Prevention of Experimental Pseudotuberculosis Infection by Immunization with Porin from *Yersinia pseudotuberculosis*

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Protective properties of two molecular forms of porin, a protein from the outer membrane of *Yersinia pseudotuberculosis,* are studied in animals infected with pseudotuberculosis and plague agents. The effect of adjuvant on the protective activity of porin is investigated. The possibility of using porin as a component of chemical antipseudotuberculosis vaccine is demonstrated.

Key Words: *porin; protective activity; pseudotuberculosis*

The outer membrane of gram-negative pathogens plays an important role in the parasite-host relationships. Membrane components participate in adhesion and invasion of bacterial cells, protection from humoral and cellular defense of the host, and exert toxic effect [8,9].

Recent studies demonstrated protective properties of the outer membrane proteins of gram-negative bacteria associated with peptidoglycan (PG). Porins as immunogenic components of chemical vaccines are used for preventing infectious diseases caused by *Pseudomonas aeruginosa, Neisseria meningitidis, Neisseria gonorrhoeae, Salmonella typhimurium, Proteus mirabilis,* and *Shigella.* Porin-based vaccines possess numerous advantages over vaccines containing other cellular immunogens. The immune response to lipopolysaccharide, flagella, and other surface components of the outer bacterial membrane is type- or strain-specific, while porins induce the production of species-specific antibodies. Moreover, these proteins are nontoxic and therefore preferable for vaccinations [9,10].

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The development of specific preventive agents against pseudotuberculosis (extraintestinal yersiniosis) caused by *Yersinia pseudotuberculosis* is an important trend in research of this disease [7,8]. We investigated the protective properties of two molecular forms of porin, an outer membrane protein of *Y. pseudotuberculosis* (yersinin), in albino mice and guinea pigs infected with *Y. pseudotuberculosis* and *Y. pestis.*

MATERIALS AND METHODS

Thermolabile (TL) protein was obtained by extracting the complex with 0.5 N NaCl at 37°C in the presence of 1% sodium dodecyl sulfate (SDS) and thermostable (TS) protein by dissociation of the PG-protein complex at 100°C in the presence of 2% SDS [5].

Noninbred albino mice and guinea pigs weighing 18-20 and 350-400 g, respectively, were used. The animals were immunized with yersinin twice at 14-day intervals in doses of 12.5 and 25 μg. The protein was dissolved in physiological saline and injected subcutaneously to mice (0.2 ml) and guinea pigs (0.5 ml). In another series, guinea pigs were immunized with TL and TS yersinin in a single dose of 25 μg subcutaneously with incomplete Freund's adjuvant.

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For elucidating the protective activity of yersinin preparations, the mice and guinea pigs were infected with *Y. pseudotuberculosis*, serovar 1 (strain 147) or serovar 3 (strain 326) and *Y. pestis* (strain 1300). The strains were obtained from the collection of bacterial cultures of Institute of Microbiology. Both strains of *Y. pseudotuberculosis* contained virulence plasmid with a molecular weight of 47 MD. *Y. pestis* possessed plasmids typical of this bacterium: pFra/Tox (61 MD), pCad (43 MD), and pPst (6 MD). *Y. pseudotuberculosis* cultures were grown at 4°C for 5 days in Hottinger's agar and *Y. pestis* at 27°C for 2 days in the same medium.

Lethal doses and confidence intervals were calculated with a probability of 95% [1,2]. The resistance index was determined as the ratio of LD_{50} for immunized and intact animals.

RESULTS

Yersinin is the dominating outer membrane protein (36.7 kD) of *Y. pseudotuberculosis* strain 512, serovar 1 [5]. This protein belongs to thermodependent pore-forming PG-associated proteins [4]. Previously using different methods of dissociation of the PG-protein complex, we isolated three molecular forms of yersinin: oligomer (trimer), monomer, and denatured monomer with apparent molecular weights of 105-120, 33.5, and 40 kD, respectively [5]. Oligomer and monomer are thermolabile: 5-min heating at >70°C in SDS yields a denatured monomer (40 kD). Unlike trimer, two immunizations with TS monomer yersinin protected 82.1-92.3% animals from *Y. pseudotuberculosis* infection (serovars 1 and 3), in doses corresponding to 10-30 LD_{50} [10] (intraperitoneal inoculation). We compared the protective effects of 2 yersinin monomers (TL and TS).

The animals immunized with TS and TL proteins were divided into 2 groups. Twenty-one days after booster immunization, group 1 animals were infected subcutaneously with *Y. pseudotuberculosis* in doses of 10^2, 5×10^2, 2.5×10^3, 1.25×10^5, and 6.25×10^5 live bacterial cells, and group 2 animals were infected with *Y. pestis* in doses 1, 5, 25, 1.25×10^2, and 6.25×10^2 live bacterial cells.

Repeated immunization with yersinin (TS and TL forms) induced the formation of specific immunity (Table 1).

Repeated immunizations with 12.5 μg TL yersinin provided a considerably higher resistance index than immunization with the same dose of TS yersinin (3-fold for *Y. pseudotuberculosis* serovar 1 and 2-fold for serovar 3).

TABLE 1. Protective Activity of Yersinin Preparations towards *Y. pseudotuberculosis* in Subcutaneous Infection

Group of animals	Estimated $LD_{50}^{x}:K_{95}$, live bacteria	
	albino mice	guinea pigs
Strain 147, serovar 1		
Control	523^{x}:1.4 (1)	700^{x}:1.5 (1)
Repeated immunization with:		
TS yersinin	33394^{x}:1.8 (63.9)	1824^{x}:1.4 (2.6)
TL yersinin	93407^{x}:1.5 (178.6)	291^{x}:1.7 (0.42)
Strain 326, serovar 3		
Control	10062^{x}:1.4 (1)	4188^{x}:1.5 (1)
Repeated immunization with:		
TS yersinin	157233^{x}:1.6 (15.6)	149.72^{x}:1.6 (3.6)
TL yersinin	322100^{x}:1.3 (32)	366^{x}:1.4 (0.08)

Note. Here and in Table 2: the resistance index is given in parentheses.

TABLE 2. Protective Activity of Yersinin Preparations towards *Yersinia pestis* (Strain 1300) in Subcutaneous Infection

Group of animals	Estimated $LD_{50}^{x}:K_{95}$, live bacteria	
	albino mice	guinea pigs
Control	7^{x}:1.2 (1)	8^{x}:1.5 (1)
Repeated immunization with:		
TS yersinin	9^{x}:1.5 (1.1)	7^{x}:1.3 (0.8)
TL yersinin	8^{x}:1.3 (1.1)	6^{x}:1.4 (0.7)

Guinea pigs infected with TL yersinin did not develop specific immunity to pseudotuberculosis. Moreover, they became more susceptible to *Y. pseudotuberculosis* serovar 3, probably due to sensitization. Nevertheless, repeated immunization of guinea pigs with TS yersinin significantly increased the LD_{50} in comparison with the control: 2.6 and 3.6 times for provoking inoculation with *Y. pseudotuberculosis* serovars 1 and 3, respectively.

Incomplete Freund's adjuvant sharply enhanced the protective activity of TS yersinin in guinea pigs. Isolated porin protected the animals from 10-50 LD_{50} [9], while the adjuvant potentiated this protective effect 50-150 times.

Y. pseudotuberculosis porin is a genus-specific *Yersinia* antigen [3], and therefore, we tested the protective effect of yersinin towards another representative of this genus, *Y. pestis*. However, repeated immunization of albino mice and guinea pigs with yersinin did not protect them from infection with the plague agent (Table 2).

Therefore, porin, an outer membrane protein of *Y. pseudotuberculosis*, induced the formation of specific immunity in animals, and therefore it can be used as a component of chemical vaccine.

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