BIOLOGICAL METHODS FOR CLINICAL USE

Use of Porine from *Yersinia pseudotuberculosis* Outer Coat for Serological Diagnosis of Pseudotuberculosis

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Porine from the outer coat of *Y. pseudotuberculosis* is shown to be a species-specific agent of pseudotuberculosis. Porine-based enzyme immunoassay permits the detection of antibodies to all serological variants of *Y. pseudotuberculosis.* The possibility of diagnosing the disease in the early stages of infection is one advantage of the proposed method.

Key Words: *pseudotuberculosis diagnosis; porine; enzyme immunoassay*

Pseudotuberculosis, or Far-Eastern scarlatiniform fever caused by *Yersinia pseudotuberculosis,* is an infectious disease with a polymorphous clinical picture, which makes its diagnosis particularly difficult. For example, it is hard to differentiate between pseudotuberculosis and infectious viral hepatitis, particularly upon early examination of patients. At present, the indirect hemagglutination test (IHAT) with a commercial diagnostic agent based on *Y. pseudotuberculosis* serovar IB lipopolysaccharide (LPS) is widely used for the clinical diagnosis of Far-Eastern scarlatiniform fever [2,3]. Some authorities report the development of enzyme immunoassay systems and of immunoglobulin preparations for the rapid diagnosis of pseudotuberculosis and enteric yersiniosis [4,6,8]. However, the use of type-specific antigens (LPS and Boivin's antigen) limit the potentialities of these diagnostic methods, for the disease may be caused not only by serovar I agents, but by serovar II, III, IV, and V agents as well [4,9].

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This report presents data on the development of a test system based on species-specific *Y. pseudotuberculosis* antigen, a pore-forming protein from the bacterial outer coat, which permits the detection of antibodies to different serological variants of the pseudotuberculosis agent.

MATERIALS AND METHODS

Porine isolated from the outer coat of *Y. pseudotuberculosis* (strain 598, serovar IB) was used as antigen. The porine sample contained 75 to 80% protein and no more than 3-4% LPS. Enzyme immunoassay (EIA) was performed routinely using Dynatech plates. The test was carried out in buffered normal saline, pH 7.4, with 0.1% sodium dodecyl sulfate. When selecting the optimal sensitization regimen, we tried different a) antigen doses (0.625 to 20 μg/ml); b) buffer pH values (7.4 to 9.0); c) times and temperatures of antigen fixation on the solid phase; and d) reagents and durations of blocking of nonspecific antibody binding on the plates. Conjugates manufactured by the Gamaleya Research Institute of Epidemiology and Microbiol-

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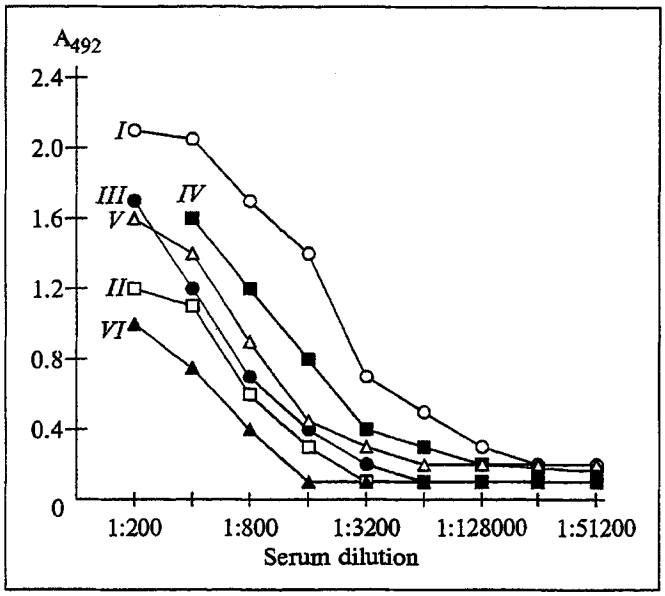

Fig. 1. Titration of rabbit antisera to *Y. pseudotuberculosis* serovars I–VI with *Y. pseudotuberculosis* porine. Here and in Fig. 2, *a*: the ordinate shows optical density at 492 nm.

ogy, Russian Academy of Medical Sciences (Moscow), and o-phenylenediamine (Sigma) as substrate were used in the test. The results of staining were recorded with a Multiscan Plus spectrophotometer at 492 nm and expressed in optical density units. The results were statistically processed by traditional methods [1] with estimation of the mean values and errors, and the reliability of the results was assessed using Student's tables.

The specificity of the proposed test system was assessed using type rabbit antisera to different serovars of pseudotuberculosis agent (I-VI) and to other representatives of Enterobacteriaceae, specifically to *Salmonella* and *Shigella*.

A total of 114 sera from 65 patients with the characteristic clinical picture were tested using porine-based EIA; 46 of these patients had bacteriologically confirmed pseudotuberculosis (the agents belonged to serovars I, III, and IV). Some of the sera were tested over time during the first to third weeks after clinical manifestation of the disease.

The minimal diagnostic titer was determined on the basis of 79 blood samples from healthy donors and 64 blood samples from patients with various diseases other than pseudotuberculosis (26 cases of yersiniosis, 18 of salmonellosis, and 20 of viral hepatitis). Normal donor sera were obtained from the Vladivostok Blood Bank, while patient sera were provided by the Department of Infectious Diseases at the State Medical Institute (Vladivostok) and by the Research Institute of Microbiology, Siberian Division of the Russian Academy of Medical Sciences (Vladivostok).

RESULTS

Study of immunobiological properties of porine from *Y. pseudotuberculosis* outer coat revealed that antibodies to it are present in sera obtained by immunizing experimental animals with whole bacterial cells and in sera of pseudotuberculosis patients. Moreover, previously [5] we demonstrated the possibility of using porine as a diagnostic antigen and of using antiserum to it for the detection of pseudotuberculosis agents in the environment. These data served as the basis for using *Y.*

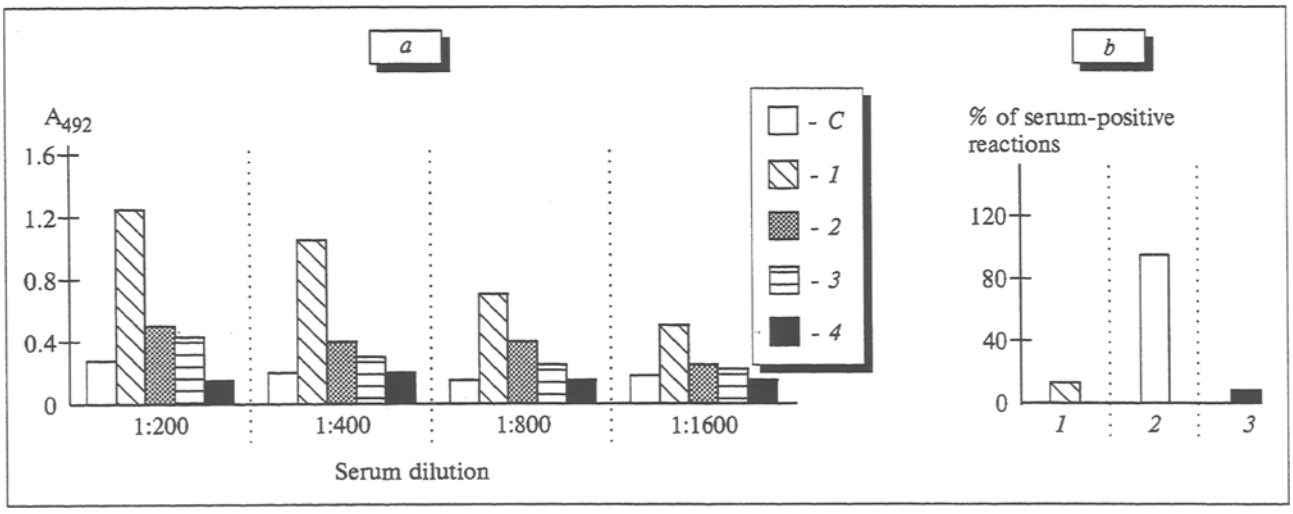

Fig. 2. Screening of patient and donor sera for antibodies to *Y. pseudotuberculosis* in EIA using *Y. pseudotuberculosis* porine. *a*) mean antibody titers in the sera of patients with pseudotuberculosis (*1*), yersiniosis (*2*), salmonellosis (*3*), and hepatitis (*4*), and in healthy donors (*C*) in various dilutions. *b*) comparative assessment of specificity of test system with *Y. pseudotuberculosis* porine in titering antibodies in patient and donor blood sera: *1*) pseudotuberculosis patients with a bacteriologically confirmed diagnosis; *2*) donors; *3*) patients with diseases other than pseudotuberculosis.

pseudotuberculosis porine for the serological diagnosis of pseudotuberculosis.

Antibodies in immune sera were measured by EIA. The experimentally chosen optimal conditions for porine adsorption on the plates were as follows: a) porine concentration 20 μg/ml; b) buffer pH 9.0; c) 2-hour sensitization of the plates at 37°C; and d) 16-hour blocking of nonspecific binding with 0.1% Tween-20 at 4°C.

The species specificity of porine is demonstrated by comparing the curves reflecting the titration of type rabbit antisera to different serovars of pseudotuberculosis agent (I-VI). Figure 1 shows that antibodies to porine are present in all the tested antisera: the direction of the titration curves coincides, and the titers differ by no more than one dilution, except for the homologous antiserum (to IB serovar), which has higher final titer in comparison with the heterologous antisera (to serovars II-VI). This result cannot be due solely to the presence of LPS in porine samples, because we showed that in certain concentrations LPS interacts with the tested sera at the background level. Hence, porine is a species-specific antigen and may be used for the diagnosis of *Y. pseudotuberculosis* of all serovars.

The specificity of the proposed test system was assessed using a kit of standard rabbit antisera to Salmonellae (*S. typhimurium, S. paratyphi B, S. anatum, S. enteritidis, S. choleraesuis,* and *S. heidelbergi*) and Shigellae (*S. flexneri, S. sonnei,* and *S. dysenteriae*) causing enteric diseases. Cross-reactions were observed only with antisera to *S. sonnei, S. flexneri,* and *S. choleraesuis.*

The possibility of using the developed test system for the diagnosis of pseudotuberculosis in man was verified in examinations of 114 sera of patients with typical clinical manifestations of the disease and 79 donor sera. The marked polymorphism of pseudotuberculosis infection prompted us to examine patients with infectious diseases clinically similar to pseudotuberculosis (salmonellosis, yersiniosis, and hepatitis) when carrying out trials of the new test system.

To determine the minimal diagnostic titer, we compared the ratio of the mean optical density values of sera from pseudotuberculosis patients to the negative reference normal donor sera diluted 1:200, 1:400, 1:800, and 1:1600. These values were 4.0, 4.6, 4.5, and 3.5, respectively. The results indicate that the 1:400 or 1:800 dilution may be taken as the minimal diagnostic titer in EIA (Fig. 2, *a*).

Comparative study of blood sera from three groups of individuals with a bacteriologically confirmed diagnosis of pseudotuberculosis, diseases other than pseudotuberculosis, and normal subjects demonstrated a fairly high specificity of EIA with porine from the outer coat of *Y. peudotuberculosis* used as antigen (Fig. 2, *b*). Despite the fact that sera from patients with yersiniosis and salmonellosis interact with porine, the optical density values of pseudotuberculosis sera were almost three times higher in diagnostic dilutions (Fig. 2, *a*).

It should be noted that testing of donor sera by the proposed system revealed a positive reaction to pseudotuberculosis in 13% of subjects. This result correlates with the data on an increased general background level of antibodies to pseudotuberculosis in residents of regions where *Y. pseudotuberculosis* is in constant circulation.

The next step of our study was to compare statistically reliable [1] results of serological screening of pseudotuberculosis patients using the proposed test system and IHAT with commercial diagnostic agent. Forty-six blood samples from persons with a bacteriologically confirmed diagnosis were tested for this purpose. The efficacy of EIA proved to be almost twice as high. For example, a positive reaction in IHAT was observed in only 52% of cases, whereas in EIA 98% of samples were positive; note that the disease was not detected by the commercial diagnostic agent if the *Y. pseudotuberculosis* agents belonged to serovars III or IV. Moreover, IHAT testing of sera during the first and second weeks of the disease showed negative results in all cases, whereas EIA confirmed pseudotuberculosis in 57% of patients with bacteriologically documented pseudotuberculosis during the same period (days 7-10 from the first clinical manifestations).

Hence, EIA with porine, a species-specific antigen from the *Y. pseudotuberculosis* outer coat, is an effective, specific, and reliable method of serological diagnosis of pseudotuberculosis, suitable for clinical use.

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REFERENCES

1. I. P. Ashmarin and A. A. Vorob'ev, *Statistical Methods in Microbiological Studies* [in Russian], Moscow (1962).
2. A. M. Korolyuk, *Serological Diagnosis of Pseudotuberculosis (Scarlatiniform Fever)* [in Russian], Thesis of Cand. Med. Sci., Leningrad (1971).
3. *Microbiological Diagnosis of Yersiniosis in the Soviet Army and Navy. Methodological Recommendations* [in Russian], Moscow (1984).
4. O. D. Novikova, E. V. Urikh, N. F. Timchenko, *et al.*, in: *Natural Focal Diseases of Siberia and the Far East* [in Russian], Novosibirsk (1985), pp. 33-38.

5. V. B. Sboichakov, A. M. Korolyuk, V. N. Verbov, *et al.*, *Zh. Mikrobiol.*, № 7, 83-86 (1986).
6. N. V. Sorokina, E. M. Gavrilova, A. M. Egorov, and N. A. Koltovaya, *Ibid.*, № 12, 68-72.
7. N. F. Timchenko, *Data on the Microbiology of the Agent of Far-Eastern Scarlatiniform Fever (Human Pseudotuberculosis)* [in Russian], Thesis of Cand. Med. Sci., Vladivostok (1972).
8. G. F. Shpilyuk, T. N. Goloveshkina, I. K. Volodina, *et al.*, *Zh. Mikrobiol.*, № 6, 92-93 (1993).
9. M. Ynone, H. Nakashima, T. Yshida, and M. Tsubokura, *Zentralbl. Bakteriol. Mikrobiol. Hyd.*, **186**, 504-511 (1988).

Antibodies to *Bacillus megaterium H.* Glycoprotein in Pregnant Women with a Pathological Course of Pregnancy

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The humoral immune response to *Bac. megaterium H.* glycoprotein was studied in women with normal and abnormal pregnancies. The level of antibodies to bacterial glycoprotein was elevated in women with chronic foci of infection during the second and third trimesters. Elevated levels of antibodies to *Bac. megaterium H.* reflect the presence of pathological changes in the fetus during intrauterine development and may be used as prognostic criteria of intrauterine disease.

Key Words: *antibodies; glycoprotein; pregnant women*

The levels of antibodies to *Bac. megaterium H.* are elevated in patients with tumors of various localization, due to the presence of common antigenic determinants in tumor cells and in bacterial glycoprotein [4]. Fetal and tumor cells are known to possess common antigens, and fetal antigens induce an immune response in cancer patients [3,5,7]. For this reason we decided to study the humoral immune response to *Bac. megaterium H.* in pregnant women and analyze the data in comparison with the health status of the newborns.

MATERIALS AND METHODS

Sera of 148 pregnant women divided into 3 groups depending on the health status of their newborns were tested. The main criterion of infant health was the status of the central nervous system, the involvement of which is the most frequent pathology during the neonatal period and infancy, reflecting intrauterine disease of the fetus. Group 1 consisted of 12 pregnant women whose infants were considered to be healthy, group 2 was made up of 71 women who gave birth to children with manifest disorders of the central nervous system which persisted in one form or another after the age of 2, and group 3 consisted of 65 women whose infants presented with less pronounced abnormalities of the CNS which disappeared by the age of 2 as a result of therapy. All groups were matched for age and obstetric history.

Glycoprotein with a molecular weight of 65 to 70 kD isolated from *Bac. megaterium H.* was used as antigen. Antibodies in the sera of pregnant women were detected by enzyme immunoassay (EIA).

Glycoprotein solution (100 μl) in carbonate-bicarbonate buffer, pH 9.0±0.2 was pipetted into plates in a concentration of 100 μg/ml and incu-

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