of shedding and the intensity of the local immune response.

A previously described enzyme-linked immunosorbent assay for detecting IgG and IgA antibodies to bacterial products was used to detect specific antibody activity in intestinal loop secretions (8). Standard solutions with IgG and IgA activity against *S. typhi* 643W, *S. typhi* 5076-IC, and *S. sonnei* 9774 were prepared as previously described for *Shigella flexneri* (8). Results of standards were normalized to a fixed number, and the values of unknown specimens were corrected to these normalized standards. Results were expressed as optical density at 405 nm per 100 min.

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TABLE 1. Specificity of enzyme-linked immunosorbent assay for bacterial strain LPS

Antisera[a] raised to:	Specificity[b] for bacterial strains[c]:		
	S. typhi 643W	*S. sonnei* 9774	*S. typhi* 5076-IC
S. typhi 643W	4.366	0	3.906
S. sonnei 9774	0	0.696	1.824
S. typhi 5076-IC	1.908	0.901	3.882

[a] Diluted 1:1000 in phosphate-buffered saline with Tween 20.

[b] Results expressed as the change in optical density of IgG at 405 nm per 100 min.

[c] LPS from these bacteria used to coat plates for enzyme-linked immunosorbent assay.

To determine whether cross-reactivity would occur, antisera raised against *S. typhi* 643W, *S. typhi* 5076-IC, and *S. sonnei* 9774 (2) were reacted with wells coated with Westphal lipopolysaccharide (LPS) preparations from each strain. Antisera raised against *S. typhi* 643W reacted with the immunizing strain and with the *S. typhi* 5076-IC transconjugant (Table 1). Antisera raised against *S. sonnei* 9774 also reacted with the immunizing strain and the *S. typhi* 5076-IC transconjugant. Finally, antisera raised against *S. typhi* 5076-IC transconjugant reacted with all three bacteria. These data indicate that the *S. sonnei* form I antigen is expressed by *S. typhi* 5076-IC.

After the first intraloop dose of *S. typhi* 5076-IC, IgA responses were detectable by day 6 post-immunization (Table 2). Only a weak IgA response was found to the *S. sonnei* 9774 LPS antigen at this time. These responses declined after a peak on day 8.

After a booster intraloop dose of *S. typhi* 5076-IC on day 10, vigorous IgA responses were found to this microorganism and to *S. typhi* 643W from day 14 through the end of the study (day 24). An increase was also found in the IgA response directed against the plasmid-encoded *S. sonnei* form I antigen, as compared with that observed after the first dose of the antigen (Table 2).

No local IgG antibodies were found with activity against any of the above microorganisms.

A vigorous IgA response in the absence of IgG activity against mucosally administered *S. typhi* 5076-IC is consistent with our previous findings against mucosally administered shigella (11, 12). The lack of specific IgG in the secretions is probably not due to rapid intraluminal degradation (14).

A booster effect was seen in the activity of IgA after the second intraluminal administration of antigen on day 10. We have previously seen such a booster effect in this model system with shigella antigens (9). Furthermore, with live *Shigella* X16 (a genetic hybrid of *S. flexneri* and *Escherichia coli*) three weekly oral doses were able to prime for a mucosal IgA anamnestic response lasting as long as 60 days after the last oral dose of antigen (13).

The IgA response to the *S. sonnei* 9774 LPS antigen was weaker than the IgA responses against the salmonella strains. This was true both in the local IgA responses shown in Table 2 and in the serum IgG responses listed in Table 1. This may reflect the density of the shigella antigen expressed on the surface of both *S. sonnei* 9774 and the transconjugant *S. typhi* 5076-IC. Whether it is due to the density of antigen or to the ability of this antigen to elicit a

TABLE 2. Local IgA response after mucosal immunization with transconjugant strain *S. typhi* 5076-IC

Day[a]	*n*[b]	Local IgA response[c]		
		S. typhi 5076-IC	*S. typhi 643W*	*S. sonnei 9774*
0	6	0.017 ± 0.008	0.122 ± 0.052	0.065 ± 0.024
2	6	0.039 ± 0.013	0.033 ± 0.011	0.091 ± 0.014
4	6	0.081 ± 0.022	0.077 ± 0.027	0.083 ± 0.011
6	6	0.277 ± 0.123	0.308 ± 0.154	0.133 ± 0.033
8	6	0.544 ± 0.218	0.530 ± 0.315	0.163 ± 0.013
10	6	0.310 ± 0.100	0.362 ± 0.189	0.109 ± 0.038
12	6	0.207 ± 0.100	0.150 ± 0.035	0.125 ± 0.018
14	6	1.004 ± 0.203	1.343 ± 0.614	0.174 ± 0.049
16	6	2.797 ± 1.040	2.679 ± 1.011	0.347 ± 0.121
18	5	1.988 ± 1.063	1.470 ± 0.784	0.213 ± 0.076
20	5	1.165 ± 0.453	1.371 ± 0.630	0.324 ± 0.143
22	4	1.078 ± 0.392	1.253 ± 0.613	0.225 ± 0.076
24	4	0.892 ± 0.348	1.189 ± 0.669	0.113 ± 0.036

[a] Day after initial mucosal stimulation with strain 5076-IC. All animals were stimulated on days 1 and 10 after surgery.

[b] *n*, Number of individual assays.

[c] Results expressed as the change in optical density of IgA at 405 nm per 100 min ± standard error of the mean.

vigorous immune response is unclear at the present time.

Live shigella vaccines administered orally are effective in eliciting protection in both experimental animals and humans. However, most attenuated shigella vaccine strains can revert to virulence. The present study indicates that the genetically stable transconjugant *S. typhi* 5076-IC is effective in stimulating the local IgA response to both the parent salmonella and the plasmid-borne *S. sonnei* form I antigens. Therefore, this bivalent oral vaccine offers a means to elicit local immunity to both intestinal pathogens by peroral immunization.

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