Preparation and Characterization of Murine Monoclonal Antibodies to *Salmonella typhimurium* K-Antigen

M. V. Raevskaya, Yu. A. Belaya, N. V. Koval'chuk, L. N. Chernousova, and V. G. Petrukhin

UDC 615.371:579.842.14].012

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 117, № 6, pp. 633-635, June, 1994
Original article submitted January 25, 1994

Monoclonal antibodies to *S. typhimurium* K-antigens representing IgG1 immunoglobulins with a "kappa"-type light chain are prepared and characterized. They are highly active in indirect enzyme immunoassay with purified K-antigens and whole fixed *S. typhimurium* cells and virtually do not cross-react with O-antigen of the same bacterial species or with *E. coli* antigenic structures. The kinetics of binding and ability to agglutinate whole bacterial cells are studied and epitope analysis is carried out, which shows that antigenic determinants of *S. typhimurium* K-antigen qualitatively differ from those of O-antigen of the same bacterium.

Key Words: *monoclonal antibodies; Salmonella antigen; enzyme immunoassay*

Acute enteric infections are among the most pressing medical and socioeconomic problems due to their wide prevalence and the high mortality they cause [9]. Therefore, early detection of their causative agents is particularly important.

The aim of the present research was to prepare monoclonal antibodies (MAB) to *Salmonella* K-antigen. K-antigen is a surface-somatic thermolabile phosphoryl glycoprotein present in all *Salmonella* bacteria, its concentrations correlating with the virulence of strains [5,7]. Diagnostic use of MAB to K-antigen will help not only assess the level of contamination of samples with *Salmonella*, but estimate the virulence of detected strains as well.

MATERIALS AND METHODS

Formalin-fixed or heat-killed preparations of *Salmonella typhimurium* 3379 and *Escherichia coli* 055 and purified K-antigen preparation were made at the N. F. Gamaleya Research Institute of Epidemiology and Microbiology. Mutant *S. typhimurium* n 30 red was used to isolate K-antigen. MAB were prepared as follows: 5 female BALB/c mice aged 6 to 10 weeks were intraperitoneally immunized with heat-killed *S. typhimurium* in a dose of 2×10^6 cells with complete Freund's adjuvant. Immunizations were carried out at 1-month intervals. Three days before hybridization a booster intravenous immunization was performed with 100 μg of K-antigen in 0.25 ml of phosphate saline (PSB). Animals whose sera had the highest titers in enzyme immunoassay (EIA) were used for hybridization. Hybridization and culturing of hybrid cells were carried out using standard methods. To obtain antibodies in high concentrations the hybrid cells were grown *in vivo* as ascitic tumors [8]. The MAB were initially purified by salting out with ammonium sulfate [1]. Protein was measured spectrophotometrically. Further purification of the globulin fraction was performed using ion-exchange chromatography on DEAE-cellulose and gel filtration in a column packed with Sephacryl S-200 (Pharmacia) [1]. MAB were then affine-purified:

Department of Cell Physiology and Immunology, Biological Faculty, M. V. Lomonosov Moscow State University; N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences; Institute of Tuberculosis, Moscow. (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0006-0637$12.50

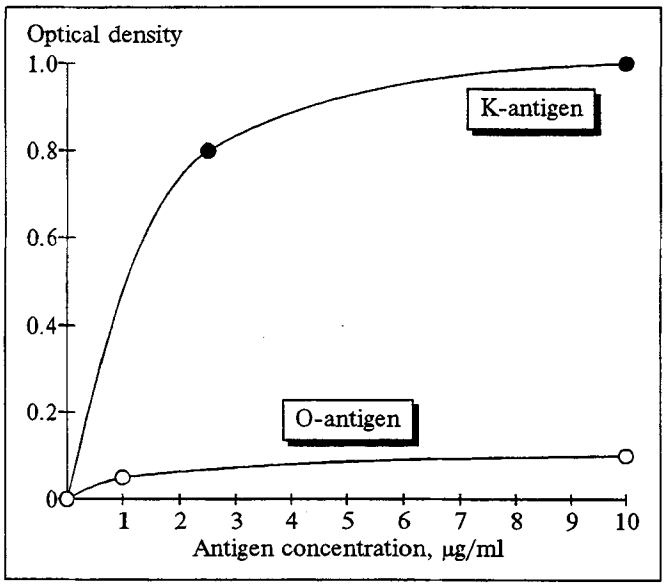

Fig. 1. Interaction of E6 MAB with *S. typhimurium* K– and O–antigens.

K-antigen in a concentration of 5 mg/ml was used as ligand, and BrCN-activated sepharose as sorbent. Covalent suturing was performed as recommended by the manufacturer (Pharmacia). Antibodies were adsorbed overnight at +4°C, washed 3 times on a glass filter with PSB, and eluted with Bio-Rad 6 M urea for 5 min. The eluate was then layered onto the column with Sephadex G-25 to separate the urea. The separation was monitored spectrophotometrically. The class of MAB secreted by hybrid clone cells was determined by immunodiffusion after Ouchterlony [2] with rabbit antibodies to murine immunoglobulins of various classes (ICN)

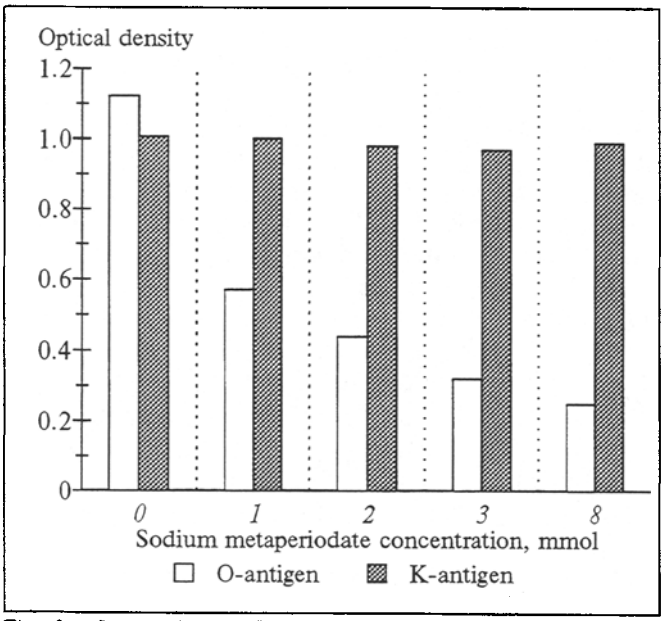

Fig. 2. Interaction of E6 MAB with *S. typhimurium* K– and O–antigens pretreated with sodium metaperiodate. Antigen concentration 5 μg/ml, supernatant dilution 1/20.

and by EIA with caprine biotinylated antibodies to various immunoglobulin chains (Sigma). The capacity of MAB for agglutinating whole bacterial cells was tested as recommended elsewhere [6]. Indirect EIA was carried out using the standard procedure [4]. K- and O-antigens were dissolved in a concentration of 1 mg/ml, divided into aliquots, and stored frozen at -20°C. EIA was performed in 96-well Dynatech Microelisa, Nunclon, and Lenmedpolimer polystyrene plates. Antigens in concentrations of 0.01, 0.1, 0.5, 1.0, 2.5, 5, and 10 μg/ml in PSB (working volume 50 μl) were used for saturation of plates. Immune murine sera in dilutions 1/2 to 1/64 were placed in wells in a double step, then supernatants of hybrid cultures in dilutions 1/2 to 1/128 in a double step, and then ascitic fluids in 14 successive dilutions. Conjugate of rabbit antimurine immunoglobulins with horseradish peroxidase (N. F. Gamaleya Institute of Epidemiology and Microbiology) in dilution 1/200 was used. Orthophenylenediamine in citrate-phosphate buffer was used as the substrate [4]. The results were assessed from the value of optical density using Multiscan-Elisa at wavelength 492 nm. The kinetics of MAB binding with antigen was studied as recommended previously [10]. For EIA with whole fixed cells, 50 μl of a 0.1% solution of poly-L-lysine were placed in polystyrene plates for microtitration and incubated for 1 h at 37°C. Fixed bacteria were washed free of formalin once in cold PSB and sedimented by centrifugation at 2000 g for 40 min. A bacterial cell suspension in concentrations 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, and 10^{10} cells/ml was placed in wells, working volume 50 μl, incubated overnight, centrifuged for 30 min at 300 g, and washed in PSB, after which 50 μl of 2% glutaraldehyde were added. The mixture was incubated for 1 h at room temperature. The subsequent steps were carried out as described before. For characterization of sugar residues in the binding epitope of K-antigen periodate oxidation of antigen was performed using a sodium periodate solution in concentrations 1, 2, 4, and 8 mmol. Further treatment was carried out as described elsewhere [3]. The antigen thus treated was used for adsorption on polystyrene plates for EIA.

RESULTS

Sixteen hybrid clones were obtained; clone E6, which produced MAB to K-antigen, was selected as the producer clone. MAB secreted by clone E6 were referred to the IgG1 class with a "kappa"-type light chain. The yield of purified specific antibodies from 1 ml of supernatant was 79.8 μg/

ml. The antibodies could not agglutinate whole bacterial cells.

Analysis of E6 MAB in EIA showed that they were specifically directed to *S. typhimurium* K-antigen, and cross-reactions with *S. typhimurium* O-antigen occurred in 8.1% of cases (Fig. 1), the mean square error being 6.87%.

Treatment of K-antigen with sodium metaperiodate in ascending concentrations did not reduce binding, whereas similar treatment of O-antigen, according to our data, noticeably reduced the reaction values in inverse proportion to the concentration of added periodate at the expense of oxidation of α-glycol groups of the polysaccharide part. The results give grounds for assuming that if the binding epitope is a component of the polysaccharide, it differs qualitatively from that in O-antigen or belongs to the protein part of the K-antigen complex (Fig. 2).

Study of the binding kinetics showed that half-saturation under the specified conditions was attained in 10 min, and complete saturation in 45-50 min.

Analysis of MAB binding to whole bacterial cells showed that the MAB specifically bound to *S. typhimurium*. No cross-activity of MAB between *Salmonella* K-antigen and *E. coli* antigenic structures was observed (Fig. 3). Concentrations 10^6-10^7 cells/ml were reliably detected, whereas in EIA with polyclonal serum concentrations starting from 10^8-10^9 cells/ml were detected [5].

Hence, MAB to *S. typhimurium* K-antigen were obtained and characterized for the first time. They belong to IgG1 immunoglobulins with a "kappa"-type light chain. MAB to *S. typhimurium* K-antigen bind to whole cells of *S. typhimurium* and do not cross-react with *E. coli*, a representative of the *Enterobacteriaceae*. K-antigen binding epitope differs qualitatively from those of *S. typhimurium* O-antigen.

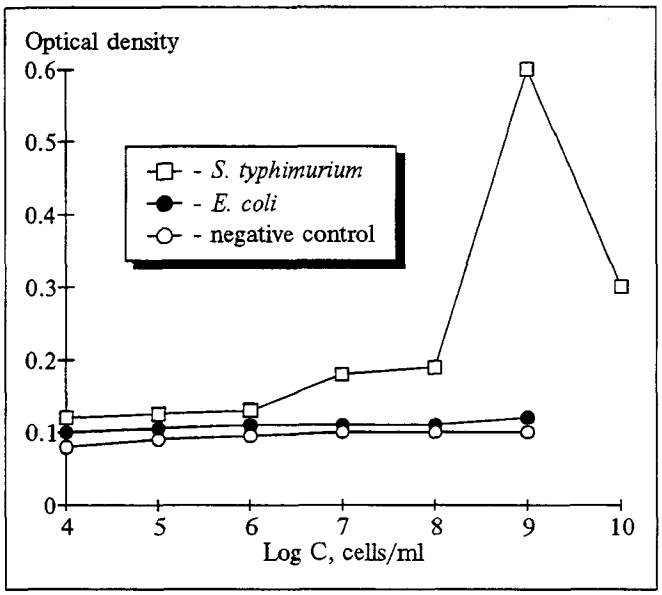

Fig. 3. Interaction of E6 MAB with whole bacterial fixed cells.

REFERENCES

1. H. Arvie and A. F. Williams, in: *Antibodies; A Practical Approach*, Ed. D. Catty, Vol. 1, IRL Press (1988).
2. E. Boehm, in: *Immunological Methods*, ed. by G. Frimel [Russian translation], Moscow (1987), pp. 73-89.
3. J. Brock, *Ibid.*, 438-439.
4. A. M. Egorov, A. P. Osipov, B. B. Dzantiev, and E. M. Gavrilova, *Theory and Practice of Enzyme Immunoassay* [in Russian], Moscow (1991).
5. N. R. Ivanov and Yu. M. Mikhailova, *Salmonelloses in Adults and Children* [in Russian], Saratov (1990).
6. *Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analysis*, ed. by R. H. Kenneth *et al.*, Plenum Press, New York (1980).
7. L. K. Stepanova, Yu. A. Belaya, and V. D. Gekker, *Current Topics in Epidemiology and Infectious Diseases (Salmonelloses)* [in Russian], Saratov (1976), pp. 28-30.
8. H. Fibich, in: *Immunological Methods*, Ed. G. Frimel [Russian translation, 1987], pp. 20-34.
9. I. S. Sharp, P. V. Collier, G. Forbes, and T. V. Hill, *Bull. WHO*, **66**, № 4, 50-55 (1988).
10. S. J. Challacombe, *ELISA and Other Solid Phase Immunoassays*, (1988), pp. 319-343 .