INFECTION AND IMMUNITY, Jan. 1987, p. 211–216 Vol. 55, No. 1
0019-9567/87/010211-06$02.00/0
Copyright © 1987, American Society for Microbiology

A Murine Monoclonal Antibody Specific for the Outer Core Oligosaccharide of *Salmonella* Lipopolysaccharide

RAYMOND S. W. TSANG,[1]* K. H. CHAN,[1] P. Y. CHAU,[1] K. C. WAN,[1] M. H. NG,[1] AND SIEGFRIED SCHLECHT[2]

Department of Microbiology, University of Hong Kong, Hong Kong,[1] and Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany[2]

Received 26 June 1986/Accepted 11 October 1986

Immunoglobulin G3 murine monoclonal antibody T6 specific for the lipopolysaccharide of *Salmonella* O serogroups A to E was established. By using R mutants of *Salmonella* spp., *Escherichia coli*, and *Shigella* spp., the major reactive epitope with T6 was tentatively identified as the terminal disaccharide, *N*-acetylglucosamine $\xrightarrow[\alpha]{1,2}$ glucose, of the core oligosaccharide. T6 was reactive with 10 clinical isolates of each of the *Salmonella* O serogroups A to E but not with 58 isolates of other gram-negative bacteria. Its selective reactivity against *Salmonella* spp. renders T6 a potentially more useful reagent than the conventional polyvalent serum for the identification of *Salmonella* spp. It may also serve as a useful molecular tool for the study of the outer core structure of all *Salmonella* and related species.

The lipopolysaccharides (LPS) of *Salmonella* spp. and other members of the family *Enterobacteriaceae* are complex molecules comprising three structurally and serologically distinct regions, i.e., a lipid moiety, a core oligosaccharide, and the O-specific side chain. The O-specific side chain is made up of oligosaccharide molecules that determine the O specificity of the organism. The core oligosaccharide may be further divided into the inner and outer core regions. The inner core is common to many enterobacterial species and is made up of heptose and 2-keto-3-deoxyoctonate (KDO), with the latter residues serving as a linkage to the lipid A moiety. The outer core polysaccharide appears to be common to all or almost all *Salmonella* species. Although there are over 2,000 serologically distinct species, it is believed that in *Salmonella* species there is only one core structure (25) which shows serological cross-reactivity with some members of the *Enterobacteriaceae* (20).

In this communication, we describe murine monoclonal antibody (MAb) T6, which selectively reacted with *Salmonella* spp. of O serogroups A to E. By using various R mutants of *Salmonella* spp. and *Escherichia coli* with known outer core structures, the antigenic specificity of T6 was studied. The results suggest that T6 reacts with the terminal di- or trisaccharide of the outer core oligosaccharide of *Salmonella* LPS. Given the specificity of T6 for this structure, the potential applications of T6 are discussed.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *Salmonella typhi* 620Ty, an aromatic-dependent mutant (7) with well-developed O, H, and Vi antigens when grown in a complex medium such as brain heart infusion, was a kind gift from B. A. D. Stocker, Department of Medical Microbiology, Stanford University Medical School, Stanford, Calif. *Salmonella* Ra to Re mutants, *E. coli* and *Shigella* rough mutants bearing the R1 to R4 and K-12 core structures, and the isogenic *Salmonella typhimurium* enterobacterial common antigen (ECA) mutants were from the strain collection of Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany. All other bacteria used in this study were recent clinical (blood, stool, urine, and pus) isolates obtained from the clinical microbiology laboratory of the University of Hong Kong, and they were identified by standard biochemical and serological methods (12). Bacteria used for immunization, whole-cell radioimmunoassay (RIA), and preparation of sonic cell lysates were grown in static culture in brain heart infusion broth (Oxoid Ltd., London, England) for 16 to 18 h at 37°C. Cells were then inactivated with 0.5% Formalin before being harvested by centrifugation at 5,000 × *g* for 15 min; harvested bacteria were washed twice with large volumes of sterile phosphate-buffered saline (PBS).

Production and characterization of MAbs. MAbs were produced by the method of Köhler and Milstein (10). Briefly, BALB/c mice were immunized intraperitoneally with a 0.1-ml suspension of acetone-fixed cells of *S. typhi* 620Ty (10^9 cells per ml). The immunization was repeated weekly for 2 months, and 3 days after the last injection the spleen cells from one of the mice that gave the highest antibody response were fused with the NSI myeloma cells by use of polyethylene glycol (molecular weight, 4,000; BDH, Poole, England). Hybridomas producing antibodies to *S. typhi* were screened by enzyme-linked immunosorbent assay (1) by using *S. typhi* 620Ty ultrasonic lysate-coated microtiter plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.) and horseradish peroxidase-conjugated anti-mouse immunoglobulin G (heavy and light chain) (Tago, Inc., Burlingame, Calif.). Hybridomas producing specific antibodies were cloned twice by limiting dilution, and purified hybridomas were either expanded for antibody production in 250-ml tissue culture flasks (Becton Dickinson Labware, Oxnard, Calif.) or injected into mineral oil-primed mice for the production of ascitic fluid. MAbs from culture supernatant or ascitic fluid were purified by 50% ammonium sulfate precipitation. The immunoglobulin class and subclass and the light-chain pattern of the MAb were determined by double immunodiffusion by using anti-mouse immunoglobulin antisera obtained from Nordic Immunological Laboratories, Tillburg, The Netherlands.

Isolation of LPS. LPS from smooth strains were isolated by the hot phenol-water method of Westphal and Jann (24); LPS from rough strains were isolated by the phenol-chloroform-petroleum ether method of Galanos et al. (2).

* Corresponding author.

TABLE 1. Study by dot RIA of the effects of different treatments on the reactivity of *S. typhi* Ty2 LPS antigen with MAb T6

Treatment[a]	T6 reactivity, mean cpm of ^{125}I-protein A bound (range)[b]
None	7,558 (7,433–7,618)
Heat	7,384 (6,954–7,911)
Heat with 4% SDS	7,562 (7,448–7,620)
Heat with 5% 2-mercaptoethanol	7,443 (7,062–7,715)
Heat with 4% SDS and 5% 2-mercaptoethanol	7,522 (7,224–7,835)
10 mM sodium *meta*-periodate oxidation	264 (241–280)

[a] Heat treatments were performed at 100°C for 10 min.
[b] Results are the means of triplicate determinations.

Whole-cell RIA. Formalin-fixed and washed cells were adjusted to an optical density of 1.20 at 600 nm and were then boiled for 30 min at 100°C or used without boiling for testing of binding with the MAb. Samples of 0.5 ml of the cell suspension were dispensed into small polystyrene vials (40 by 6 mm; Sterilin, Feltham, England), and cells were spun down in an Eppendorf centrifuge (12,000 rpm). The supernatant was removed by suction, and the cells were suspended with mixing in either 400 μl of MAb-containing culture supernatant or 400 μl of a 1:1,000 dilution (in 5% bovine serum albumin [BSA]-PBS) of ascitic fluid. After 2 h of incubation at 37°C, cells were washed four times with 0.85% saline–0.05% Tween 20 (saline-Tween). Protein A (Sigma Chemical Co., St. Louis, Mo.) was radiolabeled with ^{125}I (Amersham Corp., Aylesbury, England) to a specific activity of 800 cpm/ng by the method of Greenwood et al. (6) and was added to each vial in a 400-μl volume containing 40 ng of protein A and incubated at 37°C for 2 h. After four final washes with saline-Tween, the amount of radioactivity bound to the cells was counted in a Beckman Gamma 4000 counter (Beckman Instruments, Inc., Palo Alto, Calif.). Each test was done in duplicate, with unused culture medium or 5% BSA-PBS as a negative control. For each strain tested, the positive-to-control ratio was calculated as counts per minute bound in the presence of antibody divided by counts per minute bound in the absence of antibody.

Dot RIA. Samples of 5 μl of either LPS (1 mg/ml) or bacterial ultrasonic lysates were spotted onto dots of nitrocellulose paper held in wells of a flat-bottom microtiter plate, and the antigen solution was left to dry at room temperature. After unbound sites in the nitrocellulose paper were saturated with 5% BSA-PBS, MAb-containing culture supernatant was added (100 μl per dot) and left to react at 37°C for 2 h. After four washes with saline-Tween, 40 ng of ^{125}I-labeled protein A (800 cpm/ng) was added to each dot and the reaction was incubated at 37°C for 2 h. After four more washes with saline-Tween, the nitrocellulose paper was counted for radioactivity as described above. Controls included dots without antigen and unused culture supernatant or 5% BSA-PBS instead of MAb added to antigen-coated dots.

Methods for other relevant procedures. Protein was determined by the method of Lowry et al. (13), and sodium *meta*-periodate oxidation was performed on antigens immobilized on nitrocellulose paper by the procedures of Woodward et al. (26). BSA (fraction V), which served as the standard for protein determination, and sodium *meta*-periodate were obtained from Sigma. Sodium borohydride was obtained from E. Merck AG, Darmstadt, Federal Republic of Germany.

Heat treatment of LPS antigen was done by subjecting a 1-mg/ml antigen solution in distilled water, in 4% sodium dodecyl sulfate (SDS; Sigma), in 5% 2-mercaptoethanol (Calbiochem-Behring, La Jolla, Calif.), or in SDS–2-mercaptoethanol to a boiling water bath at 100°C for 10 min. After the solution cooled, a 5-μl volume was applied to each nitrocellulose paper dot and the reactivity of the treated antigen with T6 was assayed by dot immunoassay.

RESULTS

Characterization of MAb T6 and the nature of its reactive epitope. Murine MAb T6 was raised against *S. typhi* 620Ty. It was an immunoglobulin G3 antibody with lambda light chain, which was found to bind staphylococcal protein A.

Hot phenol-water-purified LPS from *S. typhi* Ty2 was found to react with T6, and boiling the LPS antigen in the presence of 4% SDS or 5% 2-mercaptoethanol or both did not render it nonreactive with T6. The antigenic activity for T6 was, however, destroyed when the purified LPS was subjected to sodium *meta*-periodate oxidation (Table 1).

To determine which part of the *Salmonella* LPS molecule was responsible for reaction with T6, a whole-cell RIA was performed with various *Salmonella* R mutants. Pairs of ECA isogenic mutants were used to determine whether T6 reacted exclusively with ECA. The results showed that only cells with the smooth or Ra type of LPS reacted with the MAb T6 (Table 2). Four *Salmonella* strains that have a smooth type of LPS all reacted with T6, although two strains, *Salmonella minnesota* SF 1114 and *S. minnesota* SF 10158, reacted only after the cells had been boiled for 5 min at 100°C. Cells with the Ra type of LPS, *S. minnesota* R60 and *S. typhimurium* his 386, both bound the MAb, even without prior heat treatment. *S. minnesota* R5 was an exception; it showed good binding of T6 after the cells had been boiled. This mutant, although it synthesized predominantly the Rc type of LPS, has been described as leaky (19); thus, every culture might contain cells with a complete core polysaccharide. Also evident was the lack of correlation between the presence of ECA on bacterial cells and their ability to bind T6 (Table 2).

RIA with purified LPS antigens to determine the specificity of MAb T6. The fine specificity of MAb T6 was determined by RIA by using purified LPS obtained from *E. coli* and *Shigella* rough mutants that lack O-specific side chains and whose outer core structures are known to be similar to that of *Salmonella* spp. The RIA results obtained with the purified LPS together with their corresponding chemical structures are summarized in Table 3. MAb T6 reacted with both the *S. typhi* Ty2 and the *S. typhimurium* his 386 LPS, whereas among the *E. coli* and *Shigella* rough-mutant LPS, MAb T6 reacted only with the *E. coli* F576 and F632 LPS, both of which belong to the R2 core type.

Presence of a common LPS determinant among *Salmonella* serogroups A to E and its absence among clinical isolates of various gram-negative bacteria. To screen for the presence of a common LPS determinant in clinical *Salmonella* isolates and other gram-negative bacilli, boiled whole cells were used as antigens for a binding study by RIA (Table 4). All 50 strains from *Salmonella* serogroups A to E showed good binding of T6, whereas 47 other members of the family *Enterobacteriaceae* and 11 members of the families *Neisseriaceae*, *Vibrionaceae*, and *Pseudomonadaceae* did not react.

To ascertain that the failure of the non-*Salmonella* organisms to bind the T6 was not due to the fact that the epitope was inaccessible for binding in the intact organism, ultra-

TABLE 2. Whole-cell binding of MAb T6 to isogenic *Salmonella* ECA mutants and various *Salmonella* LPS rough mutants

Strain	ECA	LPS type	LPS core structure[a]	P/C ratio for bacterial cells[b]	
				Unboiled	Boiled
S. minnesota SF10158[c]	−	S[d]	P	3.1	17
S. minnesota SF10159[c]	+	S	|	23.1	ND
S. minnesota 218[e]	+	S	Lipid A ← KDO ← KDO ← Hep ← Hep ← Glc ← Gal ← Glc	2.1	41.2
S. minnesota SF1114[f]	+	S	↗ ↗ ↑ ↑ ↗	14.9	ND
S. minnesota R60	+	Ra	KDO-P-EtN P-P-EtN Hep Gal GlcNAc	84.6	ND
S. typhimurium his386	−	Ra		80.5	ND
			P		
			|		
S. typhimurium SL1780	ND[c]	Rb_1	Lipid A ← KDO ← KDO ← Hep ← Hep ← Glc ← Gal ← Glc	1.4	0.9
			↗ ↗ ↑ ↑		
			KDO-P-EtN P-P-EtN Hep Gal		
			P		
			|		
S. minnesota R345	−	Rb_2	Lipid A ← KDO ← KDO ← Hep ← Hep ← Glc ← Gal	1.1	1.7
			↗ ↗ ↑ ↑		
			KDO-P-EtN P-P-EtN Hep Gal		
S. minnesota R5	−	Rc, Rb	Lipid A ← KDO ← KDO ← Hep ← Hep ← Glc	1.1	23.6[g]
			↗ ↗		
			KDO-P-EtN P-P-EtN		
S. minnesota R7	−	Rd_1	Lipid A ← KDO ← KDO ← Hep ← Hep	1.5	2.9
			↗ ↗		
			KDO-P-EtN P-P-EtN		
S. minnesota R4	−	Rd_2	Lipid A ← KDO ← KDO ← Hep	1.2	2.2
			↗		
			KDO-P-EtN		
S. minnesota R595	−	Re	Lipid A ← KDO ← KDO	1.1	1.3
			↗		
			KDO-P-EtN		

[a] Abbreviations: Gal, galactose; Glc, glucose; Hep, heptose; P, phosphate; EtN, ethanolamine. Structures are as presented in reference 14.
[b] Counts per minute bound in the presence of MAb/counts per minute bound in the absence of MAb. ND, Not determined.
[c] Isogenic ECA mutants.
[d] Smooth-type LPS.
[e] Parent strain to *S. minnesota* R60 and R345.
[f] Parent strain to *S. minnesota* R5, R7, R4, and R595.
[g] Results have been repeated with another batch of cells. R5 is a leaky mutant which may synthesize Ra LPS.

sonic lysates were prepared from the various nonreacting bacteria together with representatives of the reactive strains. These were then spotted onto nitocellulose paper to test for reactivity with T6 by RIA. All ultrasonic lysates had comparable protein content, and only the ultrasonic lysates prepared from *Salmonella* spp. and the *E. coli* that had the R2 LPS core type were found to react with the MAb.

Direct slide agglutination with MAb T6. Smooth *Salmonella* organisms, which reacted with T6 by whole-cell RIA, that were suspended in saline or PBS were not agglutinated by the MAb by using the slide agglutination method, even when antibodies were present in very high titers, such as in ascitic fluid. However, when cells were first incubated for 5 min at 37°C in a 10 mM Tris hydrochloride buffer (pH 8.0) with 10 mM EDTA and 1% Triton X-100, rapid and strong slide agglutination was observed with the T6 ascitic fluid. This was probably because Tris, EDTA, and Triton X-100 in the presence of EDTA have all been reported to disrupt the outer membrane (8, 11, 21), and this alteration in the organization of the outer membrane might have allowed LPS Ra core determinants to become accessible for reaction with MAb T6. No agglutination was observed when similarly treated cells were tested against an irrelevant MAb; i.e., ascitic fluid containing MAb specific for Vi antigen did not agglutinate the Vi-negative *Salmonella* cells which had been treated with the EDTA–Triton X-100–Tris hydrochloride buffer.

With this modification of prior cell treatment in the slide agglutination procedure, 25 *Salmonella* clinical isolates and 150 other gram-negative isolates were tested for agglutination with the T6 ascitic fluid. All 25 *Salmonella* strains, including 9 strains of serogroup B, 6 strains of serogroup C,

TABLE 3. Binding of MAb T6 to purified LPS antigens derived from rough mutants of *Salmonella* spp., *E. coli*, and *Shigella* spp.

LPS source/core type	LPS outer core structure[a]	P/C ratio[b]
S. typhi Ty2/Ra	GlcNAc (1.2 ↓ α) Gal (1.6 ↓ α) Glc $\xrightarrow[\alpha]{1.2}$ Gal $\xrightarrow[\alpha]{1.3}$ Glc $\xrightarrow[\alpha]{1.3}$	126
S. typhimurium his 386/Ra		150
E. coli O8$^-$:K27$^-$ (F470)/R1	Gal (1.2 ↓ α) Glc (1.3 ↓ β) Gal $\xrightarrow[\alpha]{1.2}$ Glc $\xrightarrow[\alpha]{1.3}$ Glc $\xrightarrow[\alpha]{1}$	1.8
E. coli O8$^-$:K27$^-$ (F1283)/R1		1.5
Shigella boydii type 3$^-$ (F3140)/R1		2.0
E. coli O8$^-$:K42$^-$ (F576)/R2	GlcNAc (1.2 ↓ α) Gal (1.6 ↓ α) Glc $\xrightarrow[\alpha]{1.2}$ Glc $\xrightarrow[\alpha]{1.3}$ Glc $\xrightarrow[\alpha]{1}$	46
E. coli O100$^-$:K (F632)/R2		14
E. coli O111$^-$:K58$^-$ (F653)/R3	Glc (1.2 ↓ α) GlcNAc (1.3 ↓ α) Gal $\xrightarrow[\alpha]{1.2}$ Glc $\xrightarrow[\alpha]{1.3}$ Glc $\xrightarrow[\alpha]{1}$	1.5
S. flexneri 4b$^-$ (F4130)/R3		1.7
E. coli O14:K7$^-$ (F2513)/R4	Gal (1.2 ↓ α) Gal (1.4 ↓ β) Glc $\xrightarrow[\alpha]{1.2}$ Gal $\xrightarrow[\alpha]{1.3}$ Glc $\xrightarrow[\alpha]{1.3}$	1.6
E. coli O14:K7 (F1327)/R4		1.4
E. coli K-12 (D21)/K-12	GlcNAc (1.6 ↓ β) Gal (1.6 ↓ α) Glc $\xrightarrow[\alpha]{1.2}$ Glc $\xrightarrow[\alpha]{1.3}$ Glc $\xrightarrow[\alpha]{1}$	1.7
E. coli K-12 (W3100)/K-12		1.6

[a] Abbreviations: Gal, galactose; Glc, glucose. Structures are as presented in reference 19.
[b] P/C ratio is defined in Table 2, footnote *b*.

and 10 strains of serogroup E, were strongly agglutinated, whereas the 150 other gram-negative bacteria including 60 *E. coli*, 20 *Klebsiella pneumoniae*, 15 *Proteus mirabilis*, 20 *Enterobacter aerogenes*, 2 *Citrobacter freundii*, 5 *Shigella flexneri*, 2 *Serratia marcescens*, 16 *Pseudomonas aeruginosa*, and 10 *Acinetobacter anitratus* strains were uniformly negative.

TABLE 4. Whole-cell binding of MAb T6 to clinical isolates of gram-negative bacteria

Strains	T6 reactivity with boiled whole cells	
	No. positive/ no. tested	Mean P/C ± SD[a]
Enterobacteriaceae		
Salmonella		
Serogroup A	10/10	227 ± 50
Serogroup B	10/10	171 ± 56
Serogroup C	10/10	113 ± 58
Serogroup D	10/10	117 ± 30
Serogroup E	10/10	180 ± 43
E. coli	0/25	1.5 ± 0.3
E. aerogenes	0/5	1.8 ± 0.5
C. freundii	0/3	1.7
S. flexneri	0/2	1.1
P. mirabilis	0/5	1.6 ± 0.4
K. pneumoniae	0/5	2.1 ± 0.8
S. marcescens	0/2	0.9
Pseudomonadaceae, *P. aeruginosa*	0/5	1.1 ± 0.5
Vibrionaceae, *Aeromonas hydrophila*	0/1	1.6
Neisseriaceae, *Acinetobacter anitratus*	0/5	1.3 ± 0.4

[a] P/C ratio was calculated as described in Table 2, footnote *b*. When five or more strains were tested, the standard deviation is given.

DISCUSSION

The antigen recognized by MAb T6 was probably the outer core region of *Salmonella* LPS for the following reasons. (i) MAb T6 reacted with the hot phenol-water-purified LPS from *S. typhi* Ty2. (ii) The antigen, being carbohydrate in nature, was resistant to heat, SDS, and 2-mercaptoethanol treatment. (iii) The antigen was labile to sodium *meta*-periodate oxidation. (iv) MAb T6 reacted with *Salmonella* species of serogroups A to E but not with 10 other species of gram-negative bacilli tested. By an assay with a set of *Salmonella* R mutants that were defective in the biosynthesis of the core oligosaccharide (Table 2), it was found that MAb T6 reacted with only the outermost portion of the complete *Salmonella* (Ra) core LPS. The lack of reactivity with the Rb_1 mutant suggested that α-linked *N*-acetylglucosamine (GlcNAc) was probably the immunodominant sugar in the reactive determinant.

Heat treatment of the smooth form of cells (including *S. minnesota* R5 since it is leaky) probably destroyed the integrity of their outer membrane, since an increase in permeability in such treated cells has been documented (23). Therefore, it is possible that heat treatment of cells also enables better penetration of the MAbs for reaction with the less exposed Ra core determinants (Table 2). On the other hand, LPS rough mutants have been shown to have decreased outer membrane protein content, increased LPS molecules, and increased permeability (5), all of which can account for the reaction of the *Salmonella* Ra mutants with the MAb T6, even without prior boiling.

The reactivities of purified LPS derived from the following organisms were compared (Table 3): smooth *S. typhi* Ty2; rough *S. typhimurium* his 386 mutant that only synthesized the complete Ra core; and rough *E. coli* and *Shigella* mutants that synthesized the complete R1 to R4 and K-12 core oligosaccharides sharing structural similarity with the complete Ra core of *Salmonella*. From this study, the major reactive epitope for MAb T6 was tentatively identified as involving the terminal disaccharide of the complete *Salmonella* Ra core, GlcNAc $\xrightarrow[\alpha]{1,2}$ glucose. When the terminal GlcNAc was replaced by glucose, as in the R3 core, or when the terminal GlcNAc was joined to the core through β 1,6 linkage instead of α 1,2 linkage, as in *E. coli* K-12, the antigen binding ability of T6 was completely abrogated. This indicated that not only was the presence of GlcNAc important, but how it was linked to the core oligosaccharide was also important in defining the antibody specificity. Replacement of the third distal sugar, galactose, by glucose, as in the R2 core, did not completely destroy its binding ability to T6, although a significant reduction in binding was noted. This suggested a possible involvement of the third distal sugar, galactose, in the T6 reactive epitope. The slightly lower binding activity seen with the smooth Ty2 LPS was most likely because of steric hindrance by the O-specific side chain. This was in contrast to the core glycolipid, which has been reported (4, 17) to be inaccessible for binding to antibody in the extracted smooth LPS and in the unboiled intact whole cells; thus, confirming that the reactive epitope for MAb T6 must be situated in the outermost portion of the core region.

Chemical and serological studies with polyvalent antisera indicate a structural and serological similarity between the *Salmonella* complete Ra core and that of certain enterobacterial species (20). This is particularly so with the *E. coli* R2 core structure (9). The present study with T6, however, indicated a distinct epitope in the *Salmonella* LPS not shared by nine other species of aerobic gram-negative bacilli. A total of 25 *E. coli* clinical isolates (5 from blood, 5 from pus, and 15 from urine) when tested for reactivity with T6 were uniformly negative. This suggested that *E. coli* with the R2 core LPS was rarely isolated from hospitalized patients in Hong Kong.

Because of its strict monospecificity, the present MAb would serve as a useful tool for studying the outer core structures in all *Salmonella* species, either to confirm the presence of only one common outer core or to reveal some structural diversity in this part of the LPS molecule. The reactivity of the MAb with all 50 *Salmonella* isolates of serogroups A to E might also suggest that it can be a potentially more useful serological reagent than the conventional polyvalent antiserum for the identification of these organisms. The feasibility of using MAb T6 for the identification of *Salmonella* spp. was demonstrated by the positive slide agglutination test obtained with 25 *Salmonella* clinical isolates but not with the 150 non-*Salmonella* bacteria. Other potential applications of such a MAb may include direct detection of *Salmonella* species, either in specimens or through enrichment serology by methods already well established in both food and clinical microbiology (3, 15, 16, 18, 22). In this connection, it was found that MAb T6 could detect soluble antigens in the spent medium of *Salmonella* spp. grown in brain heart infusion broth.

Although antisera against the core glycolipid have been shown to offer protection against the lethal effects of LPS from a variety of gram-negative bacteria in both animal studies (27, 28) and human subjects (29), the exact mechanism of this protection is still not understood. Recent studies (4, 17) with MAbs directed against the core glycolipid have failed to show binding of such MAbs to the intact smooth bacteria and the isolated LPS from such cells. These findings cast serious doubt on the protective role of antibodies to the LPS core glycolipid. In contrast, MAb T6 binds to isolated *Salmonella* smooth LPS, as well as to some smooth and all heat-treated *Salmonella* cells (Tables 1, 2, and 3). Therefore, it will be of interest to determine whether MAb T6 can (i) inhibit the growth of smooth *Salmonella* spp., (ii) have opsonic and bactericidal activities against *Salmonella* spp., and (iii) protect against LPS toxicity. This information will be useful for understanding the potential use of MAb T6 for immunoprophylaxis. However, any activities of MAb T6 would be directed against *Salmonella* species only.

Presently we are studying the occurrence of the epitope reactive with T6 in other *Salmonella* O serogroups and comparing MAb T6 with the conventional polyvalent *Salmonella* O antiserum in the identification of *Salmonella* species.

ACKNOWLEDGMENTS

We thank B. A. D. Stocker for the aromatic-dependent mutant of *S. typhi*.

We are grateful to the Croucher Foundation for financial support.

LITERATURE CITED

1. **Engvall, E., and P. Perlman.** 1972. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme labelled anti-immunoglobulin in antigen coated tubes. J. Immunol. **109**:129–135.
2. **Galanos, C., O. Luderitz, and O. Westphal.** 1969. A new method for the extraction of R-lipopolysaccharides. Eur. J. Biochem. **9**:245–249.
3. **Gibbs, P. A., J. T. Patterson, and J. G. Murray.** 1972. The fluorescent antibody technique for the detection of *Salmonella* in routine use. J. Appl. Bacteriol. **35**:405–413.
4. **Gigliotti, F., and J. L. Shenep.** 1985. Failure of monoclonal antibodies to core glycolipid to bind intact smooth strains of *Escherichia coli*. J. Infect. Dis. **151**:1005–1011.
5. **Gmeiner, J., and S. Schlecht.** 1979. Molecular organization of the outer membrane of *Salmonella typhimurium*. Eur. J. Biochem. **93**:609–620.
6. **Greenwood, F. C., W. M. Hunter, and J. S. Glover.** 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J. **89**:114–123.
7. **Hoiseth, S. K., and B. A. D. Stocker.** 1981. Aromatic-dependent *Salmonella typhimurium* are non-virulent and effective as live vaccines. Nature (London) **291**:238–239.
8. **Irvin, R. T., T. J. MacAlister, and J. W. Costerton.** 1981. Tris(hydroxymethyl)aminomethane buffer modification of *Escherichia coli* outer membrane permeability. J. Bacteriol. **145**: 1397–1403.
9. **Kauffmann, F.** 1961. Die Bakteriologie der *Salmonella* Species. Munksgaard International Publishers, Ltd., Copenhagen.
10. **Köhler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predicted specificity. Nature (London) **256**:495–497.
11. **Leive, L.** 1974. The barrier function of the gram-negative envelope. Ann. N.Y. Acad. Sci. **235**:109–127.
12. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
13. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. **Luderitz, O., M. A. Freudenberg, C. Galanos, V. Lehmann, E. T. Rietchel, and D. H. Shaw.** 1982. Lipopolysaccharides of gram-negative bacteria. Curr. Top. Membr. Transp. **17**:69–151.
15. **Mikhail, I. A., W. R. Sanborn, and J. E. Sippel.** 1983. Rapid,

economical diagnosis of enteric fever by a blood clot culture coagglutination procedure. J. Clin. Microbiol. **17**:564–565.

16. **Mohr, H. K., H. L. Trenk, and M. Yeterian.** 1974. Comparison of fluorescent-antibody methods and enrichment serology for the detection of *Salmonella*. Appl. Microbiol. **27**:324–328.
17. **Nelles, M. J., and C. A. Niswander.** 1984. Mouse monoclonal antibodies reactive with J5 lipopolysaccharide exhibit extensive serological cross-reactivity with a variety of gram-negative bacteria. Infect. Immun. **46**:677–681.
18. **Samborn, W. R., M. Lesmana, and E. A. Edwards.** 1980. Enrichment culture coagglutination test for rapid low-cost diagnosis of salmonellosis. J. Clin. Microbiol. **12**:151–155.
19. **Schlecht, S., and I. Fromme.** 1977. Growth of *Salmonella* R-mutants in submersed cultures. 1. Influence of aeration intensities on the yield in cell mass and lipopolysaccharide and on the chemical composition and serological behaviour of the lipopolysaccharides. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A **239**:178–188.
20. **Schmidt, G., I. Fromme, and H. Mayer.** 1970. Immunochemical studies on core lipopolysaccharides of *Enterobacteriaceae* of different genera. Eur. J. Biochem. **14**:357–366.
21. **Schnaitman, C. A.** 1971. Effect of ethylenediaminetetraacetic acid, Triton X-100, and lysozyme on the morphology and chemical composition of isolated cell walls of *Escherichia coli*. J. Bacteriol. **108**:553–563.
22. **Sperber, W. H., and R. H. Deibel.** 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. Appl. Microbiol. **17**:533–539.
23. **Tsuchido, T., N. Katsui, A. Takeuchi, M. Takano, and I. Shibasaki.** 1985. Destruction of the outer membrane permeability barrier of *Escherichia coli* by heat treatment. Appl. Environ. Microbiol. **50**:298–303.
24. **Westphal, O., and K. Jann.** 1965. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **5**:83–90.
25. **Westphal, O., K. Jann, and K. Himmelspach.** 1983. Chemistry and immunochemistry of bacterial lipopolysaccharides as cell wall antigens and endotoxins. Prog. Allergy **33**:9–39.
26. **Woodward, M. P., W. W. Young, Jr., and R. A. Bloodgood.** 1985. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. J. Immunol. Methods **78**:143–153.
27. **Young, L. S., P. Stevens, and J. Ingram.** 1975. Functional role of antibody against "core" glycolipid of *Enterobacteriaceae*. J. Clin. Invest. **56**:850–861.
28. **Ziegler, E. J., H. Douglas, J. E. Sherman, C. E. Davis, and I. A. Braude.** 1973. Treatment of *E. coli* and *Klebsiella* bacteremia in agranulocytic animals with antiserum to a UDP-gal epimerase-deficient mutant. J. Immunol. **111**:433–438.
29. **Ziegler, E. J., J. A. McCutchan, J. Fierer, M. P. Glauser, J. C. Sadoff, H. Douglas, and A. I. Braude.** 1982. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. N. Engl. J. Med. **307**:1225–1230.