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Experimental Validation of the Possibility of Serological Diagnosis of Pseudotuberculosis in Humans by Immunoblotting

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Pseudotuberculosis is diagnosed by immunoblotting. The method is highly effective in serological diagnosis of pseudotuberculosis and differential diagnosis of pseudotuberculosis and enteric yersiniasis in clinically obscure cases, when the causative agent could not be isolated from the patient.

Key Words: *pseudotuberculosis; enteric yersiniasis; immunoblotting; plasmid-specific proteins*

The similarity of symptoms of pseudotuberculosis (PT) to the symptoms of other diseases prompts bacteriological and serological analyses to confirm clinical diagnosis [1,2,4]. Based on the analysis of clinical manifestations of PT in *Papio hamadryas,* we proposed the immunoblotting (IB) technique for serological diagnosis of PT when it was impossible to isolate the causative agent [3].

In the present study IB was used to test the sera of patients hospitalized with polymorphous clinical symptoms and a tentative diagnosis of PT. Bacteriological and serological analyses for PT at clinical laboratories gave no positive results.

MATERIALS AND METHODS

Patients' sera were tested by IB. Electrophoresis of cell culture lysates was carried out in 1.5-mm polyacrylamide gel (5 to 15% linear gradient) in the presence of sodium dodecyl sulfate [7]. Sigma proteins with molecular weights of 14.4 to 67.0 kD were used as markers. Cell lysate of test strain culture (150 μl) was applied onto gel strip, this amount corresponding to 480-500 μg of protein. The order of applying lysates of agar cultures of bacterial test strains was universal (left to right): 1) *Yersinia pseudotuberculosis* strain 326 grown at 37°C and 2) *Yersinia enterocolitica* strain 657 grown at 37°C. After electrophoresis, separated proteins were applied onto Sartorius SM-11307 nitrocellulose as described elsewhere [10]. Proteins were incubated for 18-20 h with blood sera and protein A conjugates with Sigma peroxidase at ambient temperature [8]. Reactive antibodies were detected using a substrate-buffer mixture on the basis of 3,3'-diaminobenzidine with nickel chloride [5]. Other details of electric transfer and subsequent treatment of blots are described elsewhere [6].

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RESULTS

Thirty-four individual sera from patients were tested in 1994-1995. Indirect hemagglutination test with the erythrocytic pseudotuberculosis antigenic dry diagnostic agent (St. Petersburg Research Institute of Vaccines and Sera) was negative. IB diagnosed PT in 3 cases and enteric yersiniasis (EY) in 6. The results of tests of 2 sera from patients with clinical diagnosis of systemic lupus erythematosus (female patient M., 26 years) and arthralgic PT with relapsing course (male patient K., 20 years) are presented.

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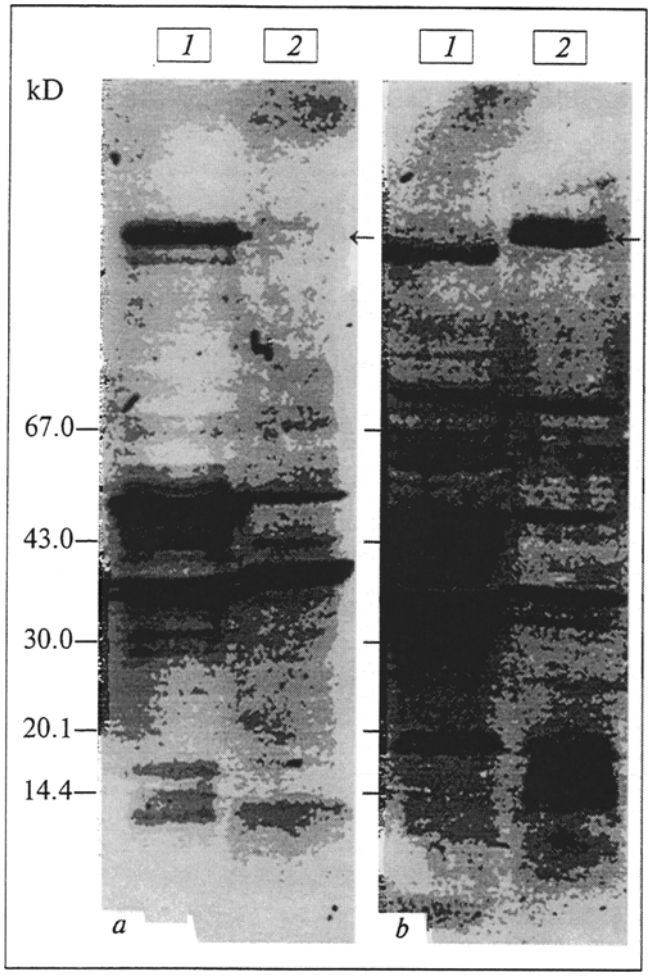

Fig. 1. Immunoblotting of blood sera of patients M. (*a*) and K. (*b*). Left: molecular weights of marker proteins.

Blood was collected on day 28 of the disease in patient M. and on day 59 in patient K. An acute onset of the disease, intoxication, dyspeptic symptoms, rash, arthralgia, enlargement of the liver, ECG changes, and abdominal pain typical of the polymorphic clinical picture of PT were observed in both patients. Enteric yersiniasis with clinical symptoms similar to those of PT was also probable. Therefore, we tested the patients' sera with cell lysates of strains *Y. pseudotuberculosis* 326 and *Y. enterocolitica* 657 (both strains are calcium-dependent) characterized by the maximum expression of plasmid-specific protein *YopA*. Preliminary tests with sera of monkeys convalescenting after PT and EY showed negligible cross-reactivity between *YopA* proteins from PT and EY agents [3]. Moreover, these proteins differ by molecular weight: the molecular weight of *YopA* protein from PT agent is 200 kD, which is 20-30 kD less than that of *YopA* protein from EY agent [9]. This permits differential diagnosis of PT and EY by IB of patients' sera.

The attempts to use other plasmid-determined *pCad* proteins failed, since all these proteins were immunologically related in the PT and EY agents and only *YopA* protein was clearly specific.

Figure 1, *a*, presents the IB of blood serum from patient M. Antibodies to *Y. pseudotuberculosis*, including those to protein *YopA* (shown with an arrow), are clearly seen, which indicates the diagnosis of PT. In patient K. (Fig. 1, *b*), IB revealed response to *Y. pseudotuberculosis* protein *YopA* and an expressed response to *YopA* protein of *Y. enterocolitica*, which indicates the presence of both PT and EY. In this case we can speak not about a relapse of PT in this patient, but about infection with the PT agent after EY.

Diagnosis of PT by IB in patients M. and K. helped carry out effective etiotropic therapy.

Our results confirm the diagnostic validity of IB for the diagnosis of PT and differentiation between PT and EY. This method can be used in intricate and obscure cases, when the diagnosis of PT or EY cannot be confirmed by bacteriological analyses. Immunochemical test systems for serological diagnosis of PT and EY on the basis of *YopA* proteins are a promising tool of differential diagnostics.

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