การประยุกต์ใช้โมโนโคลนัลแอนติบอดีที่จำเพาะต่อโปรตีนของ *Salmonella typhi* เพื่อการวินิจฉัยโรคไข้ทัยฟอยด์

ปัทมา เอกโพธิ์, ปร.ด. (จุลชีววิทยา)
สุภิญญา พงษ์สังข์, วทม. (จุลชีววิทยา)

บทคัดย่อ คณะผู้วิจัยได้ผลิตโมโนโคลนัลแอนติบอดีที่จำเพาะต่อโปรตีนส่วนหางของ *S. typhi* จากเซลล์ลูกผสมจำนวน 10 นิคม โดยโมโนโคลนัลแอนติบอดีเหล่านี้มีความจำเพาะต่อโปรตีนขนาด 52,000 ดัลตัน ของโปรตีนส่วนหางของ S. typhi โดยไม่ทำปฏิกิริยาข้ามกับบักเตรีที่ทำให้เกิดอาการคล้ายไข้ทัยฟอยด์ ซึ่งได้แก่ *S. paratyphi A, S. paratyphi C, S. choleraesuis, S. enteritidis, S.krefeld, S.panama,. S.typhimurium, E.coli, Ps. pseudomallei* และ *Y. enterocolitica*

โมโนโคลนัลดังกล่าวได้นำมารวมกันเพื่อใช้ประโยชน์ในการตรวจหาโปรตีนส่วนหางของ *S. typhi* โดยวิธี double antibody sandwich ELISA ด้วยวิธีดังกล่าว คณะผู้วิจัยได้พัฒนาจนสามารถตรวจหา โปรตีน ส่วนหางของ *S. typhi* ที่มีจำนวนน้อยถึง 6.25 นาโนกรัมต่อมิลลิลิตร ได้ และได้นำวิธีตรวจนี้ไปประยุกต์ใช้เพื่อตรวจหาโปรตีนส่วนหางในซีรั่มคนไข้ต่าง ๆ แต่ก็ไม่สามารถตรวจพบโปรตีนส่วนหางนี้ได้เลย

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

Pattama Ekpo, Ph. D. (microbiology)*
Supinya Pongsunk, MSc. (microbiology)*

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.

* ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ ประสานมิตร
Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Prasarnmit.

Introduction

Typhoid fever is caused by enteric bacteria, namely, *Salmonella typhi.* The disease is still a public health problem in developing countries.[1] The clinical manifestations of the disease are usually nonpathognomonic.[2,3] Thus, the diagnosis is based on clinical suspicion and supported by positive culture and serological finding of paired serum samples.[4] 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.[5,6] 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[7-10] and antibody detection.[11,12] However, there is no single immunodiagnostic test the 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.*[13, 14] 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.[14] 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.[15]

In this present study, we try another espect 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 sreum 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[16] 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.[13] 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 iwoffi*, *Citobacter diversus*, *E. coil*, *Enterobacter sp.*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas sp.*, gram positive bacteria (18 patients) such as *Bacillus sp.*, *Clostidium 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* flagellia 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.

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

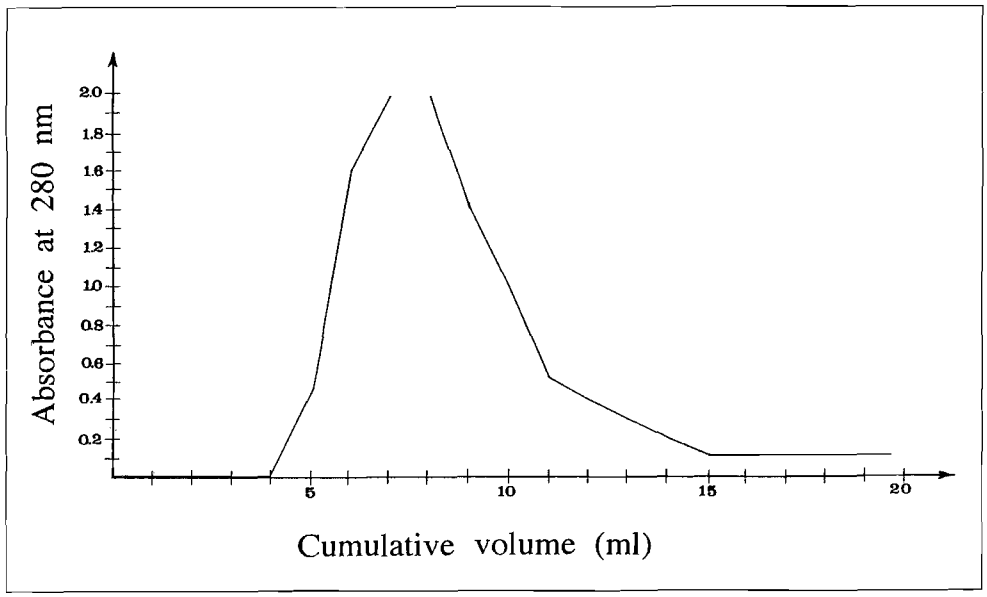

Fig. 1 *Fractionation of rabbit IgG containing antibody against S. typhi flagellin protein antigen on a DEAE - cellulose column.*

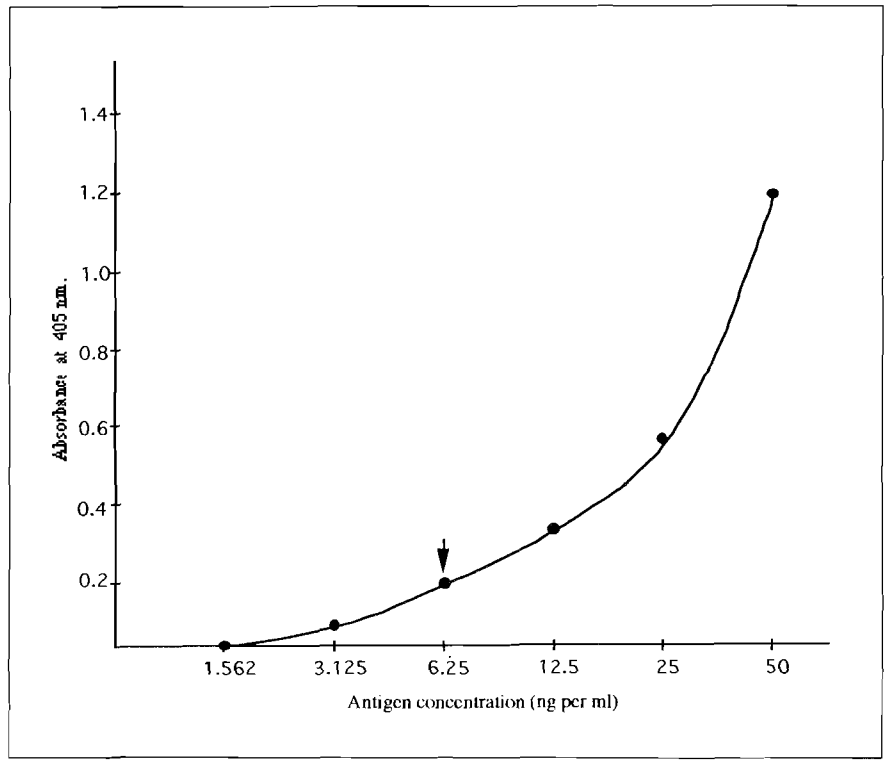

Fig. 2 *Dose response curve obtained from the assay of flagellin protein antigen of S. typhi at various concentrations with double antibody sandwich ELISA. This assay could detect approximately 6.25 ng ml of the antigen (arrow).*

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

1. Abraham G, Teklu B, Gedebu M, Salassie GH, Azene G. Diagnostic Value of the Widal test. Trop Geogr Med 1981; 33:329 - 33.
2. Stuart BM, Pullen RL. Typhoid clinical analysis of three hundred and sixty cases. Arch Intern Med 1946; 78 : 629 - 35.
3. Wicks ACB, Holmes GS, Davidson L. Endemic typhoid fever. Q J Med 1971; 40 : 341 - 5.
4. Wallace JG. Letter Br Med J 1987; 1 : 389 - 90.
5. Levine MM, Grados O. Diagnostic value of the Widal test in endemic area for typhoid fever. Am J Trop Med Hyg 1978; 27 : 795 - 800.
6. Pang T, Puthucheary SD. Significance and value of the Widal test in the diagnosis of typhoid fever in an endemic area. J Clin Pathol (suppl) 1983; 36 : 471 - 5.
7. Sadallah F, Brighouse G, Giudice GD, Dayal RD, Hocine M, Lambert PH. Production of specific monoclonal antibodies to *Salmonella typhi* flagellin and possible application to immunodiagnosis of typhoid fever. J Infect Dis 1990; 161 : 56 - 64.
8. Chaicumpa W, Kuangkunaporn P. Diagnosis of typhoid fever by antigen detection of *Salmonella typhi* antigen in urine. J Clin Microbiol 1992; 30 : 2513 - 15.
9. Sivadasan K, Kurien B, John TJ. Rapid diagnosis of typhoid fever by antigen detection. Lancet 1984;134 - 5.
10. Taylor DN. Harris JR, Barrette TJ, et al. Detection of urinary Vi antigen as a diagnostic test for typhoid fever. J Clin Microbiol 1983 ; 18 : 872 - 6.
11. Ortiz V, Isibasi A, Garcia OE, Kumate J. Immunoblot detection of class specific humoral immune response to outer membrane proteins isolated from *Salmonella typhi* in human with typhiod fever. J Chin Microbiol 1989 ; 27 : 1640 - 5.
12. Nalan CM, Feeley JC, White PC, Hamble EA, Brown EL, Wong KH. Evaluation of a new assay for Vi antibody in chronic carriers of *Salmonella typhi*. J Clin Microbiol 1980; 12 : 22 - 6.
13. Ekpo P, Sarasombath S, Banchuin N, Sirisinha S. Monoclonal antibodies to 52 Kilodalton protein of *Salmonella typhi*. J Clin Microbiol 1990 ; 28 : 1818 - 21.
14. Sukosol T, Sarasombath S, Mongkolsuk S, et al. Molecular cloning and expression of Salmonella typhi flagellin: characterization of 52 kDa specific antigen of *S. typhi*. Asian Pac J Allergy Immunol 1993; 11 : 57 - 69.
15. Sukosol T, Sarasombath S, Songsivilai S, Ekpo P, Rungpitarangsi B, Pang T. Fusion protein of *Salmonella typhi* flagellin as antigen for diagnosis of typhoid fever. Asian Pac J Allergy Immunol 1994 ; 12 : 21 - 5.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RS. Protein measurement with the Folin phenol reagent. J Biol Chem 1951 ; 193 : 265 - 75.