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Production and Characterization of Serovar-Specific Monoclonal Antibodies to Serovars 4, 8, and 9 of *Mycobacterium intracellulare*

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Serovar-specific monoclonal antibodies against *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex serovars 4, 8, and 9 were prepared. Nine, four, and one monoclonal antibodies, respectively, to the serovars were prepared by the usual cell fusion technique. All nine monoclonal antibodies to serovar 4 were monospecific for their homologous serovar and reacted with several native glycopeptidolipids (GPLs) and one major deacylated GPL from the homologous serovar. One of the four monoclonal antibodies to serovar 8 seemed to be monospecific for its homologous serovar, but the others cross-reacted with serovar 6 because serovar 6 organisms contain the same components as does the major deacylated GPL from serovar 8. One monoclonal antibody to serovar 9 was monospecific for its homologous serovar and reacted with one of the two major deacylated GPLs from this serovar. These antibody preparations proved useful for serovar identification.

The *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex (MAIS complex) is a clinically and hygienically important atypical mycobacteria causing tuberculosislike diseases in humans and mycobacteriosis in swine. Identification of serovars within the MAIS complex is worthwhile for microbiologists, epidemiologists, and physicians (14, 20). The MAIS complex forms smooth colonies and exhibits serovar-specific surface antigens, some of which can cross-react with antisera against other serovars within the same complex (1, 19, 22); this property is not shared by mycobacteria that form rough colonies, such as *Mycobacterium tuberculosis*. On this basis, Schaefer developed a seroagglutination test for identification and classification (18). This test was successful in distinguishing 31 serovars within the MAIS complex (20).

However, the results of the tests were sometimes confused due to cross-reactions within the complex, and further absorption of the cross-reacting antibodies was needed (19). Brennan and co-workers found that the serovar-specific antigens were alkali-stable C-mycoside glycopeptidolipids (GPLs) and recommended thin-layer chromatography (TLC) of the lipid extracts for serovar identification (4, 6, 20). An enzyme-linked immunosorbent assay (ELISA) using GPLs was also developed by Yanagihara et al. (22). However, their results proved not to be better than the seroagglutination test or TLC because of the use of cross-reactive polyclonal antisera.

Recently, serovars 4, 8, and 9 of the MAIS complex have frequently been isolated from tuberculoid lesions of diseased pigs in Japan (14, 25). With this in mind, we prepared serovar-specific monoclonal antibodies to develop a rapid serological identification method. However, monoclonal antibodies to serovar 8 showed cross-reaction with serovar 6, as reported for rabbit antiserum (22); other monoclonal antibodies monospecific for serovar 4 or 9 were obtained. The results were similar when we used alkali-stable GPL antigens from each serovar.

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MATERIALS AND METHODS

Bacteria and preparation of suspensions. MAIS complex serovars 1 to 20 were obtained from an original collection of W. B. Schaefer (18), and serovars 21 to 43 came from an authenticated collection of A. Y. Tsang and co-workers (20). They were maintained in Ogawa egg medium (20a) and serotyped by the method described by Schaefer (18) and Brennan et al. (4). They were grown for about 3 weeks at 37°C. The organisms were scraped from the surface, suspended in 10 ml of distilled water, killed by heating for 10 min at 80°C, and stored at 4°C after addition of 0.5% phenol. Bacterial suspensions of *M. tuberculosis* Aoyama B, *Mycobacterium phlei* ATCC 354, *Mycobacterium paratuberculosis* ATCC 19698, *Corynebacterium renale*, and *Corynebacterium pseudotuberculosis* were also used to determine the cross-reactivity of antibodies.

Preparation of GPLs. Serovars 4, 8, and 9 were grown for 2 weeks at 37°C on 7H11 agar plates (6), harvested, and lyophilized. One gram of dry powder was extracted with 80 ml of chloroform-methanol (2:1, vol/vol) for 18 h at 50°C. The native lipid extracts were deacylated by treatment with 0.2 N NaOH in methanol at 37°C for 20 min as described by Brennan et al. (6).

Preparation of monoclonal antibodies. Each organism was suspended in 0.2 M phosphate buffer (pH 7.0) containing 137 mM NaCl and 2.68 mM KCl (phosphate-buffered saline [PBS]) at a concentration showing McFarland no. 2 turbidity, and 0.1 ml of the suspension was intravenously injected into adult female BALB/c mice. Injections of the same volume were repeated seven times at 3-day intervals as described by Schaefer (18). Within 1 month after the last injection, the mice were boosted with one injection and sacrificed 3 days later. The spleen cells (10^8) were fused with 2.5×10^7 mouse myeloma cells (P3-X63-Ag8-U1) in 50% polyethylene glycol 4000. The cells were suspended in 100

ml of Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% heat-inactivated fetal bovine serum (GIBCO), 1 mM pyruvate, 2 mM L-glutamine, 0.1 mM hypoxanthine, 4.0×10^{-4} mM amimopterin, and 1.6×10^{-2} mM thymidine. Portions (100 μl) of the suspension were seeded on 96-well microtiter plates (Costar, Cambridge, Mass.) which had been seeded with 2×10^3 peritoneal macrophages as feeder cells 1 day before the fusion (12). The culture plates were incubated at 37°C in a 5% CO_2 atmosphere. After several hybridomas had grown sufficiently, antibody production was screened by ELISA as described below. Each hybridoma was cloned twice by a limiting dilution technique or soft agar method by using the same feeder cells. More than 10^6 hybridoma cells were intraperitoneally injected into a syngeneic mouse pretreated with 0.5 ml of 2,6,10,14-tetramethylpentadecane. Two or three weeks later, ascitic fluids were harvested. The antibody-containing globulin fractions of the ascitic fluids were precipitated by addition of ammonium sulfate at 50% saturation. The immunoglobulin class and subclass of each antibody were determined by an immunodiffusion precipitation test with specific antiserum for each class or subclass (Miles Laboratories, Inc., Elkhart, Ind.).

ELISA. ELISA with whole bacterial cells was used to detect antibodies in culture supernatants and determine their titer and cross-reactivity. The bacterial stock solution was centrifuged and suspended in 15 mM sodium carbonate–34.9 mM sodium bicarbonate buffer (pH 9.6) at a concentration showing McFarland no. 1 turbidity. As an antigen-negative control, an Ogawa egg medium extract of the same turbidity was used. In the ELISA with GPL as an antigen, 200 μg of GPL was suspended in 1 ml of the same buffer containing 5% sodium taurodeoxycholate (Sigma Chemical Co., St. Louis, Mo.). As a negative control, the taurodeoxycholate-containing buffer was used. The bacterial or GPL antigenic solution (50 μl) was added to each well of 96-well polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.), and the plates were coated by incubation at 4°C overnight. Nonspecific binding sites were blocked by incubation with 1% bovine serum albumin (BSA)-containing carbonate-bicarbonate buffer solution (100 μl) for 1 h at room temperature. After the plates were washed three times with PBS containing 3.1 mM NaN_3 and 0.05% Tween 20, culture supernatant or ascitic fluids diluted with 1% BSA-PBS (50 μl) were added to each well and incubated for more than 1 h at room temperataure, followed by three washes. As a second antibody, the immunoglobulin G (IgG) fraction of goat anti-mouse IgG serum (Cooper Biomedical, Inc., West Chester, Pa.) was labeled with alkaline phosphatase (Sigma) by using glutaraldehyde, as described by Voller et al. (21). The antibody solution was diluted 500-fold with 1% BSA-PBS, and 50 μl of the solution was added to each well. Incubation was performed for an additional 1 h at room temperature. After the plates were washed three times, 100 μl of 4-nitrophenyl phosphate (Sigma) dissolved in 10% diethanolamine hydrochloride buffer (pH 9.8) at a concentration of 1 mg/ml was added to each well and incubated for 30 min at room temperature. After the 30-min incubation, the A_{405} of the developed color was read with an ELISA reader (Auto Reader MR580; Dynatech Laboratories, Inc., Alexandria, Va.).

Agglutination test. Portions (50 μl) of culture supernatants or ascitic fluids diluted with 3.1 mM Veronal (Daiichi Pure Chemicals Co., Tokyo, Japan) buffer (pH 7.5) containing 0.15 M NaCl and 0.1% gelatin were added to wells of V-bottom microtiter plates. Each bacterial suspension (50 μl) at a concentration showing an A_{525} of 0.3, corresponding roughly to no. 2 turbidity of the McFarland scale (19), was mixed by shaking and incubated at 37°C with a cover seal. Agglutination results were read 5 and 24 h later and graded as shown in Table 1.

TLC and TLC-enzyme immunostaining. Lipids were applied to high-performance TLC plates (Si-HPF plates; J. T. Baker Chemical Co., Phillipsburg, N.J.) and developed with chloroform-methanol-water (60:15:2 or 65:25:4, vol/vol/vol). All GPLs were visualized by spraying with 0.1% orcinol in 40% H_2SO_4 and heating at 110°C for 20 min (6). Antigenic GPLs were visualized by immunostaining by the methods of Higashi et al. (10) and Kannagi (11). Briefly, the plates were soaked overnight at 4°C in 5% BSA-PBS and then placed in a plastic bag containing each monoclonal antibody (ascites form) diluted 400-fold with 0.5% BSA-PBS. After 1 h of incubation at 37°C, the chromatograms were washed three times with 0.5% BSA-PBS renewed every 10 min and soaked again for 1 h at 37°C in 5% BSA-PBS. The plates were reincubated similarly with horseradish peroxidase-conjugated protein A (Sigma) at a concentration of 500 ng/ml and washed as before. The plates were stained with a substrate solution consisting of 4-chloro-1-naphthol and hydrogen peroxide for 20 min at room temperature and then rinsed with 50 mM Tris hydrochloride buffer–200 mM NaCl (pH 7.0). The chromatograms were dried and stored in a dark cool place.

RESULTS

Monoclonal antibodies to serovar 4, 8, or 9 were prepared by the usual cell fusion technique. The ELISA reactivities of each monoclonal antibody with serovars 1 to 43 are shown in Table 1. Nine antibodies against serovar 4 reacted only with the homologous organisms, but 2A2 reacted with all serovars. Sixteen antibodies to serovar 8 were divided into six major groups. The first group (6GA) reacted only with the homologous serovar; their reactivities with serovar 6 antigen were slightly higher than the other negative values. The second group (1B2 and two other antibodies) cross-reacted definitely with serovar 6. The third group (2Cl and 2CB) cross-reacted with serovar 21 and slightly with serovar 6 antigen. The fourth group (4D3 and six other antibodies) cross-reacted with serovars 6 and 21. The fifth group (6C4 and 7B5) cross-reacted differently with serovars 4 and 17, and the sixth group (4D7) reacted with serovar 1 and 15 other serovars. Four antibodies were prepared against serovar 9. 5F7 specifically reacted with the homologous serovar, 2F3 cross-reacted with serovar 8, and BD1 and 9G3 reacted with almost all the serovars. The specificities of these antibodies in the agglutination test were also similar except for 2A2, 6C4, 7B5, 4D7, 2F3, BD1, and 9G3. The 2F3 and BD1 antibodies cross-reacted with heterologous serovars in the ELISA but agglutinated only serovar 9. The other antibodies (2A2, 6C4, 7B5, 4D7, and 9G3) did not agglutinate any serovar.

Most of the antibodies did not cross-react with *M. tuberculosis, M. phlei, M. paratuberculosis, C. renale,* or *C. pseudotuberculosis* in the ELISA; 2A2 and 9G3 were the exceptions. 2A2 antibodies cross-reacted with the mycobacteria, and 9G3 antibodies cross-reacted with the mycobacteria and *C. pseudotuberculosis*.

Next, the antigenic molecules that had been recognized by each monospecific monoclonal antibody were identified. For antigenic candidates, native and deacylated lipid fractions were prepared from each antigenic serovar by the method of

TABLE 1. Reactivity of monoclonal antibodies with 31 serovars of MAIS complex in ELISA and agglutination test

Monoclonal antibody	ELISA titer[a]	Reactivity in ELISA with serovar[b]:																													Agglutination of serovar[c]:				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	41	42	43	4	6	8	9	21
Anti-serovar 4																																					
1CA (IgM)	ND	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
1H4 (IgG3)	10,240	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
2A4 (IgG3)	ND	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
2F3 (IgG3)	10,240	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
4CB (IgG3)	40,960	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
5CC (IgM)	640	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
6A2 (IgG3)	2,560	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
8C4 (IgG3)	640	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
AH5 (IgM)	640	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
2A2 (IgM)	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
Anti-serovar 8																																					
6GA (IgG3)	2,560	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
1B2 (IgG3)	40,960	−	−	−	−	−	*	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
3AC (IgG2a)	163,840	−	−	−	−	−	*	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
5C4 (IgG2a)	10,240	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
2C1 (IgG3)	ND	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+
2CB (IgG3)	ND	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+
4D3 (IgM)	640	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
5E4 (IgM)	10,240	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
8BC (IgM)	2,560	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
9A3 (IgM)	ND	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
9E5 (IgM)	10,240	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
AA4 (IgM)	ND	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
AEB (IgM)	ND	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
6C4 (IgG1)	ND	−	−	−	*	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7B5 (IgG1)	40,960	−	−	−	*	−	−	−	+	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4D7 (IgG1)	2,560	*	*	*	−	−	*	−	*	−	−	−	+	−	−	−	+	−	+	−	−	*	−	*	−	*	+	−	+	+	*	*	−	−	−	−	−
Anti-serovar 9																																					
2F3 (IgM)	10,240	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
5F7 (IgG3)	10,240	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
BD1 (IgM)	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*	−	−	*	*	−	−	−	−	*	−	−	−	−	+	−
9G3 (IgM)	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−

[a] The highest dilution of mouse ascitic fluids (globulin fraction) produced by injection of each hybridoma that gave an A_{405} of more than 0.1 in the ELISA with the homologous serovar. ND, Not determined.

[b] Culture medium supernatants were assayed at their original concentration, and the results are expressed in terms of A_{405} as follows: −, <0.05; *, 0.05 to <0.01; +, ≥0.1.

[c] Culture medium supernatants were used. +, Complete agglutination; −, partial or no agglutination.

Brennan et al. (6). For monoclonal antibodies, 1H4 and 5F7, which were monospecific for serovars 4 and 9, respectively, were chosen. 3AC, which reacted with serovar 8 but slightly cross-reacted with serovar 6, was also chosen so that a cross-reacting molecule could be studied. Native lipids consist mainly of apolar and polar GPLs (Fig. 1). After deacylation, apolar GPLs moving on the solvent front in TLC changed into three major bands in each serovar, but none of them was antigenic. Polar GPLs, each of which has a different number of acetyl groups (nuclear magnetic resonance data will be published elsewhere), changed into one or two major GPL(s) after deacylation. All native polar GPLs and the deacylated polar GPL from serovar 4 (GPL-4) were antigenic to 1H4 antibody. Serovar 6 organisms had a minor component at a position identical to that of the major deacylated polar GPL from serovar 8 (GPL-8), and these components were at a lower position than that of a major deacylated polar GPL from serovar 6 (GPL-6). Both GPL-8 and the minor component from serovar 6 were immunostained with 3AC, indicating that GPL-8 may exist in a serovar 6 as a minor component. Deacylated polar GPLs from serovar 9 were separated into two major GPLs (GPL-9-I and GPL-9-II) as previously described (3), but only GPL-9-I was antigenic to 5F7 antibody. The reactivities of all antibodies in the ELISA, using the deacylated GPLs, were similar to the results of the agglutination test.

DISCUSSION

The seroagglutination test for the MAIS complex is an easy diagnostic method, but it requires serovar-specific antisera. Usually, rabbit antisera to whole bacterial cells contained large amounts of nonspecific antibodies, and repeated absorptions were required to prepare a serovar-specific antiserum. Marks et al. (13) found that members of the MAIS complex share an array of closely related lipid antigens; subsequently Brennan et al. (2) found that there is a serovar-specific GPL antigen in each serovar. Immunization with the GPL antigen seems more ideal to elevate serovar-specific antibodies than immunization with whole bacterial cells (1). However, for mammalian glycosphingolipid (GSL) antigens, immunization with a complex of a GSL and a carrier protein such as methylated BSA was not suitable for preparing monoclonal antibodies in mice. The laboratory of Hakomori (9, 23, 24) and another laboratory (8) have usually used *Salmonella minnesota* coated with a GSL

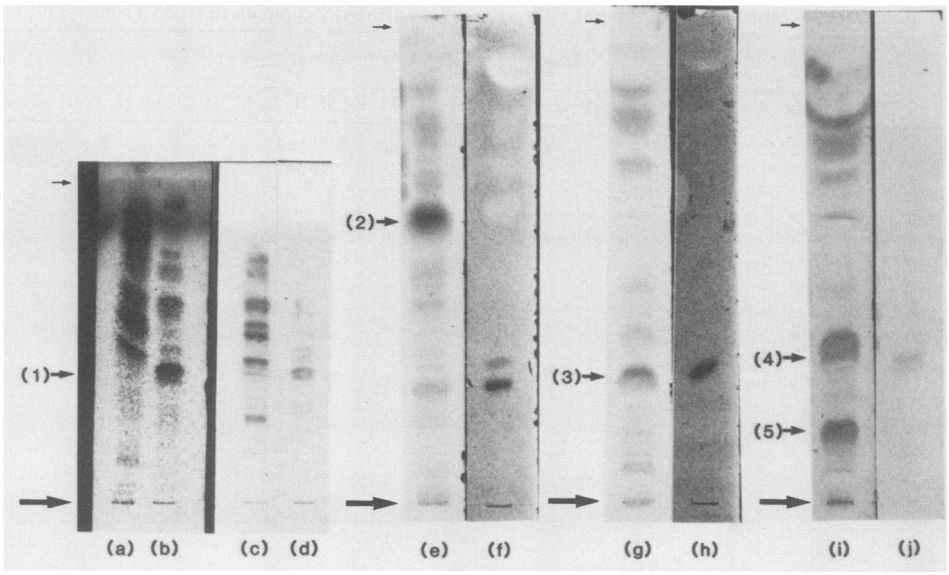

FIG. 1. TLC-enzyme immunostaining of native GPLs and deacylated GPLs. Native lipids from serovar 4 were applied to lanes a and c. In the remaining lanes, deacylated lipids were applied as follows: lanes b and d, serovar 4; lanes e and f, serovar 6; lanes g and h, serovar 8; lanes i and j, serovar 9. Lipids from serovar 4 were developed with chloroform-methanol-water (60:15:2, vol/vol/vol); the other lipids were developed with chloroform-methanol-water (65:25:4). Plates of lanes a, b, e, g, and i were sprayed with orcinol reagent and heated at 110°C for 20 min to visualize GPLs. The other plates were immunostained with monoclonal antibodies as follows: c and d, 1H4; lanes e and g, 3AC; lane j, 5F7. The numbers 1 to 5 indicate the major serovar-specific components GPL-4, GPL-6, GPL-8, GPL-9-I, and GPL-9-II, respectively. The large arrows at the bottom indicate the origins of the compounds applied, and the small arrows at the top indicate the solvent fronts.

antigen as an immunogen. Therefore, in the present studies, we used whole organisms to immunize mice and selected serovar-specific monoclonal antibodies by screening.

All monoclonal antibodies prepared against serovar 4 were monospecific, as described by Schaefer (18), except for one preparation (2A2). This suggests that these antibodies may react with the serovar 4-specific GPL (GPL-4) that was described by Brennan et al. (4, 5, 6). In fact, one preparation (1H4) immunostained not only GPL-4 but also, to our surprise, all spots of native GPLs. Nuclear magnetic resonance studies showed that each of the native GPLs possesses a different number of *O*-acetyl groups (one to five) and that there is no acetyl group in the deacylated GPL-4 (unpublished results). If the hypothesis suggested by Brennan et al. (3, 5, 6) that hydroxy groups of sugar moieties in a native GPL are substituted with different numbers of acetyl groups is right, the monoclonal antibodies cannot react with such O-acetylated sugar moieties. O acetylation of a sugar moiety usually makes the antigenic determinant different in *Escherichia coli* capsular polysaccharide antigen (17) and GSL antigens (7, 15). The exact positions of *O*-acetyl groups in native GPLs and the complete structure of GPL-4 should be studied in the future.

Monoclonal antibodies to serovar 8 were divided into six groups based on their specificities. Most of the antibodies cross-reacted with serovar 6, because serovar 6 contains the GPL-8 compound as a minor component. Some antibodies lacked cross-reactivity with serovar 6. It can be considered that such antibodies have a low affinity for GPL-8; therefore, they can not react with limited amounts of the antigen on serovar 6 organisms, whereas they can react with abundant amounts of the antigen on serovar 8 organisms. An example of such antibodies was previously demonstrated by us (16) with human blood group P^k antigen. Specific antibodies to serovar 6 should recognize GPL-6, so there should be no problem in distinguishing serovar 6 from serovar 8 in serotyping.

One monoclonal antibody specific for serovar 9 was obtained. Serovar 9 possesses two major GPLs (GPL-9-I and GPL-9-II) after base treatment. The chemical structure of the former GPL was proposed by Brennan et al. (2, 3) as a serovar specific component. Serovar 3 shares the latter GPL as a minor component (22). The fact that 5F7 reacted only with GPL-9-I coincides with these previous findings.

Although we have to check the reactivity of these monospecific monoclonal antibodies with many other mycobacteria and non-acid-fast bacteria to confirm the monospecificity, they distinguished only homologous strains from five species of other mycobacteria and non-acid-fast bacteria, demonstrating that the serovar-specific antigens were not shared by mycobacteria that form rough colonies (2), thus increasing the worth of these antibodies. Monoclonal antibodies to the other serovars will be prepared in the near future. A set of monoclonal antibodies to each member of the MAIS complex will make typing easier, more rapid, and more sensitive.

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