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Production of Monoclonal Antibody Specific to *Campylobacter jejuni* and Its Potential in Diagnosis of Campylobacter Enteritis

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SUMMARY A monoclonal antibody (MAb 3G6) specific for *Campylobacter jejuni* and *Campylobacter coli* was produced by immunizing BALB/c mice with a local strain of *C.jejuni* (28.1). No cross-reactivity was observed with *Enterobacteriaceae* controls. By immunoprecipitation, MAb 3G6 identified a major protein band of molecular weight 45 kDa and also gave a slight reactivity with 30 and 55 kDa proteins. Using an indirect enzyme-linked immunosorbent assay, MAb 3G6 was able to detect *C. jejuni* suspended in stool without cross-reactivity to 14 other enteropathogenic bacteria suspended, normal flora in fecal suspension, or fecal debris. In the analysis of fifty clinical specimens, MAb 3G6 detected most positive samples with the exception of one which possessed very low *Campylobacter* concentration and gave no reactivity to negative samples, demonstrating its high specificity to *C. jejuni and C. coli*. MAb 3G6 may be suitable as a new tool for the development of diagnostic method for *Campylobacter* infection.

Campylobacter jejuni and *Campylobacter coli* are important gastrointestinal pathogens causing acute diarrhea with enteritis in humans, particularly in developing countries.[1-5] They are prevalent in infants under 1 year old[6-9] and rank as the third most common cause of acute diarrhea after Ratavirus and enterotoxigenic *Escherichia coli*.[10,11] In Thailand, a report from the Children Hospital, Bangkok indicated that of 30% bacterial isolation rate, approximately 12% were *Campylobacter* spp. whereas about 10% and 7% were *Shigella* spp and *Salmonella* spp, respectively.[12] Taylor *et al.* detected *Campylobacter* from stool of 105 (18%) of 586 children under 5 years with acute diarrhea in Bangkok.[4] Of these 105 samples, *Campylobacter* spp were isolated as the single pathogen in 50 samples (40%). Most *Campylobacter* strains isolated in developing countries are biochemically and serotypically similar to strains isolated in developed countries and there is little strain variation.[4,13-15] These *Campylobacter* spp may be both enteroinvasive leading to bloody diarrhea and enterotoxigenic causing watery diarrhea.[16] The various clinical patterns suggest that *Campylobacter* spp possess more than one virulence factor. In addition, *C.jejuni,* unlike *C. coli,* are more often associated with symptomatic infections and with bloody diarrhea.[17,18]

Since *Campylobacter* enteritis is a considerable world health problem contributing to morbidity in developed countries and to high mortality rates in children in developing countries, it is of clinical importance to develop a specific and rapid diagnostic assay to identify *Campylobacter* in stool of enteritis patients. The available culture methods for detecting the organism generally are extremely time consuming and costly. Several immunoassay have been developed to detect *Campylobacter* infection. For epidemiological purposes, immunological reagents using polyclonal antibodies[19] and monoclonal antibodies (MAbs) against formalin-treated *C. jejuni* are available for characterization and typing of *C.jejuni* and

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C. coli.[20] However, these methods all require *Campylobacter* colonies isolated from stool culture. An immunological approach for the detection of *C. jejuni* utilizing stool suspension has not been reported.

In this study, MAbs that are highly specific for veronal extracted antigen (VE-Ag) of *C. jejuni* have been prepared. These MAbs reacted with proteins of 20, 45 and 55 kDa and had the ability to react with intact cells of *Campylobacter.* The preliminary assessment of sensitivity and specificity of the MAb was also performed as a mean of determining its usefulness as an immunodiagnostic marker. Furthermore, clinical specimens obtained from diarrheal patients were analyzed to evaluate the real potential use of the MAb in diagnosis.

MATERIALS AND METHODS

Bacterial strains and growth conditions

C. jejuni isolates 28.1, 29.8, 3.8, 51.8 and 29.9, *C. coli* isolates 19 and 23.8 and other *Campylobacter* isolates were kindly provided by Dr. Udom Leksomboon (The Children Hospital, Bangkok, Thailand). *C. jejuni* and *C. coli* standard strains (ATCC 43502 and 43482, respectively) were obtained from the American Type Culture Collection, Rockville, Maryland. The bacteria were inoculated onto blood agar plate and placed in 37°C incubator with an atmosphere of 89% N_2, 5-10% CO_2 and 5% O_2. After 24-48 hours of incubation, bacteria were suspended in brucella broth (Difco Laboratories, Detroit, MI) and spreaded onto columbia agar (Gibco, BRL, Bethesda, MD) supplemented with 10% human blood. After 48 hours the bacteria were stained with Gram stain and examined under light microscope for a seagull-shape bacteria. *E. coli, Proteus mirabilis, Salmonella choleraesuis, Salmonella enteritidis, Salmonella typhimurium, Salmonella paratyphi* A, *Salmonella paratyphi* B, *Salmonella panama, Shigella* spp, *Yersinia enterocolitica, Vibrio parahaemolyticus, Streptococcus pyogenes, and Streptococcus agalactiae* were obtained from Dr Suttipant Sarasombath, Department of Immunology, Faculty of Medicine Siriraj Hospital, Bangkok.

Preparation of antigens

C. jejuni and *C. coli* VE-Ag were prepared as originally described by Barber, Vladoianu and Dimache with some modifications.[21] In brief, each bacterial isolate was cultured on columbia agar and allowed to incubate for 48 hours in 37°C incubator with 5-10% CO_2. The bacterial cells were harvested and 3 volumes of acetone were added. The suspension was centrifuged at 10,000 x g, 4°C for 5 minutes and a pellet was washed three times with acetone. Ten grams of acetone-dried cells were suspended in 200 ml of 0.15 M veronal buffer, pH 8.4 and the suspension was allowed to stand with continuous stirring at room temperature overnight. After centrifugation at 12,000 x g at 4°C for 20 minutes the supernatant was removed and dialysed against distilled water at 4°C for 2 days. The dialysed supernatant was filtered through a 0.45 μm Seitz filter (Gibco, BRL) to remove residual bacteria and other cellular debris. Other bacterial antigens were prepared the same way. Protein content was determined by Folin-Ciocalteau method.[22]

Immunization of mice

VE-Ag of *C. jejuni* 28.1 was selected to immunized mice because a good antibody response was obtained with low toxicity. Three six week-old female BALB/c mice were immunized subcutaneously with 100 μg of antigen emulsified in complete Freund's adjuvant (Difco). On day 14 and 21, 100 μg of the same antigen suspended in incomplete Freund's adjuvant were injected intraperitoneally. Animals were bled and antibodies against VE-Ag were quantitated by indirect enzyme-linked immunosorbent assay (ELISA) before fusion. Five days before fusion, a booster dose of 250 μg of antigen in saline was injected intraperitoneally.

Fusion Procedure

Fusion of the splenocytes of a high responding BALB/c mouse (anti-VE-Ag titre, 1/28,000) with the plasmacytoma cell line P3-X63.Ag 8.653 was performed in a ratio of 10:1 in a solution containing 37% (wt/vol) polyethylene glycol (Gibco, BRL) by the method of Margulies[23] with modifications. Fused cells at a concentration of 1×10^5 cells/ml were dispensed in 96-well tissue culture plate (NUNC, Denmark) which contained a feeder layer of 10^4 murine peritoneal macrophages. Cells were grown in RPMI 1640 supplemented with 15% fetal calf serum (Flow Lab, Mississauga, Ontario, Canada), 2 mM L-glutamine and 1% hypoxanthine-aminopterin-thymidine (HAT) (Gibco, BRL).

Medium was changed on day 7, substituting HAT with hypoxanthine-thymidine (Sigma Chemical Co, St. Louis, MO). Approximately on day 15, supernatants of wells containing growing clones were tested for MAb directed against VE-Ag. The antibody producing cells were twice recloned by limiting dilution. A clone designated 3G6 was one of the three selected for further study.

Determination of immunoglobulin class

Ig isotyping was done by an antibody capture ELISA using isotype and class-specific antisera (Immuno Select™ Isotyping System) (Gibco, BRL). Briefly, 50 μl of a monoclonal rat anti-mouse isotype antibody was used to coat the surface of a microtitration plate and acted as the capture antibody. Nonspecific binding sites were blocked with 3% bovine serum albumin (BSA) in phosphate bufffered saline (PBS) (0.01 M sodium phosphate buffer pH7.4, 0.14 M NaCl) and 150 μl of hybridoma culture supernatant to be tested was added. After incubation and washing, 150 μl of rat anti-mouse immunoglobulin-alkaline phosphatase conjugate were added to each well and a color development using alkaline phosphatase substrate p-nitrophenyl phosphate was performed. The intensity of the color is proportional to the amount of antigen in the test sample.

ELISA procedure

Screening of hybridoma culture supernatants and detection of *Campylobacter* in clinical specimens were done by indirect ELISA. U-bottom microtitration plates (Immunolon II, Dynatech Industries, Inc., Alexandria, VA) were coated with 100 μl of 10 $\mu g/ml$ *C. jejuni* VE-Ag or whole-cell suspension (10^6-10^{10} cfu/ml) or 10% fecal suspension in carbonate buffer, pH 9.6, and kept for either 4 hours at 37°C or 16 hours at 4°C Any remaining protein binding sites in the plate were saturated by adding 100 μl of 5% milk (Carnation) in PBS. The plates were washed 3 times with PBS containing 0.05% Tween 20 (Sigma) (PBS-T). Culture supernatants (150 μl) from the fusion plates were added. After incubation for 4 hours at 37°C, the plate were washed 3 times with PBS-T. One hundred microlitres of goat anti-mouse immunoglobulin horseradish peroxidase conjugate (Dakopatts, Copenhagen, Denmark) diluted 1:2,000 in PBS-T were added. The plate were incubated for 1 hour at 37°C. After washing, 100 μl of 3,3' 5,5'-tetramethylbenzidine solution (0.1 mg/ml in 0.1 M sodium acetate-0.1 M citric acid (pH 5.7) with 0.005% H_2O_2) were added to each well and incubated for 20 minutes at room temperature. The color was developed in the dark for 20 minutes at room temperature and the reaction was stopped by adding 25 μl of 4 M H_2SO_4. The optical density was determined on ELISA reader (Minireader II, Dynatech) at 450 nm. An optical density >25% of maximum reading of positive control was considered positive.

SDS-PAGE

Sodium dodecyl sulfate-10% polyacrylamide gels were prepared as described by Laemmli[24] using 0.75 mm-thick gels. Molecular weight standards (14-205 kDa) (Bio-Rad Laboratories, Richmond, CA) were included in each gel. The gel system consisted of a 10% separating gel and 3% spacer gel. Samples of veronal extracted antigens and immunoprecipitations were dissolved in sample buffer, boiled for 3-5 minutes and then applied to the gel. Electrophoresis was carried out with a constant current of 30 mA.

Antigen labelling and immunoprecipitation

Campylobacter antigens were radiolabelled with ^{125}I (Amersham Internationl Limited, UK) by the chloramine-T technique originally described by Greenwood *et al.*[25] In brief, pH of antigen suspension was adjusted by adding 1/19 volume of 20x concentration of Tris-HCI pH 8.0. Radioactive NaI was added to the antigens at a ratio of 2 $\mu Ci/\mu g$ protein. Then chloramine T was added (0.2 $\mu g/\mu g$ protein) and the reaction mixture was incubated at 4°C for 30 seconds. The labelling reaction was stopped by adding excess amounts of sodium metabisulfite (0.2 $\mu g/\mu g$ chloramine T), NaI radioactive carrier (approximately 10 times the amount of radioactive NaI used) and 1 μg of BSA/μg protein.

Immunoprecipitation of ^{125}I-labelled *C.jejuni* antigens was performed using a technique slightly modified from that described by Ivarie and Jones.[26] To reduce non-specific binding, the ^{125}I-labelled antigens were first preadsorbed with a 50% slurry protein-A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden). After centrifugation at 10,000 x g at 4°C for 1 minute, the supernatant was carefully removed and divided equally into 5-ml snap cap tubes (Falcon, Becton Dickenson, Lincoln Park, NJ). Then the supernatant containing labelled antigens was allowed to react with concentrated (6X) MAb, pooled irrelevant hybridoma culture supernatants and pooled immune sera from mice immunized with VE-Ags of *C. jejuni*. The reaction was incubated at 37°C for 1 hour and then kept overnight in the refrigerator. Immune complexes were separated from non-reacted antigens by the addition of a precalibrated amount of protein A-Sepharose. After 1 hour incubation at room temperature, the immune complexes were washed once with NETT (0.1 M NaCl, 0.5 M EDTA and 0.1 M Tris-HCI pH 7.4), twice with NETT buffer and twice with 1% BSA in NETT buffer. The Sepharose beads with the attached immune complexes was suspended in SDS-PAGE buffer and analyzed by SDS-PAGE. Autoradiography was performed using X-OMAT XR film (Eastman Kodak Co, Rochester, NY)

Clinical specimens

Fecal specimens from patients with acute diarrhea (50 samples) were specimens routinely submitted to the Children Hospital, Bangkok. The specimens were collected in brucella broth containing 0.1 mg/ml of soybean trypsin inhibitor in 50 mM EDTA. Control feces consisted of 5 samples from children (age range 3 months to 2 years). A 10% fecal suspension was made and 100 μl was used to coat U-bottomed microtitre plate (Dynatech) for *Campylobacter* detection by indirect ELISA. A filtration culture method adapted from that of Steele and McDermott[9] was also performed in parallel at the Children Hospital Laboratory, Bangkok since this method is used routinely to diagnose *Campylobacter* infection. In brief, several drops of fecal suspension were applied directly onto a sterile 0.45 μm membrane filter (Millipore Corp, Bedford, MA) placed on columbia blood agar base without an-tibiotics. The filter was removed after 15 minutes and the plate was incubated microaerobically at 42°C for 24 to 48 hours. *Campylobacter* were able to dart through the filter to produce colonies on the agar surface. Subsequently, Gram stain was performed to search for seagull-shaped bacteria. In addition, specimens were inoculated onto culture plates with antibiotic containing media.[27]

RESULTS

Antibody-producing hybridomas were obtained from 2 different fusions. A total of 736 culture supernatants were screened and only 3 hybridomas producing specific antibodies reacting with VE-Ag and intact cells of *Campylobacter* were detected. One stable hybridoma designated 3G6 which produced high antibody titre reacted specifically with *C.jejuni* and with lower binding capacity to *C. coli* but not with other *Enterobacteriaceae* used in the initial screening.

Isotyping revealed that MAb 3G6 is of IgG1 class and has a kappa light chain. Using an indirect ELISA, only 0.5 μg of MAb 3G6 could successfully detected intact *Campylobacter* cells. The MAb was highly reactive to *Campylobacters* as with an O.D. of >1.0 for all isolates (suspended in PBS) tested. The specificity of MAb was determined by ELISA using sonicated antigens and intact cells of other several strains of pathogenic enteric bacteria. None of the enteric bacteria tested reacted with MAb 3G6 (O.D. less than 0.1).

In immunoprecipitation analysis, MAb 3G6 was shown to recognize 30, 45 and 55 kDa polypeptide bands as shown in Fig. 1. The MAb reacted strongly with the 45 kDa antigen and showed slight reactivity with the 30 and 55 kDa proteins.

To evaluate whether MAb 3G6 has potential in rapid diagnosis for *Campylobacter* infection, different concentrations of whole intact cells of *C. jejuni* and *C.coli* were suspended in 10% stool suspensions from 3 normal children aged 3 months to 4 years old. Stool suspensions with 10^5-10^{10} cfu/ml of *C.jejuni* and *C.coli* gave positive reactions detected by indirect ELISA when compared to stool suspension alone (Fig. 2). However, the mean optical densities of samples containing stool suspension mixed with *Campylobacter* were constantly less than those of samples containing the bacteria suspended in PBS (Fig. 2). In addition, MAb 3G6 could recognize various

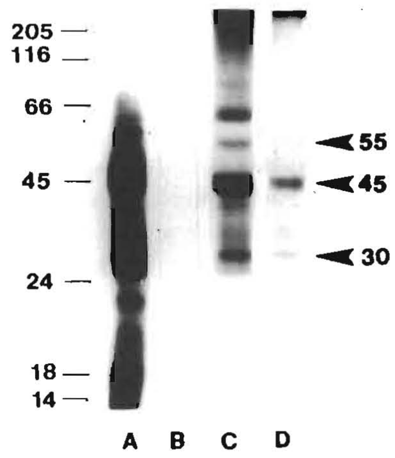

Fig. 1. Molecular weight determination of specific antigens bound to anti-*C. jejuni* antibodies. Autoradiographic patterns of ^{125}I-labelled sonicated antigens of *C. jejuni* are shown in lane A. The ^{125}I-labelled antigens were immunoprecipitated with different anti-*C. jejuni* antibodies and subsequently separated on SDS-PAGE. Lane B, the labelled antigens reacted with irrelevant hybridoma culture supernatants represents negative control; lane C, the labelled antigens reacted with polyspecific immune sera; lane D, the labelled antigens reacted with MAb 3G6. Numbers on the left indicate the molecular weight standards. Arrows show 30, 45 and 55 kDa protein bands.

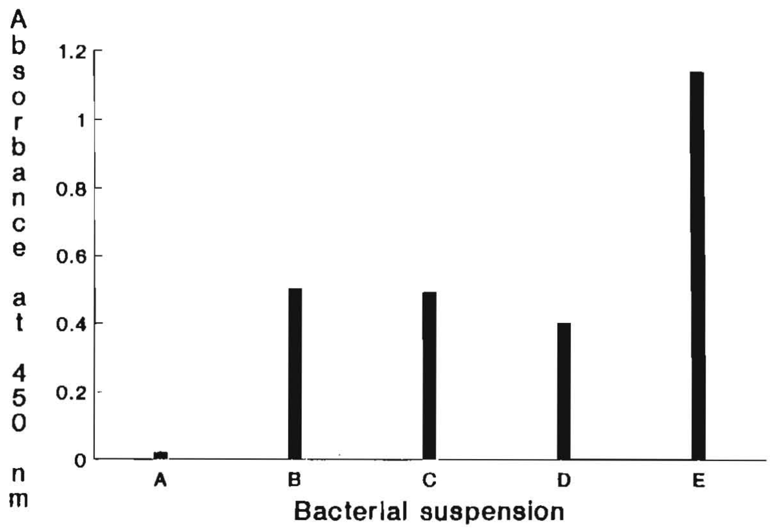

Fig. 2. Detection of *C. jejuni* and *C. coli* by ELISA ELISA results demonstrate mean optical density of reaction between MAb 3G6 and *C. jejuni* suspended in 3 different normal stool suspension (B, C, D) and in PBS (E). The stool suspension without *C. jejuni* was used as negative control (A).

isolates of *Campylobacter* in 10% stool suspension as exemplified for seven of them in Fig. 3.

To exclude the possibility that MAb 3G6 would react with pathogenic enteric bacteria in stool suspension, stool was suspended with approximately 10^5 cfu/ml of each of 14 pathogenic enteric bacteria tested. Mean optical densities at 450 nm ranged from 0.02 to 0.08 for other enteric bacteria whereas those of *Campylobacter* were more than 1. Hence, no cross reactivity of MAb 3G6 with 14 other species of enteric bacteria was detected.

A double blinded study was performed to evaluate the potential application of MAb 3G6 in diagnosis of *Campylobacter* infection. Fifty stool specimens were obtained from the Children Hospital from November to December 1991. *C. jejuni* and *C. coli* were isolated using direct culture on antibiotic containing medium in 7 and by filtration method in 4 of 50 specimens. The indirect ELISA results demonstrated that 6 of these 7 samples were positive (Fig. 4). Three stool specimens which had very low *Campylobacter* concentration ($<10^3$ cfu/ml) were negative using filtration method. Of these three samples, one failed to be detected by indirect ELISA. All samples negative on direct culture, gave mean optical density readings less than 0.25. In addition, the optical density readings at 450 nm correlated to the number of *Campylobacter* found in stool.

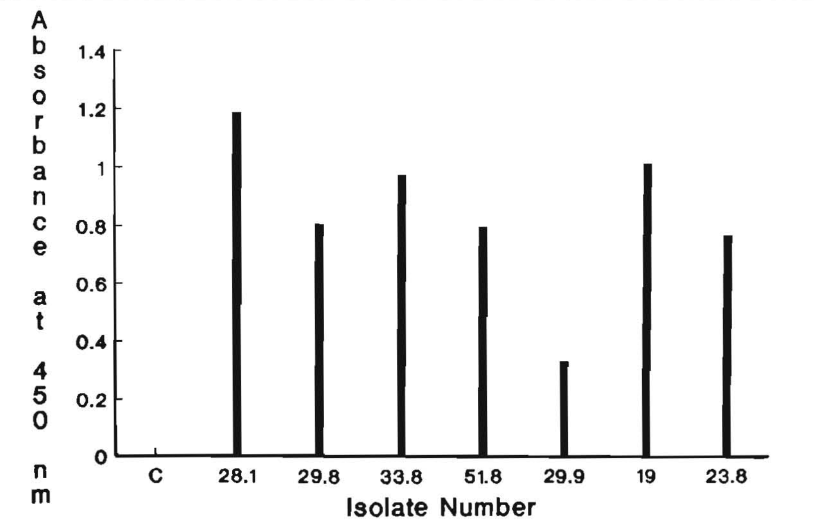

Fig. 3. The ability of MAb 3G6 in detecting different isolates of *Campylobacter*. ELISA results show mean optical density at 450 nm of specific binding of MAb 3G6 to *C. jejuni* (strains 28.1, 29.8, 33.8, 51.8, 28.9) and *C. coli* (19.1, 23.8) suspended in a normal stool suspension. The stool suspension without *Campylobacter* was used as negative control (C).

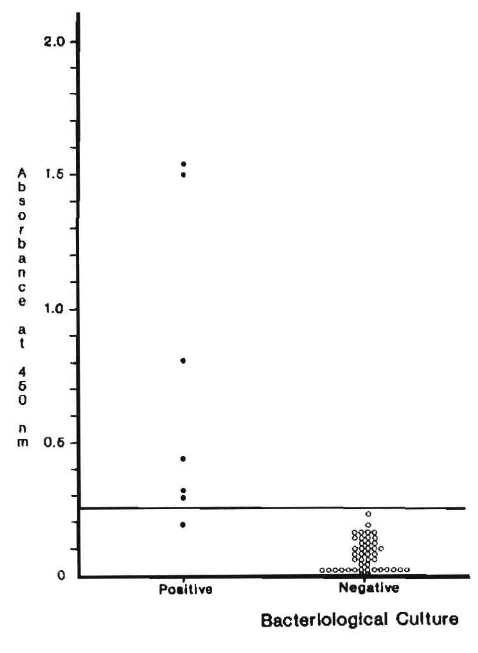

Fig. 4. Evaluation of the potential application of MAb 3G6 in diagnosis of *Campylobacter* diarrhea. Distribution of mean optical density readings of fifty stool samples from children with diarrhea, obtained from the Children Hospital, Bangkok, Thailand, during November to December 1991 were analysed by indirect ELISA. Seven of 50 stool specimens were positive by culture on the antibiotic selective media. Negative means stool samples that gave negative results when cultured on the selective media. Horizontal line is the ELISA cut-off value (O D =0.25).

DISCUSSION

In this study, we immunologically identified a specific polypeptide of 45 kDa of *C. jejuni* protein. A MAb (3G6) that reacted with 45 kDa band did not react with other 14 strains of enteric bacteria. These isolates were selected for testing because they are known to cause gastroenteritis and might interfere in an immunologic assay for detection of *Campylobacter* in stool specimens.

The MAb recognized intact cells of *Campylobacter*. Therefore, the determinants or epitopes to which MAb binds on the antigen appear to be exposed or partially exposed on the cell surface. Preliminary data suggest that the antigen may be major outer membrane proteins of *C. jejuni*.[28-32] The major outer membrane proteins of different isolates of *C. jejuni* have reported molecular weight of 41-45 kDa.[29,33] Other proteins identified such as protein of relative molecular weight of 30 kDa which contains cross-reactive epitopes with the major outer membrane protein were also identified by Logan and Trust.[34] However, reactivity of MAb 3G6 to outer membrane will need to be confirmed.

Potential application of MAb 3G6 in diagnosis of *Campylobacter* diarrhea was evaluated. For specificity, indirect ELISA results demonstrated that MAb 3G6 gave no cross-reactivity with 14 other species of enteropathogenic bacteria and reacted with all isolates of *Campylobacter* tested. However, when suspending *Campylobacter* in stool of normal children, we found that the ability of MAb 3G6 to detect *Campylobacter* decreased, compared to those suspended in PBS. The decline in optical density readings may be due to the competitive binding of *Campylobacter* with other fecal flora or stool components to the limited surface area in the U-bottomed well. Although competitive surface saturation might not cause measurable effect for stool specimens from patients age under 12 months due to low concentration of fecal flora and fecal debris, a double antibody sandwich ELISA was performed in an attempt to elimiate the problem. Unfortunately, high background was observed when using biotinylated-polyclonal and -monoclonal antibodies to detect bacteria in stool samples.

The results of indirect ELISA using MAb 3G6 suggested that it is preferable to utilize MAb in the detection of *Campylobacter* in stool because of its high specificity. Previous report also stated the cross-reactivity of polyclonal antibodies raised against *Campylobacter* antigens with other fecal flora and enteric bacteria.[35]

Additionally, MAb 3G6 was unable to distinguish strains of *C. coli* from those of *C. jejuni*. This demonstrated how closely related these two bacterial species are. Our results are in agreement with a previous study by Mills *et al.*[31,36] which showed that antigen preparations of *C. jejuni* and *C. coli* gave very similar characteristic pattern when analyzed by SDS-PAGE. Furthermore, the two bacterial species produce similar symptoms and are sensitive to the same antibacterial agents. Therefore, it is unnecessary to differentiate *C. jejuni* from *C. coli* for clinical diagnosis, although it will be important for epidemiological studies. It is also important to note that this study was focused only on *C. jejuni* and *C. coli* infections because they are the most common species of the genus *Campylobacter* that have been isolated from stool specimens of patients.[19] Other species such as *C. laridis, C. fetus* subsp. *fetus* and *C. upsaliensis* are rarely isolated.[5]

Our results demonstrated that MAb 3G6 has significant diagnostic potential for detection of whole cells of *C. jejuni* and *C. coli*. Indirect ELISA failed to detect *Campylobacter* when its concentration in stool was less than 10^3 cfu/ml. Nevertheless, such low number of bacteria may not be associated with diarrhea, as in developing countries there are multiple pathogens encountered.[4] Furthermore, there is no reported clear-cut association between the number of *Campylobacter* and disease pattern. There are, however, frequent isolation of *Campylobacter* from asymptomatic infection.[4] It remains to determine the specificity of MAb 3G6 to various species and isolates of *Campylobacter* using clinical specimens from patients suffering from bacterial enteritis.

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REFERENCES

1. Chowdhury MNH. *Campylobacter jejuni* enteritis:a review. Trop Geoge Med 1984;36:215-22.
2. Skirrow MB. *Campylobacter* enteritis-a 'new' disease. Br Med J 1977;2:9-11.
3. Smilbert RM. The genus *Campylobacter*. Ann. Rev. Microbiol. 1978;32:673-709.
4. Taylor DN, Escheverria P, Pitarangsi C, Seriwatana J, Bodhidatta L, Blaser MJ. Influence of strain characteristics and epidemiology of *Campylobacter* infections in Thailand. J Clin Microbiol 1988; 26, 5:863-8.
5. Walker RI, Caldwell MB, Lee EC, Guerry P, Trust TJ, Ruitz-Palacis GM. Pathophysiology of *Campylobacter* enteritis. Microbiol. Rev. 1986;50:81-94.
6. Bokkenheuser VD, Richardson NJ, Bryner JH *et al.* Detection of enteric campylobacteriosis in children. J Clin Microbiol 1979;9:227-32.
7. Glass RI, Stoll BJ, Huq MI, Struelens MJ, Kibriya AKMG. Family studies of *Campylobacter jejuni* in Bangladesh: implications of pathogenesis and transmission. In Peterson AD, Skirrow MB, Rowe B, Davies JR, Jones DM, eds., *Campylobacter* II: Proceedings of the Second International Workshop on *Campylobacter* Infections. London: Public Health Laboratory Service. 1984:141-2.
8. Glass RI, Stoll BJ, Huq MI, Struelens MJ, Blaser M, Kibriya AKMG. Epidemiologic and clinical features of endemic *Campylobacter jejuni* infection in Bangladesh. J Infeect Dis 1983;148:292-6.
9. Steele TW, McDermott SN. The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. Pathol 1984;16:263-5.
10. Klipstein FA, Enert RF, Short H, Schenk EEA. Pathogenic properties of *Campylobacter jejuni* : assay and correlation with clinical manifestations. Infect Immun

1985;50:43-9.

11. Olusanya O, Adebayo JO, Williams B. *Campylobacter jejuni* as a bacterial cause of diarrhea in Ile-Ife, Nigeria. J Hyg 1983;91:77-80.

12. Leksomboon U, Jiamwatanasuk N, Sriwattana N, Prommalee C, Ritarangkla V. Mode of transmission of *Campylobacter* diarrhea in urban children. Bull Dept Med Serv 1989;14:615-26.

13. Georges-Courbt MC, Baya C, Beraud AM, Meunier DMY, Georges AJ. Distribution and serotypes of *Campylobacter jejuni* and *Campylobacter coli* in enteric *Campylobacter* strains isolated from children in the Central African Republic. J Clin Microbiol 1986;23:592-4.

14. Karmali MA, Penner JL, Flemin PC, Williams A, Hennessy JN. The serotype and biotype distribution of clinical isolates of *Campylobacter jejuni / coli* over a three year period. J Infect Dis 1983;147: 243-6.

15. Patton CM, Barrett TJ, Morris GK. Comparison of the Penner and Lior methods for serotyping *Campylobacter* spp. J Clin Microbiol 1985;22:588-565.

16. Duffy MC, Benson JB, Rubin SJ. Mucosal invasion in *Campylobacter* enteritis. Am J Clin Pathol 1980;73:706-8.

17. Cadranal S, Rodesch P, Butzler JP, Dekeyser P. Enteritis due to related *Vibrio* in children. Am J Dis Child 1957;126:152-5.

18. Newman A, Lambert JR. *Campylobacter jejuni* causing flare-up in inflamatory bowel diseases. Lancet 1980;2:919-21.

19. Nachamkin I, Barbagallo S. Culture confirmation of *Campylobacter* spp by latex agglutination. J Clin Microbiol 1990;28: 817-8.

20. Kosunen TU, Ban BE, Hurme M. Analysis of *Campylobacter jejuni* antigens with monoclonal antibodies. J Clin Microbiol 1984;19:129-33.

21. Barber C, Vladoianu IR, Dimache G. Contributions to the study of *Salmonella:* immunological specificity of proteins separated from *salmonella typhi*. Immunol 1966;11:287-96.

22. Kabat E, Mayer M. Estimation of protein with the Folin-Ciocalteau phenol reagent. In: Thomas CC, ed, Experimental immunochemistry. Springfield, USA, 1961: 556-8.

23. Margulies DH. Regulation of immunoglobulin expression in mouse myeloma cells. Cold Spring Harbor Symposium on Quantitative Biology 1977;41:781-91.

24. Laemmli U. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.

25. Greenwood FC, Hunter WM, Glover JS. The preparation of I-131 labelled human growth hormone of high specific radioactivity. Biochem J 1963;89:114-23.

26. Ivarie RD, Jones PP. A rapid, sensitive assay for specific protein synthesis in cells and cell-free translation: use of *Staphylococcus aureus* as an adsorbent for immune complexes. Anal Biochem 1979;97:24-35.

27. Doyle MP, Roman DJ. Recovery of *Campylobacter jejuni* and *Campylobacter coli* from inoculated foods by selective enrichment. Appl Environ Microbiol 1982; 43:1343-53.

28. Austen RA, Trust TJ. Outer membrane protein composition of *Campylobacter* In: Newell DG, ed., Proceedings of the International Workshop on Campylobacter Infections. Reading, England: MTP Press, 1982:225-9.

29. Blaser MJ, Hoppins JA, Berka RM, Vasil ML, Wang WL. Identification and characterization of *Campylobacter jejuni* outer membrane proteins. Infect Immun 1983; 42:276-84.

30. McCoy EC, Wiltberger HA, Winter AJ. Major outer membrane protein of *Campylobacter fetus* : physical and immunological characterization. Infect Immun 1976;13:1258-65.

31. Mill SD, Bradbury WC. Human antibody response to outer membrane proteins of *Campylobacter jejuni* during infection. Infect Immun 1984;43:739-43.

32. Nachamkin I, Hart AM. Western blot analysis of the human antibody response to *Campylobacter jejuni* cellular antigens during gastrointestinal infection. J Clin Micrbiol 1985;21:33-8.

33. Newell DG, McBride H, Pearson AD. The identification of outer membrane proteins and flagella of *Campylobacter jejuni*. J Gen Microbiol 1984;130:1201-8.

34. Logan SM, Trust TJ. Molecular identification of surface protein antigens of *Campylobacter jejuni*. Infect Immun 1983;42:675-782.

35. Wu SL, Pacheco ND, Oprandy JJ, Rollwagen FM. Identification of *Campylobacter jejuni* and *Campylobacter coli* antigens with mucosal and systemic antibodies. Infect Immun 1991;59:2555-9.

36. Mills SD, Bradbury WC, Penner JL. Isolation and characterization of a common antigen in *Campylobacter jejuni* and *Campylobacter coli*. J Clin Microbiol 1986;24:69-75.