Dynamics of Immune Response to Porine from *Yersinia pseudotuberculosis* Outer Membrane

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 10, pp. 437-440, October, 1999
Original article submitted March 1, 1999

The dynamics of immune response to pore-forming protein isolated from the outer membrane of *Yersinia pseudotuberculosis* was studied on CBA and BALB/c mice. Experiments revealed a waveform curves of antibody levels to different porin forms reflecting the successions of immunoglobulin classes and superposition of independent responses to various antigen determinants. The dynamic of immune response to porin depends on the molecular form and dose of the antigen.

Key Words: *porins of outer membrane of gram-negative bacteria; immune response; dynamics of antibody level*

The dynamics of humoral response to protein and corpuscular antigens is well studied [12]. Normally, changes in antibody level are described by a curve consisting of segments corresponding to logarithmic increase, plateau, and gradual decrease of specific immunoglobuline (Ig) content. This response starts with IgM production followed by IgG synthesis. High level of antibodies may persist for weeks or months after immunization depending on the nature and dose of the antigen and on the state of the immune system [11].

Outer membrane of pathogenic gram-negative bacteria plays an important role in host-parasite interrelations. Pore-forming proteins of the outer bacterial membrane attract much attention as antigens inducing the production of protective, bactericidal, and opsonizing antibodies [13,15]. Porins are immunogenic components of chemical vaccines against *Pseudomonas aeruginosa, Neisseria meningitidis, N. gonorrheae, Salmonella typhimurium, Proteus mirabilis,* and some *Shigella* species [5-10].

Earlier, a protein yersinin with molecular weight of 37.6 kD was isolated from the outer membrane of *Y. pseudotuberculosis* [4]. This protein is a thermosensitive pore-forming protein associated with peptidoglycan (PG) [2]. Dissociation of PG-protein complex yeilds 2 molecular forms of yersinin: oligo- (tri-) and monopeptide with apparent molecular weight of 110 and 40 kD, respectively [4]. Oligomer is thermolabile (TL) and can be converted into thermoresistant (TR) yersinin monomer by heating to 70°C during 5 min in the presence of SDS.

We compared the dynamics of immunoglobulin production in CBA and BALB/c mice after immunization with TL and TR yersinin. The study of antibody formation to porin helped to reveal the most effective molecular form of the antigen for new vaccines and diagnostic preparations.

MATERIALS AND METHODS

Porin-containing protein fractions were isolated from the outer membrane of *Y. pseudotuberculosis* as described elsewhere [14]. TL yersinin (tripeptide) was obtained by extraction of PG-protein complex with 0.5 N NaCl at 37°C in the presence of 1% SDS. TR yersinin (monomer) was obtained by dissociation of PG-protein complex at 100°C in the presence of 2% SDS [4].

The study was carried out on 4 groups of BALB/c and CBA mice (5-6 mice in each group) weighing 18-20

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0007-4888/99/0010-1035$22.00

g. The animals were immunized once or twice (on days 1 and 9) subcutaneously into the tail base with various doses of TL and TR yersinin (10 and 100 μg/mouse) with complete Freund's adjuvant. The blood was obtained from the caudal vein every 3 days during 1-2 months.

Indirect enzyme-linked immunosorbent assay was performed using peroxidase-labeled antispecies antibodies against IgG, IgM, and total immunoglobulin fraction. The results were recorded on a Multiscan Plus spectrophotometer at 492 nm using 0.04% phenylenediamine as the chromogen. Protein preparations used for immunization served as the antigens for immunoenzyme assay. The results were estimated by the ratio of optical densities in the wells with specific and normal sera. The response was considered positive if this ratio was equal or exceeded 2.1. Peaks of antibody production were regarded as significant at $p<0.05$.

RESULTS

Immunoenzyme assay showed that maximum optical densities of specific sera of CBA and BALB/c mice were 1.0 and 1.2, respectively. Optical density of the normal serum did not exceed 0.2.

The induction of antibodies to monomeric and oligomeric porins followed a waveform curve and did not depend on the molecular form and dose of the antigen.

It should be noted that CBA mice showed more gradual changes in the antigen level than BALB/c mice (Fig. 1).

Dose dependency of the immune response to both porin forms was observed in both mouse strains, being more pronounced for trimer. In this case higher doses of the antigen not only enhanced immune response, but also changed the pattern of antibody formation (Fig. 1, *a*, *b*). Increasing the dose of monomer did not change the dynamics, but increased the magnitude and duration of immune response. In BALB/c mice the high level of antibodies persisted during 1.5 months after immunization (Fig. 1, *c*, *d*).

Several mechanisms can underlie dose dependency and oscillations of the immune response to porin. One of these mechanisms can be the independent

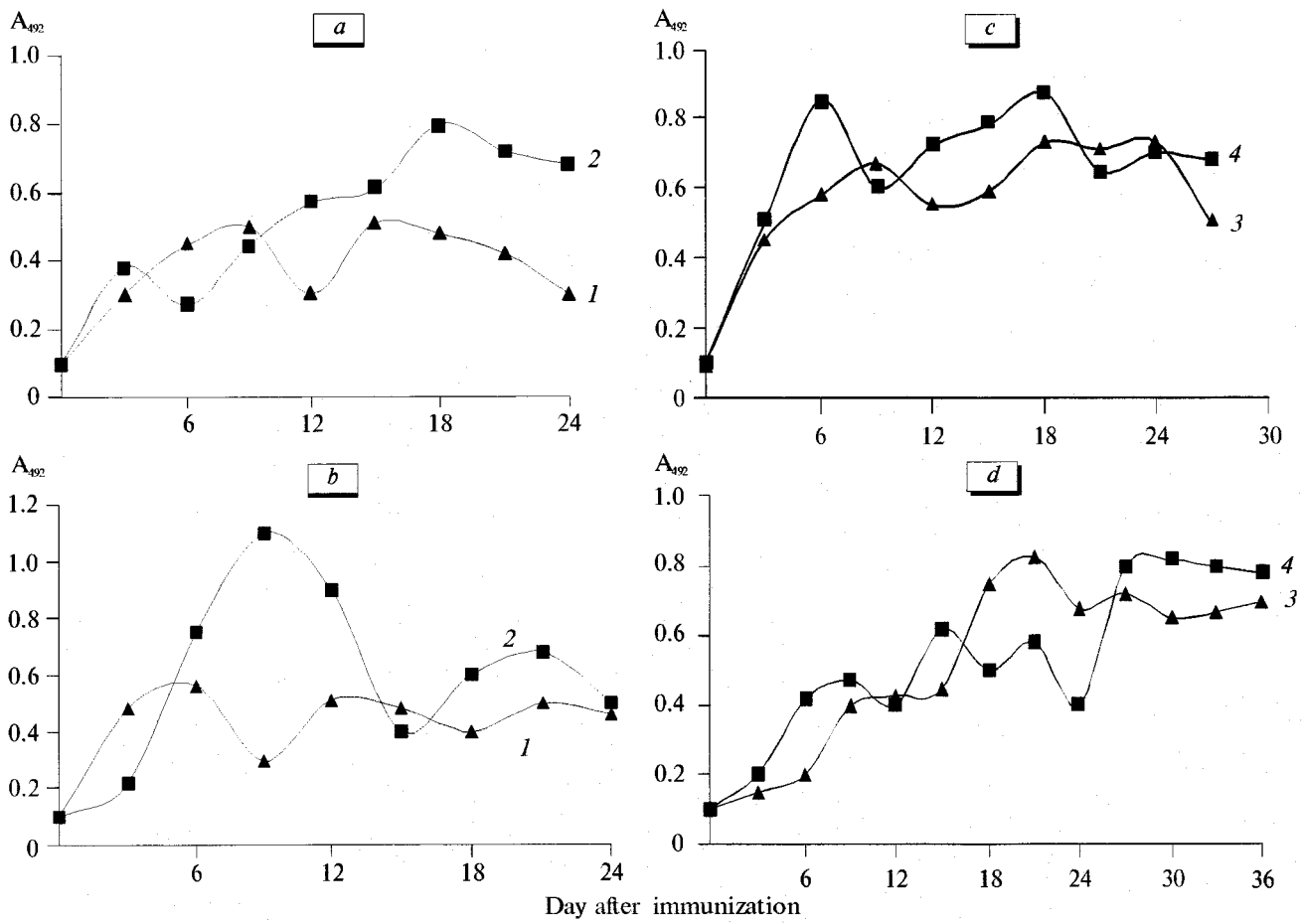

Fig. 1. Dynamics of antibody formation in CBA (*a*, *c*) and BALB/c (*b*, *d*) mice. Immunization with 10 (*1*, *3*) and 100 (*2*, *4*) μg of thermolabile (*a*, *b*) and thermoresistant (*c*, *d*) protein forms.

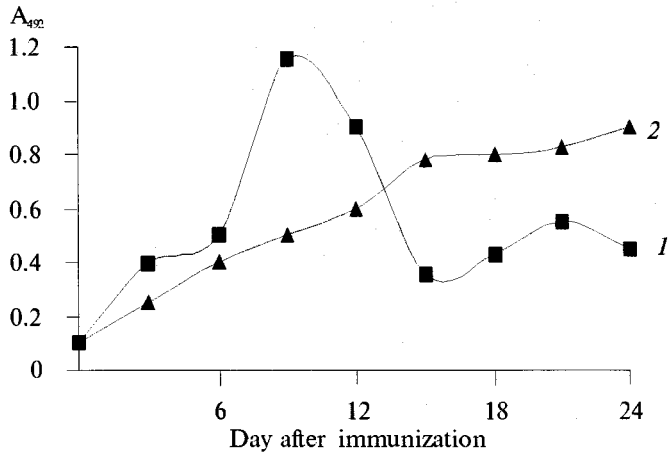

Fig. 2. Dynamics of antibody specificity in BALB/c mice. *1*) reaction of the serum obtained from mice immunized with thermolabile protein (100 μg) with thermolabile protein antigen; *2*) interaction of the same serum with thermoresistant protein antigen.

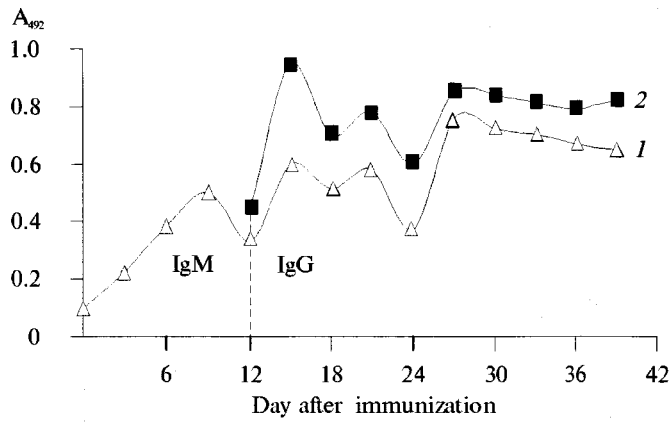

Fig. 3. Dynamics of class-specific immunoglobulin induction in BALB/c mice during single (*1*) or double (*2*) immunization with thermoresistant form of yersinin (100 μg). Second immunization of day 9.

and heterochronous recognition of antigen determinants in tri- and monomeric forms of the protein. For example, during development of the immune response to yersinin, the level of antibodies to oligomer (conformation determinant) decreased, while the level to monomer (linear determinant) increased (Fig. 2). Antibodies to trimer prevailed during the initial 2 weeks and peaked on days 9-10 after immunization.

Quantitative analysis of antibody formation to *Y. pseudotuberculosis* porin showed that only 25-30% antibodies in rabbits immunized with yersinin trimer recognized conformational determinants typical of the trimer [4]. Antibodies to linear determinants determined by primary protein sequence constituted about 65-70% of the total antibody content.

Experiments on BALB/c mice showed that early stages of immune response (before day 12) are characterized by primary IgM production, while IgG are induced at later stages (Fig. 3). Repeated antigen injection induced no IgM production. It should be noted that significant increase in antibody level 3-4 weeks after immunization is induced only by TR antigen.

Antibodies formed in response to protein antigens can induce synthesis of antiidiotypes stimulating the production of antibodies to the antigen [1], thus triggering an autooscillating process: each new wave depends on new cell generation and antibody isotype changes from IgM to IgG and within the IgG class. Thus, the resultant curve of the immune response to yersinin is a superposition of independent responses to different antigen determinants, successions of antibody isotypes, and idiotype-antiidiotype regulation. Switching over IgG isotypes on day 12 after immunization probably determines the oscillations of yersinin-induced antibody level.

Previous studies of the protective effect of different yersinin forms showed higher efficiency of monomer [3]. Our findings also suggest that TR porin is more suitable for vaccine, since it induces higher antibody level and more prolonged and dose-independent immune response as compared to the TL form.

Immunoblotting of the total peptide fraction of *Y. pseudotuberculosis* with blood serum from naturally infected patients showed that antibodies to porin predominate among antibodies to surface antigens of these bacteria (Fig. 4, *a*).

Immunoblotting of the patient sera with purified TL and TR porins revealed qualitative changes in the composition of specific antibodies in the course of

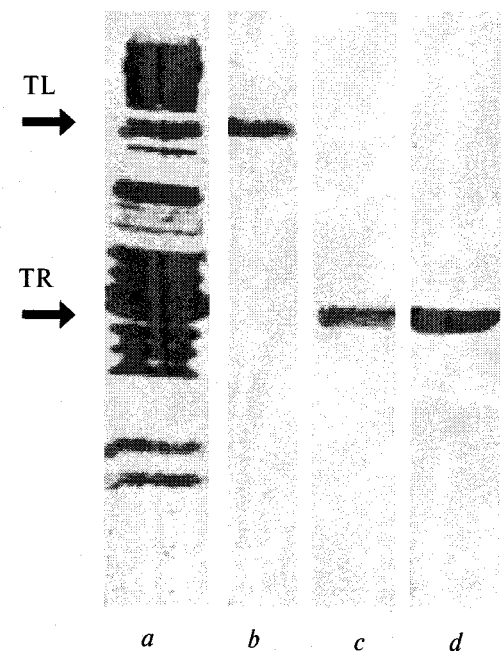

Fig. 4. Immunoblotting of protein fraction of *Yersinia pseudotuberculosis* with sera from patients obtained on days 7 (*a-c*) and 21 (*d*) of the disease. Preparations applied to rows: lysed *Y. pseudotuberculosis* cells (*a*); thermolabile (*b*) and thermoresistant (*c, d*) forms of yersinin.

infectious process. During the first week of the disease, specific antibodies to both yersinin forms were observed (Fig. 4, *b, c*), while during advanced stages only antibodies to monomeric TR form were found (Fig. 4, *d*).

Thus, the analysis of human sera corresponds to the dynamics of immune response to porin after experimental infection of mice. These data confirm the conclusion that monomeric yersinin is the most effective as a diagnostic antigen.

The authors are grateful to M. V. Skok (A. V. Palladin Institute of Biochemistry, Ukrainian Academy of Sciences) for comprehensive assistance in preparation of this material.

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