Variability of Protection Against Different Strains Induced by a Culture Filtrate (CF) Obtained from *Salmonella enteritidis*

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Key words: CF of Salmonella enteritidis, Cross-protective immunity, Antibody titers, Delayed footpad test

Abstract

Cross-protective ability against the challenge of different strains of culture filtrate (CF) obtained from *Salmonella enteritidis* was investigated. Immunization with CF from various smooth strains with the exception of strain 3551 which is a rough strain of *S. enteritidis* induced protective immunity against challenge with strains 2547 and 116M. However, when strains 2822, 3551 and 3975 were used as the challenge strain, no protective effect was observed. The agglutination titers against whole cells of strain 2547 or 2822 of sera taken from the 2547 CF-immunized mice were almost the same as those of sera from the 2822 CF-immunized mice. Furthermore, delayed footpad reactivity was observed when heat-killed cells of strain 2547 or 2822 were transferred into the footpad of 2547 CF- or 2822 CF-immunized mice. These results show that the capability of inducing protective immunity is similar between the 2547 CF and 2822 CF, and protection against infection with *S. enteritidis* differs in accordance with different challenge strains.

Introduction

Mouse infections with *Salmonella typhimurium* and *S. enteritidis* have been studied as a model of *S. typhi* infections in humans. It is known that living vaccines induce a cellular immune response, and are more effective than non-viable vaccines for the induction of immunity to *Salmonella* infections¹⁾²⁾. However, there are many reports showing that non-viable vaccines give excellent levels of protection as compared with living vaccines^{3)~5)}.

Earlier studies in this laboratory have shown that immunization with Soluble Protective Antigen (SPA) separated from the culture fluids of *S. enteritidis* strain 2547 induced high levels of resistance against the challenge of 10,000 LD₅₀ of the same organism strain⁶⁾. The protective capacity of SPA has been attributed to the ability to elicit both a cellular and humoral immune response in the host⁷⁾⁸⁾. In previous studies, chemical and biological analyses of SPA indicated that SPA contains a lipid A moiety, and possesses the properties of lipopolysaccharides (LPS) isolated from *S. enteritidis* by chemical procedures⁹⁾¹⁰⁾. However, the LPS from *S. enteritidis* strain 2547 afforded only 80% protective immunity against the challenge of 100 LD₅₀ of the homologous bacteria. These results suggested that SPA differs markedly in physiochemical capabilities from the LPS isolated by chemical procedures, although it is certainly part of the LPS released into the liquid environment by solubilization of bacterial cell wall material.

In the present study, cross-protection tests against the challenge of different strains of CF from *S. enteritidis* were conducted to determine whether protective antigen (SPA) is released only from strain 2547 into the culture fluid. Further comparisons were made concerning the ability of inducing protective

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immunity of CF from *S. enteritidis* strains 2547 and 2822.

Materials and Methods

Animals

Female mice of ddy strain were purchased from the Shizuoka Laboratory Animal Center, Hamamatsu, Japan. Mice were used at the age of 7–8 weeks.

Bacterial strains

S. enteritidis strains 2547, 2822, 3551, 3975, 4129 and 116M were supplied by Niigata and Mie Prefectural Institute of Public Health, respectively. Strains 183Fu and SR144G were obtained from Fukui and Gifu Prefectural Institute of Public Health, respectively. All strains were passaged once in ddy mice before use in this study. Fresh isolates were obtained from the spleen on day 2 postinoculation. *S. enteritidis* was cultured in liver and beef papain digestion agar medium at 37°C for 18 hr.

Preparation of CF

Culture filtrate (CF) was prepared as described in our previous paper⁶⁾. Bacterial strains were inoculated on a synthetic TSA medium without protein, cultured at 37°C for 4 days, and the culture medium was filter-sterilized by a Seiz EK filter.

Determination of cross-protective immunity

Groups of five mice were immunized intraperitoneally (i.p.) with 0.5 ml of CF obtained from each strain, and challenged i.p. with various doses of each strain in 0.5 ml of 5% (W/V) mucin 14 days after immunization. The protective ability was demonstrated by differences between the 50% lethal dose (LD₅₀) of control mice and the LD₅₀ of immunized mice.

Measurement of antibody titers

Two groups of mice were immunized i.p. with 0.5 ml of 2547 CF or 2822 CF. These mice were sacrificed 14 days following immunization and the serum separated. Whole-cell agglutination (WCA) titers in serum were determined by a microagglutination test as reported by Eisenstein *et al*¹¹⁾. Heat-killed cells of strain 2547 or 2822 prepared by heating at 70°C for 60 min were used as antigens.

Delayed footpad test

Two groups of mice were immunized i.p. with 0.5 ml of 2547 CF or 2822 CF, and tested 14 days following immunization for the presence of delayed-type hypersensitivity (DTH) measured by footpad swelling. Heat-killed cells of strain 2547 or 2822 were used as antigens for eliciting the footpad response. The eliciting antigens (7×10^5 colony-forming units) suspended in 0.05 ml saline were injected into right hind footpad, while saline was injected into the left hind footpad as a control. The thickness of the footpad was measured with dial-gauge calipers (KSM-150, KANON, Japan) just before injection of antigen and at 24 hr following injection. The thickness of the saline-injected footpad was subtracted from that of the antigen-injected footpad to yield the difference in footpad thickness for each mouse. DTH in nonimmunized mice was analyzed in a similar manner.

Results

Cross-protective immunity. CF obtained from various strains was tested for its ability to protect mice against challenge using different strains of *S. enteritidis* (Table 1). When strain 2547 or 116M were used as the challenge strain, protective effects were apparent in groups of mice immunized with CF from various smooth strains with the exception of strain 3551 (a rough strain of *S. enteritidis*). No protective effect by CF was observed with the challenge of strains 2822, 3551 and 3975.

Antibody response. Sera was collected from mice immunized 14 days previously with 2547 CF or 2822 CF, and the antibody titer to whole cells of strains 2547 and 2822 was determined. Table 2 indicates that 2547 CF and 2822 CF gave very similar agglutination titers against the whole cells of strain 2547 or 2822.

Table 1 Cross-protection test for immunization of culture filtrate (CF) obtained from *S. enteritidis*

Challenge strain	Protective ability ^{a)} of <i>S. enteritidis</i> CF immunization							
	2547	116M	2822	3551	3975	4129	183Fu	SR144G
2547	>2.8	>2.8	>2.8	1.4	>2.8	>2.4	>2.6	>1.8
116M	>1.6	>2.2	>2.6	0.6	>2.4	>2.6	>2.2	1.6
2822	0.8	<0.4	0.6	0.8	0.8	1.0	0.8	0.8
3551	0.8	0.8	1.0	0.4	1.0	0.8	0.2	0.2
3975	0	0.6	0.6	0	0.4	0.4	>1.0	0.2

a) Protective ability was demonstrated by differences between the LD₅₀ of control mice and the LD₅₀ of immunized mice, (LD₅₀ of immunized mice=10ⁿ¹mg, LD₅₀ of control mice=10ⁿ²mg, Protective ability=n1-n2)

Table 2 Agglutination of immunized mice sera by culture filtrate obtained from *S. enteritidis* 2547 or 2822

Antigen		Dilution of serum								cont. ^(c)
		1	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁶		
Heat-killed <i>S. enteritidis</i> 2547	Anti-2547 CF serum ^{a)}	+	+	+	±	—	—	—	—	
	Anti-2822 CF serum ^{b)}	+	+	+	+	±	—	—	—	
Heat-killed <i>S. enteritidis</i> 2822	Anti-2547 CF serum	+	+	+	±	—	—	—	—	
	Anti-2822 CF serum	+	+	+	±	—	—	—	—	

a) b) 2547 or 2822 CF: Culture filtrate obtained from *S. enteritidis* 2547 or 2822. Mice sera were obtained 14 days after i.p. injection of 2547 CF or 2822 CF.

c) Cont.: Phosphate buffered saline

Table 3 Delayed footpad reaction in mice after immunization with culture filtrate obtained from *S. enteritidis* 2547 or 2822

Antigen	Delayed footpad reaction (×0.1mm)		
	Mice immunized with 2547 CF ^{a)}	Mice immunized with 2822 CF ^{a)}	Nonimmunized control mice
None	-0.10±0.60	1.29±0.38	-0.13±0.48
Heat-killed <i>S. enteritidis</i> 2547	4.25±0.25	4.09±0.57	1.92±0.68
Heat-killed <i>S. enteritidis</i> 2822	4.05±0.75	3.88±0.64	1.05±0.34

a) 2547 or 2822 CF: Culture filtrate obtained from *S. enteritidis* 2547 or 2822. Mice were immunized i.p. with 2547 CF or 2822 CF, and injected into the footpad 14 days later with each antigen. Delayed footpad reaction was measured 24 hr after elicitation with each antigen.

Mean±SEM

Delayed footpad reaction of 2547 CF or 2822 CF-immunized mice. Mice were immunized with 2547 CF or 2822 CF, and the delayed footpad reaction was measured (Table 3). When heat-killed cells of strain 2547 or 2822 were used as eliciting antigens, the DTH response was observed. The capacity of inducing the DTH response was similar between the 2547 CF and 2822 CF.

Discussion

There are many reports showing that non-viable vaccines have the capacity to protect mice against a

lethal *Salmonella* challenge³⁾⁻⁵⁾. Plant *et al.*¹²⁾ reported that a supernatant factor prepared from cultures of *S. typhimurium* protects mice against a subcutaneous challenge dose of 100 LD₅₀ of *S. typhimurium*. In this laboratory, it has been shown that SPA separated from the culture fluids of *S. enteritidis* strain 2547 protects effectively against a lethal challenge of the same organism strain⁶⁾. In previous studies, chemical and biological analyses of SPA revealed the presence of LPS⁹⁾⁻¹⁰⁾. Passive transfer of anti-SPA mouse serum was shown to protect mice against infection with *S. enteritidis*, whereas the protective ability was completely lost following absorption of the anti-SPA mouse serum with LPS extracted from *S. enteritidis*. In contrast to these results, SPA alone could induce significant protection against the challenge of 10,000 LD₅₀ of *S. enteritidis*, yet LPS isolated from *S. enteritidis* strain 2547 by chemical procedures afforded only 80% protective immunity against the challenge of 100 LD₅₀ of the homologous bacteria. These results suggested that LPS may be an essential component for the induction of protective immunity with SPA, however SPA appeared to differ markedly in physiochemical capabilities from the LPS isolated by chemical procedures.

In this study, cross-protective ability against the challenge of different strains of CF obtained from *S. enteritidis* was investigated. As shown in the results, when strain 2547 or 116M were used as the challenge strain, protective effects were observed in groups of mice immunized with CF from various smooth strains with the exception of strain 3551 which is a rough strain of *S. enteritidis* (Table 1). No protective effect was observed with the challenge of strains 2822, 3551 and 3975. It was found in this study that the protective antigen (SPA) separated from culture fluids of strain 2547 is liberated into the culture fluid from various smooth strains of *S. enteritidis* by solubilization. Additionally, comparisons were made of the ability of inducing protective immunity of CF from *S. enteritidis* 2547 and 2822. The CF from strain 2547 or 2822 were able to induce delayed hypersensitivity, which is regarded as a convenient indicator of cell-mediated immunity (Table 3). The degree of delayed footpad reactivity induced by the CF and 2822 CF appeared to be almost identical. Furthermore, the agglutination titers against whole cells of strain 2547 or 2822 of sera taken from the 2547 CF-immunized mice were almost the same as those of sera from the 2822 CF-immunized mice. These results suggest that the ability to induce protective immunity is very similar between the CF from various smooth strains of *S. enteritidis*.

The conclusion obtained from this study is that protection against infection with *S. enteritidis* will vary in accordance with different challenge strains.

Acknowledgments

Thanks are due to Dr T. Nikai for very helpful discussions, and to Miss Y. Nakamura for her assistance in the experimental work.

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Salmonella enteritidis から得られた培養濾液によって

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(平成2年5月17日受付)

(平成2年6月27日受理)

S. enteritidis から得られた培養濾液 (Culture filtrate: 以下 CF と略す) のあらゆる菌株の攻撃に対する交叉防御能力を調べた結果, rough strain である 3551 株以外, 用いたすべての smooth strain からの CF で免疫する事によって 2547, 116M 株の攻撃に対して感染防御が成立した。一方, 2822, 3551, 3975 株での攻撃に対しては, 防御効果はまったく得られなかった。そこで, 2547 株の CF で免疫したマウスから得られた血清の 2547, 2822 株の熱処理死菌体に対する凝集素価

を調べた結果, 2822 株の CF で免疫したマウスから得られた血清のそれとほとんど同じ値を示した。さらに, 2547 あるいは 2822 株の CF で免疫したマウスに対する遅延型足蹠反応を調べた結果, 誘出抗原としての 2547, 2822 株の熱処理死菌体に対して両方共において反応が見られ, 又, その値にほとんど差はなかった。これらの結果より, 防御免疫を誘導する能力は 2547, 2822 株の CF では違いがなく, *S. enteritidis* 感染に対する防御は, 攻撃菌株の種類によって異なるということが判った。