Abstract

Diagnostic Application of Monoclonal Antibodies Against Protein Antigen of *Salmonella Typhi*

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Ten hybrid clones producing monoclonal antibodies (MAbs) specific for *Salmonella typhi* were produced by using the affinity purified *Salmonella typhi* protein (Bp) as immunogen. The MAbs reacted specifically with the 52 kDa flagellin protein component. The MAbs were used to establish a double antibody sandwich ELISA for detection of the 52 kDa flagellin antigen in serum samples from patients with acute typhoid fever. With this assay system, 6.25 ng per ml of flagellin antigen of *S. typhi* could be detected. However, the assay system could not detect the flagellin antigen in patients sera. (MJS 1995; 2: 69 - 73)

Key words: Monoclonal antibodies, protein antigen, *Salmonella*, typhoid fever.

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Introduction

Typhoid fever is caused by enteric bacteria, namely, *Salmonella typhi*. The disease is still a public health problem in developing countries. The clinical manifestations of the disease are usually nonpathognomonic. Thus, the diagnosis is based on clinical suspicion and supported by positive culture and serological finding of paired serum samples. The isolation of *Salmonella* sp. from various body specimens provided a conclusive diagnosis, but it is a time consuming process and sometimes can give false negative result owing to prior antibiotic therapy. Serological diagnosis of *Salmonella* infection has mainly relied on the detection of antibodies. The tube agglutination, Widal test, traditionally has been used in the diagnosis of enteric fever, but several reports have seriously requested its reliability, especially in endemic area because of its cross-reactivity with other bacteria and high prevalence of antibody in normal population.

The confirmative result requires both acute and convalescent phase serum samples that are rarely collected.

Many investigators try to develop new immunological methods for improving the diagnosis of enteric fever. The methods included antigen detection and antibody detection. However, there is no single immunodiagnostic test that can be recommended for rapid, sensitive and specific diagnosis of enteric fever. The test should be directed toward detecting the *Salmonella* antigens antigens of detecting of IgM antibodies against the specific antigens in patients specimens.

In previous reports, we established MAbs which reacted specifically with a 52 kilodalton (kDa) flagellin protein of *S. typhi*. The 52 kDa antigen is a good immunogen for human. This was demonstrated by the presence of IgM antibody to this antigen in acute typhoid sera. Thus, the MAbs were then used to select specific antigen derived from recombinant DNA technology. This recombinant *S. typhi* protein is found to be very useful for detection of specific IgM antibody in acute typhoid patients sera in and endemic area.

In this present study, we try another respect to improve the diagnostic test of typhoid fever by using the specific monoclonal antibodies (MAbs) to detect the flagellin antigen of *S. typhi*. The pooled MAbs were used to establish double antibody sandwich ELISA for detection of the specific antigens in sera from patients with typhoid fever.

MATERIALS AND METHODS

Preparation of *S. typhi* flagellin antigen

Briefly, flagella were detached by exposure of the bacteria to pH 2 with 1 N hydrochloric acid (HCl). After detachment, the flagellin in the supernatant was obtained by centrifugation at 100,000 x g for 1 hour at 4°C The supernatant was adjusted to pH 7.2 with 1 N sodium hydroxide, and the flagellin was precipitated with ammonium sulfate at final concentration of 2.67 M for 16 hours at 4°C Precipitated flagellin was separated by centrifugation at 15,000 x g for 15 minutes and dialyzed against distilled water for 18 hours at 4°C The dialyzed flagellin preparations were then lyophilized and stored at 4°C.

Rabbit IgG antibody anti-*S. typhi* flagellin antigen

The specific IgG antibody was purified from the rabbit serum anti-*S. typhi* flagellin antigen (Biotechnical, Bangkok, Thailand). Briefly, the
rabbit immunoglobulins were initially purified from the rabbit antiserum by 50 % ammonium sulfate precipitation. The rabbit IgG was then further purified from the ammonium sulfate precipitated globulin fraction by DEAE anion exchange column chromatography (Whatman DE 52, Whatman) in 0.02 M phosphate buffer pH 8.0. Appropriate IgG fractions were pooled and its protein content was estimated from the optical density (OD) value at 280 nm, using E 1% 1 cm 280 nm of 14.0. The pooled IgG was dialyzed against 0.1 N ammonium bicarbonate buffer pH 7.4, lyophilized and kept at 4°C.

Protein determination
Protein concentrations were determined by the Folin method by using bovine serum albumin as a standard.

MAbs specific to 52 kDa S. typhi flagellin antigen
MAbs specific to 52 kDa flagellin protein antigen of S. typhi was produced and characterized as previously described. The MAbs reacted specifically to only S. typhi and did not cross react with others bacterial that caused enteric fever like illness. The bacteria are S. typhi, S. paratyphi A, S. paratyphi B, S. paratyphi C, S. choleraesuis, S enteritidis, S. Krefeld, S. panama, S. typhimurium, E. coli, Ps. pseudomallei and Y. enterocolitica. MAbs were partially purified from the culture supernatants by precipitation with 50 % ammonium sulfate.

Clinical specimens
Serum specimens from patients with hemoculture positive for S. typhi (41 patients), S. paratyphi A (27 patients), S. paratyphi B (5 patients), Salmonella group B (23 patients), Salmonella group C (9 patients), Salmonella group D other than S. typhi (1 patient), gram negative bacteria (31 patients) such as Acinetobacter iwoffii, Citobacter diversus, E. coli, Enterobacter sp., Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas sp., gram positive bacteria (18 patients) such as Bacillus sp., Clostridium perfringens, Staphylococcus sp. and Streptococcus sp. and 35 normal sera were included in this study for antigen detection by double antibody sandwich ELISA with our established MAbs.

Double antibody sandwich ELISA for S. typhi flagellin antigen detection
Double antibody sandwich ELISA for antigen detection was performed by coating the microelisa plate with 20 ug per ml of the rabbit IgG anti-S. typhi flagellin antigen in 0.05 M carbonate buffer pH 9.6 at 4°C overnight. After incubation, the plate was washed with 0.05 % Tween in PBS (PBST), blocked with 1 % BSA-PBST at 37°C for 1 hour and washed again. Various concentrations of flagellin protein antigen or patient serum at dilution of 1 : 100 (in 1 % BSA – PBST) was added to the plate and incubated at 37°C for 1 hour. The plate was washed again and 10 ug per ml of pooled MAbs specific to S. typhi was added into the wells and the plate was incubated at 37°C for 1 hour, then washed. The 1:250 dilution of rabbit anti – mouse immunoglobulin – alkaline phosphatase conjugate (Dakopatts) in 1 % BSA – PBST was added. The plate was incubated at 37°C for 1 hour and washed. Then the substrate (p-nitrophenyl phosphate) was added and the absorbance value was read at 405 nm. Each sample was performed in duplicate.
RESULTS AND DISCUSSION

Rabbit IgG antibody anti-*S. typhi* flagellin antigen

An approximately 1 g of pre-swollen DEAE - cellulose was used for purification of IgG normally present in 1 ml of serum. A typhi of the rabbit IgG profile eluted from DEAE anion exchange column chromatography was shown in figure 1.

![Absorbance at 280 nm vs. Cumulative volume (ml)](image)

**Fig. 1** Fractionation of rabbit IgG containing antibody against *S. typhi* flagellin protein antigen on a DEAE - cellulose column.

Double antibody sandwich ELISA for *S. typhi* flagellin antigen detection

The 10 pooled MAbs specific to *S. typhi* flagellin antigen was used to establish a double antibody sandwich ELISA for detection of the 52 kDa flagellin antigen in serum samples from patients with acute typhid fever. With this assay system, as little as 6.25 ng per ml of *S. typhi* flagellin antigen in normal serum could be readily detected.

![Dose response curve obtained from the assay of flagellin protein antigen of *S. typhi* at various concentrations with double antibody sandwich ELISA. This assay could detect approximately 6.25 ng ml of the antigen (arrow).](image)

**Fig. 2** Dose response curve obtained from the assay of flagellin protein antigen of *S. typhi* at various concentrations with double antibody sandwich ELISA. This assay could detect approximately 6.25 ng ml of the antigen (arrow).
detected (Fig.2). However, when the clinical specimens from patients with hemoculture positive for S. typhi (41 patients), S. paratyphi A (27 patients), S. paratyphi B (5 patients), Salmonella group B (23 patients), Salmonella group C (9 patients), Salmonella group D other than S. typhi (1 patient), gram - negative bacteria (31 patients), gram - positive bacteria (18 patients) and 35 normal sera were tested, no positive result could be demonstrated. It is possible that the 52 kDa flagellin epitope is blocked in the form of immune complexes or changed after being metabolized and our MAbs could not recognize.

Up to now, the fusion protein of S. typhi flagellin which was produced and used for detection of specific IgM in acute typhoid patients sera is the most candidate antigen to be used for rapid diagnosis of typhoid fever.15

For further studies, the DNA coding for the specific flagellin epitope of S. typhi will be analyzed and used as DNA probes or DNA primers for diagnosis of the typhoid fever.

References