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Production and Characterization of Monoclonal Antibodies against the O-5 Antigen of *Salmonella typhimurium* Lipopolysaccharide

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Three murine monoclonal antibodies (MAbs) were produced by fusion of P3X63-Ag8.653 myeloma cells and splenocytes of a mouse immunized with heat-attenuated (20 min, 80°C) *Salmonella typhimurium* cells. MAbs 5A5 and 5B2 were of the immunoglobulin M (IgM) class, while MAb 4A8 was IgG2a. All possessed the κ light chains. The MAbs were specific to the lipopolysaccharide (LPS) O-5 antigen of *Salmonella* B serogroup, as determined by electrophoresis followed by immunoblotting. All MAbs recognized the same epitope, as determined by an additive enzyme-linked immunosorbent assay (ELISA), although IgM MAbs exhibited higher avidity than the IgG MAb. ELISA analyses revealed that all three MAbs reacted with *S. typhimurium* (LPS O:1, 4, 5, and 12) while failing to recognize *S. typhimurium* var. copenhagen (LPS O:1, 4, and 12). The MAbs reacted equally with live and heat-attenuated *Salmonella* B serovars containing LPS O-5 antigen. The ability of the MAbs to detect live bacterial cells was further confirmed by transmission electron microscopy. Treatment of bacteria with cholic acid and extremely low pH did not affect antibody binding to *S. typhimurium*. However, when *S. typhimurium* cells were exposed to alkaline conditions prior to reaction with all three MAbs, no binding was observed. The use of MAbs to discriminate between *S. typhimurium* and *S. typhimurium* var. copenhagen in meat samples was investigated.

Salmonella typhimurium has been recognized worldwide as the most common *Salmonella* serovar incriminated in food poisoning over the last 100 years (23). The largest outbreak of waterborne salmonellosis reported in the United States was associated with this pathogen 30 years ago in California (3). Ten years ago, a massive outbreak of *S. typhimurium* caused by contaminated milk occurred in Chicago, which involved more than 16,000 cases of human salmonellosis (3). Between 1985 and 1991, *S. typhimurium* was the most prevalent pathogen implicated in foodborne salmonellosis in the United States, accounting for more than 21% of the total *Salmonella* isolates (20). Also, in Europe, the increase in prevalence of *S. typhimurium* in foodborne diseases continued to be significant (25). In Canada, this serovar accounted for the highest number of *Salmonella* isolates implicated in recent foodborne diseases from 1992 to 1993 (13). Foods of animal origin, particularly beef and turkey, are the major causes of most cases of human salmonellosis caused by *S. typhimurium* (4).

S. typhimurium can be classified into serotypes based on differences in the chemistry of their lipopolysaccharide (LPS) O antigen. By using passive hemagglutination and phage typing techniques (1, 6), two types of serological variants of *S. typhimurium* have been identified, an O-5 antigen carrier type (LPS O:1, 4, 5, and 12) and an O-5 antigen noncarrier type (LPS O:1, 4, and 12), also known as *S. typhimurium* var. copenhagen. The latter variant is believed to be involved in salmonellosis of domestic fowl. Outbreaks caused by this pathogenic strain have been reported in broiler chickens and pigeons (9, 22). Since *S. typhimurium* infections of animals play an important role in zoonosis, it has been suggested that the serological differentiation of the types of *S. typhimurium* by specific antibodies could be an effective approach for the identification of the source of infection by this pathogen (29).

Few attempts have been made to produce monoclonal antibodies (MAbs) against *S. typhimurium*. Tsang and coworkers (25) reported the production of six MAbs to *Salmonella* B serogroup. Two of them cross-reacted with *Salmonella paratyphi* A of serogroup A, while four of these antibodies were found to be specific to LPS O-4. The latter were used to serotype heat-attenuated *Salmonella* strains in the slide agglutination assay. Moreover, Lee and coworkers (17) developed an enzyme-linked immunosorbent assay (ELISA) for detection of *S. typhimurium* in food. This assay utilized a polyclonal antiserum as capture antibodies and a monoclonal detector antibody. Although the reported MAb was more specific than polyclonal antibodies, it exhibited some cross-reaction with other *Salmonella* serotypes. Recently, MAbs against heat-attenuated *S. typhimurium* reactive with the LPS O-5 antigen have been developed (29). The latter antibodies showed various reactions with the serovars that may carry the O5 antigen.

In this paper, we describe the production and characterization of three MAbs that are specific to LPS O-5 of B serogroup *Salmonella* strains. These antibodies bind with equal avidity to live and heat-attenuated *Salmonella* cells and can be used to develop an ELISA for serological discrimination between both types of *S. typhimurium* and *S. typhimurium* var. copenhagen.

MATERIALS AND METHODS

Materials. LPSs from *S. typhimurium*, *Salmonella enteritidis*, and *Escherichia coli* were purchased from Sigma Chemical Co. (St. Louis, Mo.). Alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Ig), 20-nm gold-conjugated anti-mouse Ig, and the mouse type subisotyping kit were from Bio-Rad (Richmond, Calif.). Myeloma cells P3X63.Ag8.653 were obtained from the American Type Culture Collection (Rockville, Md.). Polyethelyene glycol 4000 (catalog no. 9727) was from Merck. Falcon Microtest III polyvinyl chloride flexible 96-well plates were purchased from Canlab (Mississauga, Ontario, Canada). All other chemicals and reagents were of analytical grade.

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TABLE 1. Specificity of MAbs for *S. typhimurium* as assessed by ELISA[a]

Group	Species	Source[b]	O antigen formula	A_{405}		
				MAb 4A8	MAb 5B2	MAb 5A5
Salmonella serovars						
B	*S. typhimurium* ATCC 13311	ATCC	1,4,[5],12	1.839	1.776	1.905
	S. heidelberg	UM	1,4,[5],12	1.822	1.840	1.887
	S. stanley	UM	1,4,[5],12,27	1.856	1.823	1.887
	S. paratyphi B	UM	1,4,[5],12	1.863	1.860	1.855
	S. albert	EITC	4,12	0.061	0.095	0.046
	S. typhimurium var. copenhagen	LCDC	1,4,12	0.075	0.066	0.136
	S. kingston	EITC	1,4,12,27	0.038	0.058	0.063
	S. agona	EITC	1,4,12	0.031	0.077	0.025
	S. brandenburg	EITC	1,4,12	0.018	0.084	0.032
C_1	*S. mbandaka*	EITC	6,7	0.068	0.089	0.081
	S. thompson	UM	6,7	0.038	0.116	0.050
	S. choleraesuis	EITC	6,7	0.028	0.087	0.048
C_2	*S. muenchen*	EITC	6,8	0.036	0.069	0.040
	S. hadar	EITC	6,8	0.024	0.062	0.028
D_1	*S. pullorum* ATCC 19945	ATCC	6,8	0.051	0.178	0.049
	S. gallinarum	UM	1,9,12	0.044	0.111	0.027
	S. berta ATCC 8392	ATCC	1,9,12	0.036	0.089	0.040
	S. enteritidis PT4	LCDC	1,9,12	0.036	0.062	0.033
	S. enteritidis PT8	LCDC	1,9,12	0.031	0.071	0.056
	S. enteritidis PT13	LCDC	1,9,12	0.029	0.058	0.057
	S. enteritidis PT1	LCDC	1,9,12	0.026	0.054	0.045
	S. enteritidis PT13a	LCDC	1,9,12	0.033	0.086	0.044
D_2	*S. maarssen* ATCC 15793	ATCC	9,46	0.029	0.082	0.039
E_1	*S. anatum*	EITC	3,10	0.026	0.092	0.065
E_3	*S. thomasville*	EITC	3,15,34	0.020	0.099	0.033
E_4	*S. senftenberg*	EITC	1,3,19	0.032	0.062	0.024
F	*S. rubislaw*	EITC	11	0.020	0.065	0.033
G_2	*S. havana*	EITC	1,13,23	0.050	0.085	0.019
Atypical	*S. arizona*	UM		0.025	0.148	0.056
Other	*Yersinia enterocolitica*	EITC		0.023	0.159	0.024
	Shigella flexneri	EITC		0.015	0.099	0.013
	E. coli ATCC 25992	ATCC		0.008	0.084	0.018
	E. coli ATCC 11775	ATCC		0.019	0.129	0.039
	Citrobacter freundii ATCC 8090	ATCC		0.028	0.072	0.021
	Enterobacter cloacae	EITC		0.013	0.076	0.015
	Pseudomonas fluorescens	EITC		0.048	0.061	0.021
	Mycobacterium fortuitum	EITC		0.008	0.073	0.012

[a] Bacterial cells heat treated for 20 min at 80°C were used as the coating antigen in ELISA.
[b] ATCC, American Type Culture Collection, Rockville, Md. UM, University of Manitoba, Winnipeg, Manitoba, Canada; EITC, Economic Innovation and Technology Council, Winnipeg, Canada; LCDC, Laboratory Center for Disease Control, Ottawa, Canada.

Bacteria and growth conditions. Stock bacterial cultures were maintained on standard plate count agar (SPC) slants at 4°C until use. To prepare a sufficient amount of *Salmonella* cells, a loopful of stock culture from SPC slants was inoculated into 5 ml of nutrient broth and incubated overnight at 37°C. One milliliter of broth was then transferred into 250 ml of M-broth and incubated with gentle shaking for an additional 16 h at 37°C. Non-*Salmonella* bacteria were grown in brain heart infusion broth at 37°C. All bacteria were harvested by centrifugation (9,000 × *g*, 10 min), washed, and resuspended in 50 ml of saline (0.8% NaCl). Cells were further diluted with 0.1% peptone water and enumerated on SPC spread plates as CFU. For ELISA, the cell suspension was heat treated (20 min, 80°C) and diluted to the required concentration with 0.05 M carbonate buffer (pH 9.6). Live bacteria were prepared by washing SPC slants with saline. The number of viable cells were determined as CFU from SPC plates after 24 h of incubation. All bacterial species used in this study are shown in Table 1.

Contaminated food samples. Beef and chicken were obtained from local retail outlets and divided into 25-g samples. Each sample received 0.1 ml of sterile saline containing approximately 0, 10, 10^2, and 10^3 CFU of either *S. typhimurium* or *S. typhimurium* var. copenhagen (determined by viable plate counts). All samples were then enriched, cultured, and prepared for ELISA analysis according to the Official Methods of Analysis (2). ELISA was performed as described below.

LPS preparation. *Salmonella* LPS extracts were prepared by the modified method of Johnson and Perry (12). Briefly, 3 g of freeze-dried bacteria was resuspended in 50 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 5 mM EDTA and 0.05% sodium azide (buffer L) and sonicated five times for 45 s each at 4°C in Braun Sonic 1510 (B. Braun, Melsungen, AG) set at 300 W. The resulting suspension was incubated with pancreas RNase and DNase (0.1 μg/ml) in 20 mM $MgCl_2$ for 10 min at 37°C, followed by another 10 min at 60°C. The suspension was then mixed with an equal volume of preheated 90% phenol, incubated for 15 min at 70°C, and centrifuged at 18,000 × *g* for 1 h, yielding aqueous and phenol phases. The aqueous phase was collected and dialyzed against distilled water until no detectable phenol odor remained. The LPS extracts were then lyophilized and stored at −20°C until use.

Production of MAbs. A group of six female BALB/c mice (6 to 8 weeks old) were immunized five times with heat-attenuated (80°C, 20 min) *S. typhimurium* cells at 1-week intervals. Initially, 100 μl of cell suspension (10^8/ml) was injected subcutaneously. The following four injections were performed intraperitonally. Mice were killed 3 days after the final injection.

The fusion was performed essentially as described by Goding (8). Spleen cells were fused with P3X63-Ag8.653 myeloma cells at a ratio of 3:1 in RPMI 1640 with 50% (wt/vol) polyethylene glycol 4000 as a fusing agent. Two weeks after the fusion, hybridomas were screened for antibody production by ELISA with attenuated cells of *S. typhimurium* ATCC 13311, *S. enteritidis* PT4, and *E. coli* ATCC 25995 as antigens. Hybridomas that reacted only with *S. typhimurium* were identified and cloned at least twice by limiting dilution.

Once established, the hybridoma lines were expanded in tissue culture flasks and frozen in liquid N_2 for future use. The MAb-secreting clones 5A5, 4A8, and 5B2 were propagated either in tissue culture or as ascitic fluid by the procedure of Harlow and Lane (10).

The isotyping of MAbs was performed on MAbs from culture supernatants with a mouse monoclonal isotyping kit according to the manufacturer's instructions.

Immunochemical techniques. (i) ELISA. Screening of antisera, spent medium,

TABLE 2. Detection of *S. typhimurium* in artificially contaminated meat by MAb 4A8-based ELISA

Sample	Inoculum (cells/25 g)	A_{405}[a]	
		S. typhimurium	*S. typhimurium* var. copenhagen
Beef	0	0.024	0.024
	10	0.192	0.045
	10^2	0.550	0.041
	10^3	1.725	0.039
Chicken	0	0.026	0.026
	10	0.104	0.032
	10^2	0.194	0.016
	10^3	0.550	0.023

[a] Values are based on triplicate experiments. A value of >0.1 is considered positive.

and ascites for the presence of antibodies to *S. typhimurium* was performed by an indirect noncompetitive ELISA with polyvinyl chloride Microtest III plates (8). Plates were coated with 0.1 ml of either heat-attenuated or live bacterial cells (10^8 cells/ml) diluted in 0.05 M carbonate buffer (pH 9.6) per well. To analyze meat samples for the presence of *S. typhimurium*, microtiter plates were coated with 0.1 ml of enriched M-broth per well.

When LPS was used as an antigen, microtiter plates were coated with 0.1 ml of poly-L-lysine (20 μg/ml) in 0.01 M phosphate-buffered saline (PBS; pH 7.2) per well for 30 min at 37°C. Then, the plates were coated with 0.1 ml of LPS (10 μg/ml) in distilled water per well. Coating with all antigens was carried out overnight at 4°C. Alkaline phosphatase-conjugated goat anti-mouse Ig and sodium *p*-nitrophenyl phosphate were used as secondary antibodies and substrate, respectively.

(ii) Additive index ELISA. Additive index ELISA was performed on paired MAbs 5B2 and 5A5, 5B2 and 4A8, and 5A5 and 4A8 as described by Friguet and coworkers (7). An additivity index for each pair of MAbs was calculated according to the formula $\{[2A_{1+2}/(A_1 + A_2)] - 1\} \times 100$, where A_1, A_2, and A_{1+2} are absorbance values with antibody 1 alone, antibody 2 alone, and the two antibodies together, respectively.

(iii) Electrophoresis and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a discontinuous buffer system with a 15% separating gel as described by Laemmli (16). About 20 μl (0.5 μg/μl) of sample was used per lane. Gels were either silver stained by the method of Kittelberger and Hilbink (14) or used for electroblotting. Immediately after completion of the electrophoretic run, LPSs were transferred electrophoretically from the SDS-PAGE gel to a nitrocellulose (NC) membrane by the method of Weintraub et al. (28). The electroblotting was carried out for 16 h at a constant current (180 mA, 4°C) in a Transblot Cell (Bio-Rad, Richmond, Calif.). After electrotransfer, the NC membrane was blocked with 3% gelatin in 0.02 M Tris-buffered saline (TBS; pH 7.5) and incubated with appropriate MAbs (5A5, 5B2, and 4A8) diluted 1:10 in TBS overnight at room temperature. The NC membrane was developed with alkaline phosphatase-conjugated goat anti-mouse Ig and a Nitro Blue Tetrazolium/5-bromo-4-chloro-3-indolyl phosphate substrate. Color development was stopped by rinsing the membranes with distilled water.

(iv) Dot blot assay. Dot blotting was performed in a Bio-Dot SF microfiltration apparatus (Bio-Rad) with an NC membrane. About 100 μl of *S. typhimurium* whole-cell suspension (10^7 cells per ml) was spotted on the membrane and pulled through by passive filtration by applying gentle vacuum. After being washed three times with TBS, the membranes were incubated in 5% NaOH or in 38% HCl for 10 s or left untreated. Immunoblotting was performed as described above.

(v) Immunoelectron microscopy. Immunolabelling was performed essentially as described by Cloeckaert et al. (5) but with some modifications. Briefly, 5 μl of bacterial cell suspension in distilled water (5×10^8 cells per ml) was placed on Formvar-coated nickel grids. After being air dried for 2 h at room temperature, the grids were first blocked in PBS containing 3% bovine serum albumin (BSA) for 30 min at 37°C and then incubated with ascitic fluid diluted to 1:200 in PBS-Tween 20 for 2 h at 37°C. Next, the grids were incubated with colloidal gold (20 nm)-conjugated goat anti-mouse Ig diluted 1:25 in dilution buffer (0.02 M Tris, 150 mM NaCl, 0.1% BSA, 0.005% Tween 20, 0.4% gelatin [pH 9]) for 20 h at room temperature. Grids were viewed with the Hitachi H-7000 transmission electron microscope (Hitachi Scientific Instruments, Mountain View, Calif.).

RESULTS AND DISCUSSION

Three fusions which resulted in about 400 hybridomas were performed to produce MAbs against *S. typhimurium*. Approximately 10% of the hybridomas secreted antibodies reactive with *S. typhimurium*. Of these, only three hybridomas secreted antibodies with high specificity to *S. typhimurium*. These clones, MAbs 5A5, 5B2, and 4A8, were propagated either in tissue culture flasks or as ascitic fluid, purified, and used for further studies. MAbs 5A5 and 5B2 were of the IgM class, while MAb 4A8 was of the IgG2a type. All possessed the κ light chains, as determined by a mouse type subisotyping assay.

The specificity of the MAbs was determined by noncompetitive ELISA with various heat-attenuated (80°C, 20 min) bacteria (Table 1). Antibodies reacted prominently with four serovars belonging to serogroup B (*S. typhimurium* ATCC 13311, *Salmonella heidelberg*, *Salmonella stanley*, and *Salmonella paratyphi* B). None of these antibodies reacted with *S. typhimurium* var. copenhagen or the nine other *Salmonella* serogroups (C1, C2, D1, D2, E1, E3, E4, F, G, and atypical *Salmonella arizona*). In addition, all three antibodies showed no reactivity to several members of the family *Enterobacteriaceae* (*Yersinia enterocolitica*, *Citrobacter freundii*, *E. coli*, *Enterobacter cloacae*, and *Shigella flexneri*) and other bacteria (*Pseudomonas fluorescens* and *Mycobacterium fortuitum*).

It appeared that the MAbs were specific only towards *Salmonella* B serovars containing the LPS O-5 antigen, which is a unique factor for some B serovars such as *S. typhimurium* ATCC 13311, *S. stanley*, *S. heidelberg*, and *S. paratyphi* B. This factor is a result of chemical modification of factor 4 and contains an acetyl group that is linked to the abiquose residue (11).

The use of MAbs to discriminate among variants of *S. typhimurium* (O-5 antigen type and variant copenhagen) was studied with MAb 4A8 and artificially contaminated beef and chicken (Table 2). The viable-cell count on the inoculum used for meat samples indicated that samples were inoculated at 10 to 10^3 cells per 25 g. All samples containing *S. typhimurium* gave positive results, while meat samples inoculated with *S. typhimurium* var. copenhagen were negative by ELISA. The results show that MAbs can be useful for serological differentiation of *S. typhimurium* types.

Furthermore, all MAbs were analyzed for the antigenic specificity by SDS-PAGE (Fig. 1A) followed by immunoblotting (Fig. 1B to D). Two LPSs extracted from *S. typhimurium* (Fig. 1, lanes 1) and *S. typhimurium* var. copenhagen (Fig. 1, lanes 2) were used for electrophoretic studies. Both LPSs have the same tetrasaccharide repeating unit, but the latter LPS lacks factor 5. In addition, LPS from *S. enteritidis* (Fig. 1, lanes 3), which shows the same trisaccharide backbone as B serogroup LPSs, and LPS from *E. coli* (Fig. 1, lanes 4), which lacks

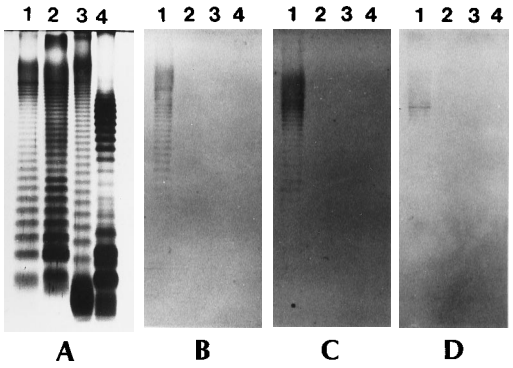

FIG. 1. SDS-PAGE and immunoblot of LPSs extracted from *S. typhimurium*, *S. typhimurium* var. copenhagen, *S. enteritidis*, and *E. coli* (lanes 1 through 4, respectively). (A) Silver-stained gel. (B to D) Immunoblots of the corresponding LPSs with MAb 5A5 (B), MAb 5B2 (C), and MAb 4A8 (D).

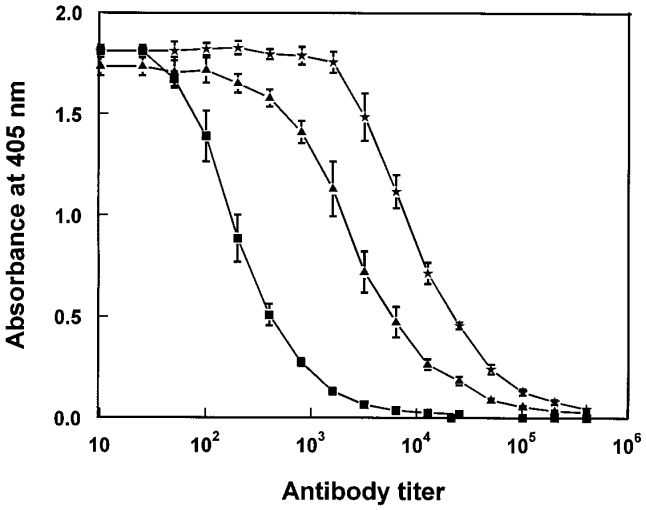

FIG. 2. Reactivities of MAbs 5A5 (★), 5B2 (▲), and 4A8 (■) with heat-attenuated (80°C, 20 min) whole cells of *S. typhimurium* ATCC 13311. Each point represents the mean of three replicates, and the bars represent the standard deviation of the mean.

the structural similarities, were used. Silver staining of SDS-PAGE gels (Fig. 1A) revealed that all LPSs are heterogeneous, with bands in a form of ladder-like patterns typical for smooth, gram-negative bacteria (30). These bands represent the LPS molecules containing increasing lengths of O antigen chains.

The specificity of MAbs for LPS O antigen was confirmed by immunoblotting (Fig. 1B to D). The antibodies reacted only with *S. typhimurium* strains that contained the O:1, 4, 5, and 12 antigen (Fig. 1B to D, lanes 1) while failing to recognize the LPS from *S. typhimurium* var. copenhagen as well as from *S. enteritidis* and *E. coli* (Fig. 1B to D, lanes 2, 3, and 4, respectively). Moreover, all three MAbs selectively bound to the LPS present in the upper region of the gel. This indicates that the binding sites were in the long-chain O antigen region bearing the O-acetyl group.

The avidity of MAbs to their epitopes was analyzed by ELISA. The titration curves obtained for the three MAbs are shown in Fig. 2. It appeared that IgM MAbs exhibited higher avidity than the IgG MAb. The pentameric character of IgM may account for this difference. It is also worthwhile to notice that at a dilution of 1:100,000, the IgM MAbs still reacted with the cells, giving an absorbance value of 0.2, while the IgG MAb gave the same absorbance at a dilution of 1:1,000.

To determine whether the MAbs recognize the same epitope, all MAbs were tested by additivity index ELISA (7). In this assay, an additivity index value close to zero indicates that two tested antibodies recognize the same or two closely associated epitopes, while a high value shows the simultaneous binding of both antibodies to distinct epitopes. Table 3 shows results of the additivity ELISA for all three MAbs. Low additivity index values for all MAbs suggest that these MAbs most likely recognized the same epitopes or epitopes of similar structures.

TABLE 3. Additivity indices for MAbs 5A5, 5B2, and 4A8 as assessed by ELISA

MAb	Additivity index		
	5A5	5B2	4A8
5A5	—	7.99	5.56
5B2	7.99	—	1.24
4A8	5.56	1.24	—

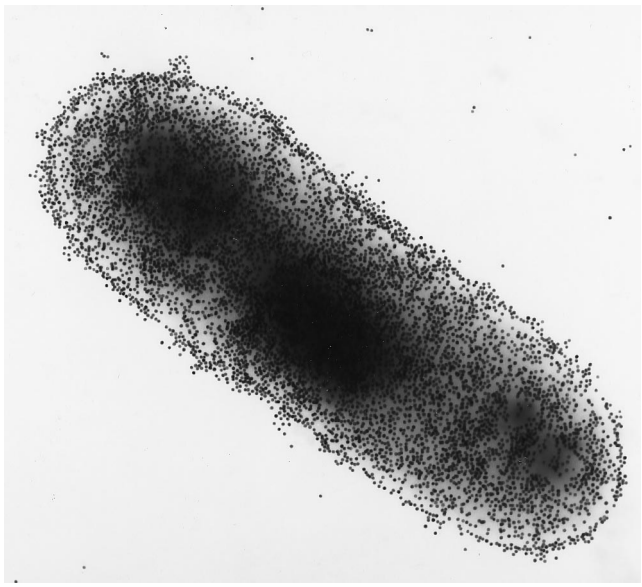

FIG. 3. Transmission electron micrograph of *S. typhimurium* ATCC 13311 probed with MAb 4A8 followed by goat anti-mouse Ig conjugated to 20-nm gold spheres. Magnification, ×17,000.

All antibodies were also highly reactive with live *Salmonella* B serovars containing O-5 antigen but failed to react with bacteria lacking the LPS O-5 factor. These findings suggest that LPS O-5 antigenic sites are easily accessible by MAbs without a need for heat treatment in order to expose the epitopes. The LPS O antigen structure of *Salmonella* serogroup B may account for this phenomenon, as a result of the protrusion of the O-acetyl group that forms factor 5 (18).

The ability of MAbs to detect live *S. typhimurium* was further confirmed by immunoelectron transmission microscopy. Figure 3 shows labelling of an *S. typhimurium* ATCC 13311 live cell by colloidal gold via MAb 4A8. When *S. typhimurium* var. copenhagen was used for immunogold staining, no labelling was observed. Similar results were obtained with the other two MAbs. A noteworthy density of gold spheres covered a whole bacterial cell, suggesting that factor O-5 is expressed at a high frequency and accounts for a significant part of the LPS external leaflet. This appeared to be consistent with other reports on the distribution of LPS within the bacterial outer membrane. Lugtenberg and Van Alphen have reported that the LPS leaflet may account for 50 to 70% of the surface of the outer monolayer (19). Moreover, the LPS O antigen in *S. typhimurium* may consist of more than 70 repeating units (21).

Binding of live *S. typhimurium* makes all three MAbs suitable for the isolation of this pathogen from food, environmen-

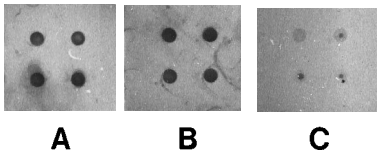

FIG. 4. Dot blot assay of whole cells of *S. typhimurium* ATCC 13311 (10^8 cells per ml) probed with MAb 5A5. (A) No treatment; (B) cells treated with 38% HCl for 10 s prior to incubation with MAb; (C) cells treated with 5% NaOH for 10 s prior to incubation with MAb. *S. typhimurium* cells were spotted on an NC membrane in quadruplicate.

TABLE 4. Effect of cholic acid on the reactivity of MAbs with whole cells of *S. typhimurium* and *S. heidelberg* and LPS from *S. typhimurium*

Cholic acid (%)	A_{405}								
	S. typhimurium			*S. heidelberg*			LPS		
	4A8	5B2	5A5	4A8	5B2	5A5	4A8	5B2	5A5
0	1.536	1.562	1.398	1.867	1.882	1.872	1.887	1.923	1.937
1	1.285	1.750	1.763	1.829	1.858	1.916	1.822	1.859	1.916
5	0.939	1.501	1.170	1.834	1.863	1.902	1.799	1.863	1.870
10	0.805	1.239	1.012	1.838	1.850	1.889	1.674	1.828	1.794
13.5	1.049	1.268	0.767	1.806	1.850	1.916	1.811	1.849	1.864

tal, and clinical samples in an ELISA capture system. MAbs immobilized on magnetic beads or dipsticks can be used directly as an immunocapture probe to isolate live *S. typhimurium* cells from samples prior to enrichment, making a selective enrichment step unnecessary (27).

Since all MAbs have been shown to be specific to bacterial LPS, the effects of various treatment such as detergent and extreme pH on the reactivity of antibodies were further investigated. The effect of cholic acid is shown in Table 4. Cholic acid and its derivatives are frequently used to treat gram-negative bacteria in order to expose LPS to binding antibodies (26). In our studies, cholic acid appeared to have mostly an adverse effect on the binding of all three MAbs to *S. typhimurium* ATCC 13311, although a slight increase in absorbance values was observed for binding of MAbs 5B2 and 5A5 to *S. typhimurium* treated with 1% cholic acid. It seemed that the decrease in the absorbance values caused by higher concentrations of cholic acid was related to the adverse effect on bacterial cells rather than LPS molecules, since free LPS extracted from *S. typhimurium* was not affected. Treatment of *S. heidelberg* with cholic acid also had no effect on binding of this pathogen to MAbs.

Furthermore, the acid treatment had a negligible effect on an enhancement of detection of *S. typhimurium* ATCC 13311 by MAbs, as assessed by dot blotting, although the antigenicity of LPS O-5 was conserved (Fig. 4). This is in disagreement with other findings that prolonged acid treatment can destroy the acetyl group (29). In contrast, when *S. typhimurium* ATCC 13311 whole-cell suspension was spotted on an NC membrane and exposed to alkaline conditions, no detection by all three MAbs was observed (Fig. 4C). Komisar and Cebra (15) suggested that alkaline pH could structurally alter the O-acetyl group linked to the abiquose residue. Since this group forms factor 5, it is most likely that alkaline pH also rendered changes in the antigenicity of this epitope. It is worthwhile to note, however, that the effect of pH on antibody binding to bacterial cells is dependent on the nature of the antibodies. Todd and coworkers (24) have reported that acid and alkaline treatment of bacterial cells was useful to expose epitopes and enhance an antigen-antibody interaction for certain anti-LPS antibodies, while it proved ineffective for other types of antibodies.

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