DETECTION OF SALMONELLA ENTEROTOXIN BY THE COAGGLUTINATION TEST

Yu. G. Parkhomenko, I. N. Emel'yanenko, and F. S. Fluer

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Salmonellas are among the most widespread agents of intestinal infectious [3, 6, 10, 11]. However, the role and relative importance of living agents and their toxins in the pathogenesis of salmonella infection are not yet clear, and it is particularly important that this be taken into account when immune preparations are produced for the prevention and treatment of this disease. Above all it is essential to have precise information about the biological characteristics of the agents which are responsible for its pathogenic properties. Whereas information on the morphology and cultural biochemical properties of salmonellas is fairly complete, only fragmentary details are available on the enterotoxigenicity of various strains on salmonellas [1, 2]. The absence of such information makes it difficult to type these microorganisms and to identify them sufficiently clearly.

The aim of this investigation was to study the ability of salmonellas of different strains to form toxin.

EXPERIMENTAL METHOD

Strains of salmonellas were obtained from the N. F. Gameleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, and had been isolated during outbreaks of acute toxicoinfections, and reference strains of salmonellas kept at the L. A. Tarasevich Institute of Standardization and Control of Medical Biological Preparations also were used. Cultures of salmonellas were grown in 10 ml of liquid nutrient medium in 40-50-ml test tubes for 24-30 h, with continuous shaking at 210 rpm and at 37°C. The material was then centrifuged at 8000 rpm for 15-20 min, the supernatant was discarded, and the residue of microbial cells was treated with 0.5 ml of distilled water and lyzed with the aid of polymixin B [4, 5]. The lysates were centrifuged at 8000 rpm for 25-20 min [sic], the residue was discarded, and the supernatant was used for further investigations in the coagglutination test. To carry out this test a staphylococcal reagent was prepared. For this purpose cells of *Staphylococcus aureus* Cowan I were sensitized with antienterotoxin serum containing specific antibodies to thermolabile enterotoxins of *E. coli* strain H-10407 with a precipitating titer of 1:16 or higher, and not containing antibodies to other *E. coli* antigens. For the control, the prepared reagents was tested for homogeneity in a drop of phosphate-salt buffer on a glass slide. In the absence of spontaneous agglutination this reagent was used in the coagglutination test. To determine the enterotoxigenicity of the salmonellas, one drop of supernatant of the test salmonella cultures was added to 2-3 drops of staphylococcal reagents on a watch glass. The drops were mixed by careful shaking for 0.5-3 min. The results of the tests were read by examining the drops above a concave mirror and the appearance of staphylococcal agglutination was recorded. The intensity of this reaction was described by a 4+ system.

The international reference enterotoxigenic strain of salmonella (*S. typhimurium* S-2-3235) and an enterotoxigenic strain *E. coli* H-10407, which give a positive coagglutination reaction, and, at the same time, a culture of *Klebsiella pneumoniae*, which does not produce an enterotoxin, were used as the control.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the intensity of enterotoxin production by the test strains of salmonellas varied from + to ++++. The investigations also demonstrated that in-

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TABLE 1. Ability of Salmonellas to Form Enterotoxin

Source of strains of salmonellas	Strain	Results of coagglutination test
N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR	S. typhimurium 415	+
	S. typhimurium	++
	S. typhimurium M	+++
	S. typhimueim 16	+
	S. typhimurium 17	++
	S. typhimurium 18	+(—)
	S. typhimurium 19	+
	S. typhimurium w 118	++++
	S. typhimurium 74	—
	S. typhimurium 32	+
	S. typhimurium 33	+++++
	S. enteritidis	—
L. A. Tarasevich Institute for Standardization and Control of Medical Preparations	S. typhimurium 978	+(—)
	S. cholerae suis 7378	+++
	S. heidelberg 287	+++
	S. anatum 1120	—
	S. bredeney 210	++
	S. ejeda 1424	+(—)
	S. goodwood 1471	—
	S. ebrie 200	—
	S. bovaire 1323.	++
	S. balboa 1886/76	++++
Control	S. enteritidis 27036	+
	Phosphate buffer	—
	K. pneumonia	—
	E. coli H-10407	++
	S. typhimurium	+++++

dividual strains of these microorganisms do not produce enterotoxin. Of 23 strains studied, 15 (65%) formed enterotoxin, three (13%) gave an uncertain reaction, and five (21%) did not form enterotoxin.

The ability of different strains of salmonellas to form enterotoxin, together with adhesiveness and skin permeability factors, is thus yet another pathogenicity factor [1, 2]. We know, for example, that the ability of salmonellas to form toxin is invariably associated in them with the presence of skin permeability factors [8]. The contradictory data in the literature on the participation of salmonella enterotoxin in the pathogenesis of disease are evidently attributable to the great difficulties in finding it. This state of affairs can be explained by its predominantly intracellular localization and its very small quantities, and also by the use of insufficiently sensitive methods for its detection. Enterotoxins likewise can be inactivated by the intestinal mucus, as a result of which its biological activity and its role in the pathogenesis of this toxicoinfection cannot be detected [9].

The results clearly show that in most cases salmonellosis is caused by strains forming enterotoxin, which is also the cause of the diarrhea that accompanies this disease. However, the role of the cytotoxin recently discovered in salmonellas [7], which gives rise to the shigellosis-like form of the disease, cannot be ruled out. The absence of enterotoxin production in 35% of the strains of salmonellas which we studied may be evidence that they form only a cytotoxin, which in this case could not be detected because the antienterotoxin serum used is without specificity for cytotoxin.

The results are undoubtedly useful in the choice of strains of salmonellas for the production of prophylactic and therapeutic preparations both for man and for animals. The additional information on the biological features of the agent and the methods of detecting enterotoxin described above will also help to discover epidemiologically important types during outbreaks of toxicoinfections.

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COUNTERFLOW IMMUNOBLOTTING

G. I. Abelev and É. R. Karamova

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KEY WORDS: isotachophoresis; electroendosmosis; immunoblotting; urinary proteins; immunoglobulin light chains

The immunoblotting (IB) method, in which proteins separated by polyacrylamide gel (PAG) electrophoresis are transferred to nitrocellulose membranes (NCM), and then developed consecutively by mono- or polyclonal antibodies and corresponding immunoenzyme conjugates, has become widely used to characterize antigens as well as monoclonal antibodies [6]. However, successive treatment of NCM with several immunoreagents and careful washing of the membranes after each treatment make IB a long and laborious method, requiring large quantities of immunoreagents, in which to immerse the NCM.

In this paper we show how it is possible to develop antigens adsorbed on NCM automatically by means of immunoreagents carried in the membrane by the flow of liquid. This form is created in NCM by isotachophoresis (ITP), i.e., electrophoresis in a heterogeneous buffer system possessing a common cation but different anions [5]. During ITP on porous membranes, an electroendosmotic counterflow (EEC) is created in them; the velocity of this counterflow in the zone of the anodal electrolyte exceeds the velocity of electrophoretic migration of any negatively charged protein, which will as a result be transferred by the flow of liquid toward the cathode [1, 2].

If ITP is carried out on NCM in which proteins separated by electrophoresis are first "imprinted," EEC will create what can be described as a conveyer belt in them, capable of carrying the immunoreagents required for successive detection of the antigens used through them. We have used this approach to detect and to determine the electrophoretic characteristics of immunoglobulin light chains (L_κ and L_λ) in human urine.

EXPERIMENTAL METHOD

Cellulose acetate membranes (CAM) of "Cellogel" type (from Chemetron, Italy), 17 cm long and from 1 to 4 cm wide, and NMC (Schleicheer und Schull, West Germany or Bio-Rad, USA) with pore diameter of 0.45 μ, were used. To develop mouse monoclonal antibodies (MCA) components or an experimental production batch of peroxidase-antiperoxidase complex from the Gabrichevskii Enzyme Research Institute, Ministry of Health of the RSFSR (Gor'kii), including goat antibodies to mouse IgG, mouse MCA to horseradish peroxidase, and peroxidase itself [3], were used. The residual protein-adsorbing capacity of the NCM was blocked with a 10% solution of the commercial preparation Bona (a milk mixture for infant feeding, made in Finland) on buffered physiological saline (BPS). Tris (base) and β-alanine, analytically pure, obtained from Serva, West Germany, were used for ITP.

Before analysis the samples of urine were filtered through filters with pore diameter of 0.22 μ (Millipore, France) and dialyzed against 0.06 M Tris-HCl, pH 6.7. As the standards for L_κ and L_λ we used lyophilized preparations of Bence-Jones (BJ) proteins of the corresponding types, generously provided by Professor E. V. Chernokhvostova, G. N. Gabrichevskii Research Institute of Enzymology, Ministry of Health of the RSFSR.

ITP of the urine was carried out by the method described previously [1, 2] on Cellogel CAM, using samples of 40-100 μl per centimeter width of the membrane, containing 30 μl of

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