MICROBIOLOGY AND IMMUNOLOGY

Preparation of Monoclonal Antibodies to O-Antigen (0-4.5) of *Salmonella typhimurium* Serogroup B

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Sixteen clones secreting murine monoclonal antibodies of various specificities to *Salmonella* antigens are obtained. One of the clones is highly specific to *Salmonella typhimurium* (serogroup B) O-antigen in enzyme immunoassay. The resultant antibodies are IgG2a immunoglobulins with a "kappa"-type light chain. The connecting epitope includes abequose-mannose-rhamnose trisaccharide. Antibodies are bound to *S. typhimurium* whole cells and do not cross-react with representatives of the *E. coli* family.

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

The production of monoclonal antibodies (MAB) to various antigens of *Salmonella* is a topic of current interest in light of the search for antigenic determinants which may be the most effective for the construction of vaccines [10] and due to their potential use for fine typing of the species and strains of these bacteria in foodstuffs and clinical samples [13]. Moreover, MAB preparations have been shown to be useful in the treatment of infected animals and humans [8]. Unfortunately, the preparation of MAB to various *Salmonella* antigens is fraught with many difficulties, the principal one of which is the high cross-reactivity not only between members of the genus *Salmonella*, but also between various representatives of the family *Enterobacteriaceae* [11,12], whereas strict specificity in respect of individual serogroups is of paramount importance for a differential diagnosis. The present research was aimed at preparing MAB to O-antigen of *S. typhimurium*, serogroup B. Despite recent changes in the quantitative ratio between *Salmonella* species and strains isolated from the environment, *S. typhimurium* still ranks first among the isolates: 34% [9].

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MATERIALS AND METHODS

Formalin-fixed or heat-killed preparations of *S. typhimurium* 3379 and *E. coli* O55 and lipopolysaccharides (LPS) of *S. paratyphi* A, *S. cholerae suis*, *S. typhi*, and *S. typhimurium* were kindly supplied by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology (Moscow).

Preparation of MAB. Five BALB/c female 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. Three immunizations were carried out at 1-month intervals. Three days before fusion a boosting intravenous immunization was performed with 100 μg LPS in 0.25 ml phosphate saline buffer (PSB). Animals whose sera had the highest titer of antibodies in enzyme immunoassay (EIA) were used for hybridization. The mice were anesthetized with ether and their spleens were removed under aseptic conditions.

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Hybridization was carried out using a standard method [5]. Double cloning by the limiting dilutions method was carried out; to obtain antibodies in high concentrations the hybrid cells were cultured *in vivo* as ascitic tumors [5]. The initial purification was done by salting out with ammonium sulfate [1], and the subsequent one by ion-exchange chromatography on DEAE-cellulose and by gel filtration in a column packed with Sephacryl S-200 [6]. The capacity for agglutinating whole bacterial cells was tested as described previously [7].

Enzyme immunoassay. O-antigens were dissolved in a concentration of 1 mg/ml, divided into aliquots, and stored frozen at -20°C. EIA was carried out in 96-well Dynatech Microelisa, Nunclon, and Lenmedpolimer polystyrene plates. For saturation of the plates antigen dilutions were used in concentrations of 0.01, 0.1, 0.5, 1.0, 2.5, 5, and 10 μg/ml in PSB, working volume 50 μl. Adsorption was carried out overnight at 4°C or for 3 h at 37°C. EIA was then performed routinely [2]. The substrate of enzymatic reaction was o-phenylenediamine. Results were recorded using Multiscan-Elisa at wavelength 492 nm. Such was the basic EIA scheme used for investigation of murine serum, ascitic fluid, and cell culture supernatants. The class of MAB secreted by hybrid clone cells was determined by EIA using caprine biotinylated sera to the light ("kappa" and "lambda") and heavy chains of murine immunoglobulins IgM, IgG1, IgG2a, IgG2b, and IgG3. The results were assessed visually. The kinetics of MAB binding to antigen in the said EIA system was studied by successive removal of MAB every 10 min after incubation was started. The remaining steps were carried out routinely. 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 suspension of bacterial cells 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, washed with PSB, and 50 μl of 2% glutaraldehyde were added. The mixture was incubated for 1 h at room temperature. The subsequent EIA steps were carried out as described above. For characterization of sugar residues in the binding LPS epitope, periodate oxidation of the antigen was used as described elsewhere [12] in our modification: to 2 ml of antigen solution in a concentration of 5 μg/ml sodium periodate solution was added to attain concentrations 1, 2, 4, and 8 mmol, left for 20 min at room temperature, and then dialyzed against PSB overnight at +4°C. Then 100 μl of sodium borhydrin solution (6 mg/ml in distilled water) were added to the dialysate, incubated for 2 h, and dialyzed overnight at +4°C. The antigen thus treated was used for adsorption in polystyrene plates for EIA.

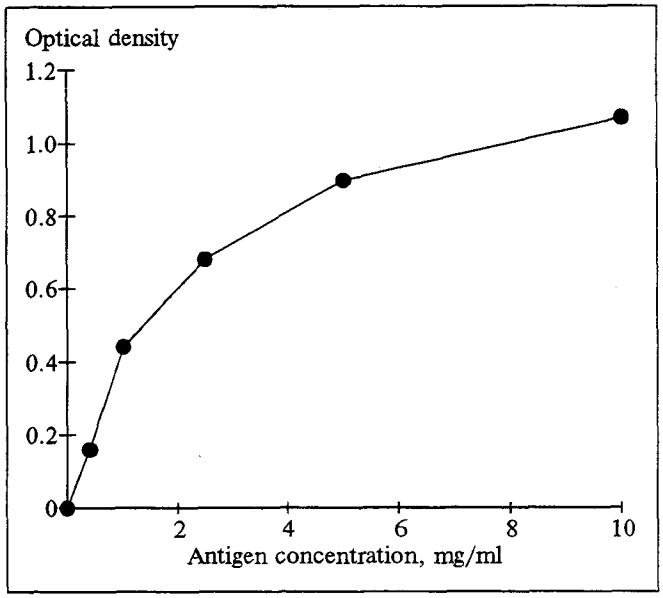

Fig. 1. Testing of samples of E9 clone supernatant. Antibodies to *S. typhimurium* O – antigen.

RESULTS

When working with bacterial antigens in enzyme immunoassay, it is advisable in general that secondary (labeled) immunoglobulins be adsorbed, because an animal might have been naturally immunized and in the event of a "shorting" (direct interaction of the conjugate and bacterial antigen), baseline values may sharply increase. During experiments with the present batch, the background values were quite satisfactory: 0.098 for O-antigen, 5.6% of the mean optical density value.

Sixteen hybrid clones stably producing antibodies of various specificities were prepared. Only

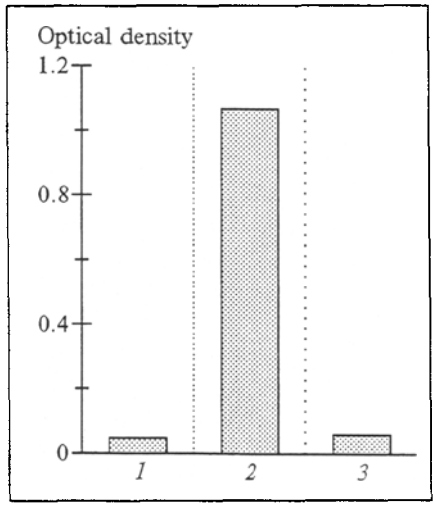

Fig. 2. Interaction of E9 clone antibodies with LPS of various *Salmonella* species. *1*) *S. cholerae suis*, 2) *S. typhimurium*, 3) *S. paratyphi*.

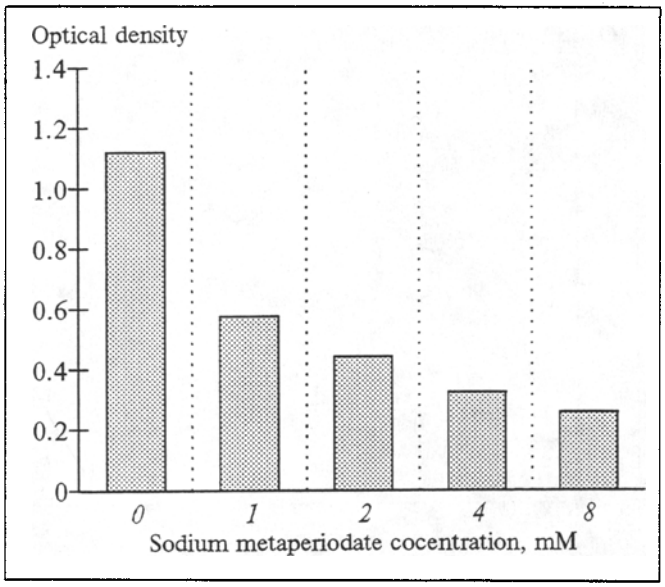

Fig. 3. Interaction of clone E9 monoclonal antibodies with *S.typhimurium* O–antigen pretreated with sodium metaperiodate. Antigen concentration 5 μg/ml, supernatant dilution 1/20.

one of them, clone E9, produced antibodies highly specific to serogroup B.

Results of the study of the activities of clone E9 antibodies in indirect EIA are shown in Fig .1 as exemplified by antigen titration starting from zero concentration to 10 μg/ml. A linear relationship was observed from 0 to 2.5 μg/ml; a plateau was reached after a 5.0 μg/ml concentration. The minimal concentration reliably detected was 10 ng/ml. It is noteworthy that the titration scheme is of a somewhat sigmoid pattern, which indicates a cooperative binding and is evidently explained by the theory of interaction between bivalent and polyvalent antigens when complexes are formed cooperating the subsequent binding [2].

A study of the binding kinetics helped reduce the period of incubation of the said system to just 30 min at 37°C.

The results of EIA indicate that antibodies secreted by the E9 clone are murine immunoglobulins IgG2a with the "kappa" light chain.

These antibodies cannot agglutinate whole bacterial cells.

Successive purification helped prepare sufficiently pure antibodies. The protein content in the resultant fraction was measured spectrophotometrically. On average, 81.7 μg/ml of pure antibodies can be obtained from 1 ml of supernatant of clone E9 hybrid cells.

The results of EIA with whole bacterial cells suggest that MAB to *S. typhimurium* O-antigen can bind to *S. typhimurium* whole cells and do not cross-react with *E. coli,* a representative of the family *Enterobacteriaceae.*

Testing of cross-reactivity with purified LPS of other *Salmonella* species, *S. paratyphi* A and *S. cholerae suis,* showed that it was virtually absent (Fig. 2), although there were common O-antigenic determinants, 1 and 12, in serogroups A and B [4], that is, the binding O-antigen epitope most likely includes the immunodominant sugar of serogroup B antigen 4(5), abeqose [3].

	O-antigen		
S. paratyphi A (A)	1,	2,	12
S. typhimurium (B)	1,	4(5),	12
S. cholerae suis (C1)		6,7	

Epitope analysis. Treatment of antigen with sodium metaperiodate in ascending concentrations was associated with a reduction of binding to MAB (Fig. 3). Periodate oxidizes only glycol groupings, that is, OH-groups located at the adjacent carbon atoms in the cis-position, i.e., on one side of the ring plane. Of the hexoses in the monomeric link of the LPS polysaccharide chain only rhamnose has such a grouping, this indicating its presence in the binding epitope. Hence, the binding epitope commonly containing 6-8 sugar residues includes the following trisaccharide: abequose-mannose-rhamnose.

In conclusion, we may recommend the antibodies produced and described here for use in further studies for investigation of antigenic structure and as a basis for the development of a differential diagnosis of *Salmonella* bacteria.

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