Immunogenic Characteristics of Recombinant OMPF-Like Porin from *Yersinia Pseudotuberculosis* Outer Membrane

O. Yu. Portnyagina, O. V. Sidorova, V. A. Khomenko, O. D. Novikova, O. P. Vostrikova, and T. F. Solovjeva

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We studied the capacity of outer membrane pore-forming recombinant protein from *Yersinia pseudotuberculosis* to initiate the development of immune response in CBA mice. Immunization with the recombinant protein induces the production of IgG antibodies with and without adjuvants. High-avidity immune serum was obtained as a result of immunization. Bactericidal activity of peritoneal macrophages from mice immunized with recombinant protein was significantly higher than that of intact mouse macrophages. The use of recombinant porin instead of native porin as the antigen in enzyme immunoassay system for the diagnosis of acute and secondary focal pseudotuberculosis does not reduce the efficiency of detection of specific antibodies in the sera of patients.

Key Words: *Yersinia pseudotuberculosis; recombinant porin; immunization; diagnosis*

Nonspecific porins forming channels for diffusion of low-molecular compounds through the membrane are the main proteins of the outer membrane (OM) in gram-negative bacteria [5]. Porins from OM of pathogenic bacterium are pathogenesis effectors, suppress the defense reactions, and act as targets for the host immune cells. Antibodies to porins are detected in the serum after vaccination and in natural development of the infection [5].

We studied the immunogenic characteristics of the monomeric form of *Y. pseudotuberculosis* OM recombinant porin.

MATERIALS AND METHODS

Recombinant porin from OM of *Y. pseudotuberculosis* was obtained by transformation of *E. coli* BL21 (DE3) cells with recombinant plasmid containing *ompF* gene fragment [6]. Since this gene fragment contained no signal sequence, the recombinant protein (RP) accumulated in *E. coli* cells as inclusion bodies and was isolated by urea extraction, purified, and renatured by chromatography on Sephacryl S-300 [3]. Recombinant protein was injected intraperitoneally to mice of four groups (CBA females, 20±2 g) in a dose of 100 μg 3 times at 7-day intervals. Group 1 animals were immunized with RP in saline, group 2 were injected with RP in mixture with Freund's complete adjuvant (1:1, v/v), group 3 animals received RP in 0.05% solution of octyl-β,D-glucopyranoside, and group 4 animals were injected with RP in 0.1% Zwittergent's solution 3-14. Rabbit antibodies to mouse IgG labeled with horseradish peroxidase (N. F. Gamaleya Institute of Epidemiology and Microbiology) served as antispecies antibodies in EIA.

The avidity index characterizing the capacity of a heterogeneous mixture of antibodies to bind to the antigen and depending on the strength of antigen/antibody complex was evaluated in EIA [7] with 6 M urea (AppliChem) as the reagent for dissociation of low-avidity antibodies.

Pacific Institute of Bioorganic Chemistry, Far Eastern Division of the Russian Academy of Sciences, Vladivostok, Russia. ***Address for correspondence:*** odd64@mail.ru. O. Yu. Portnyagina

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Peritoneal exudation cells were isolated by washing the mouse peritoneal cavity with 5 ml cold balanced Hanks' saline with 2 µl heparin. In order to evaluate the effect of RP on macrophages *in vitro*, the cells were preincubated for 1 h with RP in different concentrations, after which myeloperoxidase level was evaluated. The induced production of myeloperoxidase was detected using heat-inactivated *Y. pseudotuberculosis* cells (strain 512, serovar IB). Stimulation index was calculated as the ratio of induced to spontaneous peroxidase production.

RESULTS

Sera specific to RP were obtained by immunization of 4 groups of CBA mice. Antibody titers of immune sera were evaluated by EIA (Table 1). The sera with the highest titers were obtained by immunization of group 2 animals. The level of specific antibodies obtained by immunization was minimum in group 3.

The avidity index indicated high specificity of antibodies obtained by immunization with RP alone, RP with detergents, RP with adjuvant (Table 1). Low level of antibodies to RP in the sera of group 3 mice was virtually inessential for avidity of these antibodies.

The use of RP or synthetic peptides as components of diagnostic test systems improves their sensitivity and rules out hyperdiagnosis. We therefore studied the possibility of using RP as the antigen for the diagnosis of enteric and secondary focal pseudotuberculosis forms. Sera from patients with acute enteric pseudotuberculosis were analyzed using EIA test system. Antibodies to *Y. pseudotuberculosis* recombinant porin were detected in these sera by recombinant protein diluted 1:800; in other words, RP exhibited activity comparable to that of thermostable denatured porin

TABLE 1. Antibody Titers and Avidity Indexes of Antisera Obtained by Immunization of Mice with Different RP Samples

Group	Antibody titer, -lg	Avidity index, %
1	2.5	85
2	3.55	89
3	1.9	79
4	2.8	82

monomer isolated from *Y. pseudotuberculosis* OM traditionally used for diagnostic studies (Fig. 1, *a*). Analysis of sera from patients with symptoms of secondary focal pseudotuberculosis [4] revealed specific antibodies to *Y. pseudotuberculosis* porin in some sera from patients with locomotor involvement of different kind and with diseases of the peripheral nervous system. Recombinant protein detected specific antibodies 1.3 times more effectively than porin isolated from *Y. pseudotuberculosis* OM (Fig. 1, *b*). Presumably, higher efficiency of RP as the antigen for detection of specific antibodies can be explained by restoration of some native antigenic epitopes as a result of its refolding. These epitopes correspond to the porin conformation determinants. They are intrinsic of the tertiary structure of the protein, which is lost during thermodenaturing in the thermostable porin monomer [2].

Functional activity of mononuclear phagocytes is an important indicator of the host immune system status. Macrophage activation is paralleled by the production of highly active unstable products of oxygen reduction, including H_2O_2 serving as the main substrate for myeloperoxidase (an important component of phagocyte antibacterial system). Myeloperoxidase synthesis is more intensive in phagocytes of mice im-

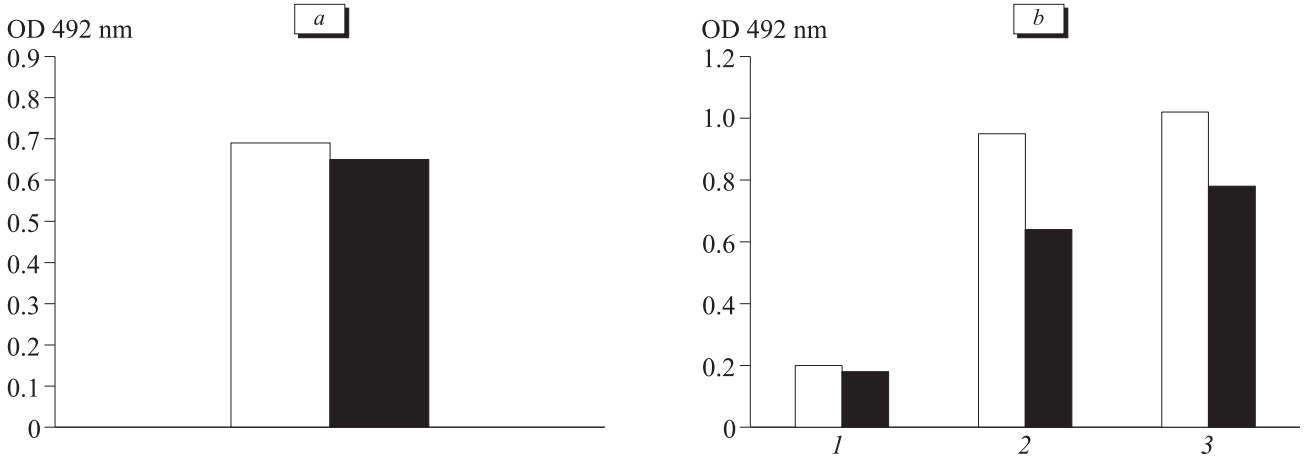

Fig. 1. Detection of specific antibodies to RP antigens (light bars) and thermostable denatured porin monomer (dark bars) in EIA with sera from patients with acute enteric pseudotuberculosis (*a*) and secondary focal pseudotuberculosis forms (*b*). *1*) donor; *2*) patient with symptoms of peripheral nervous system involvement; *3*) patient with symptoms of locomotor system involvement.

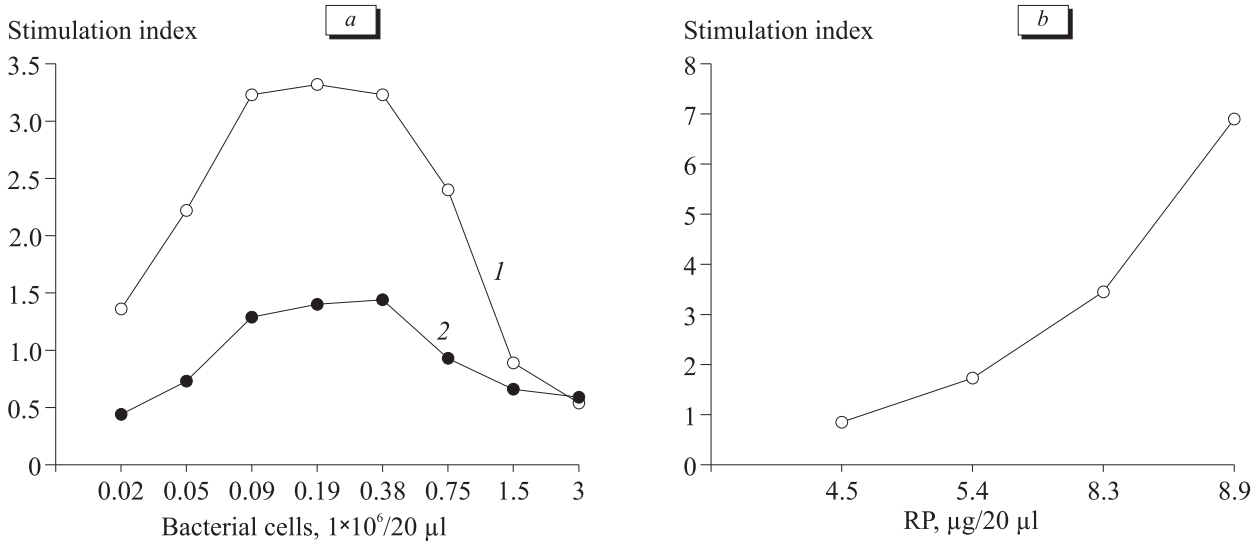

Fig. 2. Myeloperoxidase synthesis in macrophages (*a*) and RP effect on myeloperoxidase synthesis in macrophages of intact mice (*b*). *1*) immune mice; *2*) nonimmune mice.

munized with RP (Fig. 2, *a*); a similar increase of the enzyme synthesis is observed in intact animal phagocytes after preincubation of these cells with RP (Fig. 2, *b*). Since the increase in myeloperoxidase content indirectly characterizes complete phagocytosis, we can say with good grounds that RP stimulates macrophages by increasing their bactericidal activity.

Hence, *Y. pseudotuberculosis* OM RP initiates the development of humoral immune response and stimulates host immediate defense factors by stimulating oxygen-dependent bactericidal activity of macrophages. The possibility of using RP as the diagnostic antigen for detection of various forms of pseudotuberculosis is demonstrated.

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