単クローン性抗体を用いたChlamydia psittaciの抗原解析

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Monoclonal Antibodies to *Chlamydia psittaci*: Characteristics and Antigenic Analysis

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ABSTRACT. Twenty-seven hybrid cell lines producing monoclonal antibodies to *Chlamydia psittaci* were prepared by fusion of the mouse myeloma cells with spleen lymphocytes of mice immunized with *C. psittaci* pigeon or budgerigar strain. Reactions of these monoclonal antibodies were examined with five strains of *C. psittaci* and two of *C. trachomatis* serovars L2 and J by enzyme-linked immunosorbent assay, the microimmunofluorescent test and the complement fixation test. According to the reaction patterns to eight chlamydial strains, fifteen antibodies to a pigeon strain, P-1041, were divided into three groups: three genus-specific, eight subspecies-specific and four strain-specific antibodies. Twelve antibodies to a budgerigar strain, Izawa, were also tested to eight chlamydial strains and divided into two groups: two genus-specific and ten subspecies-specific antibodies. Physicochemical properties of the antigens recognized by different monoclonal antibodies were examined by KIO_4, pronase and heat treatments and by the immuno-blotting technique. Most genus-specific antibodies recognized the KIO_4-sensitive antigen, which was a pronase-resistant and heat-stable component of about 10,000 daltons. Subspecies- and strain-specific monoclonal antibodies recognized the antigens, which were pronase-sensitive and heat-labile components of about 40,000 or 90,000 daltons.—KEY WORDS: *Chlamydia psittaci,* monoclonal antibody.

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Chlamydia psittaci is distributed among numerous species of birds and mammals and causes different symptoms depending on the species of the host. *C. psittaci* infection in humans is contracted mostly from infected birds. Mammalian strains also have a potential for infecting humans [7, 18]. Recently, a new-type *C. psittaci* has reportedly been transmitted from human to human and caused respiratory symptoms [5, 15, 16]. Precise antigenic analysis of *C. psittaci* strains is extremely important for differentiating the chlamydial strains distributed among numerous species of birds and mammals and to specify the source of human infections.

The knowledge remains unclear as to how antigenic properties vary among *C. psittaci* strains from various host species and what kinds of antigenic substances exist in *C. psittaci*. The major obstacle to solving these questions may be the lack of an antibody highly specific to each strain. Antigenic analysis of *C. trachomatis* with monoclonal antibodies with different specificities, for example, has revealed that the organisms have a wide variety of antigens, each of which is specific to the genus, species, subspecies and type [19]. The biochemical nature of these antigens was also investigated [1, 11, 19]. In our previous study, we prepared monoclonal antibodies against *C. psittaci* pigeon strain and explored the antigenic characteristics of several chlamydial strains [21]. This study further extended the antigenic analysis of chlamydia using monoclonal antibodies against *C. psittaci* pigeon and budgerigar strains and examining the physicochemical properties of the antigenic components recognized by the monoclonal antibodies.

MATERIALS AND METHODS

Chlamydial strains: The chlamydial strains used in this study were as follows: *C. psittaci* strains Pigeon-1041 (P-1041), Pigeon-1313 (P-1313) [3], Budgerigar No. 1 (Bud-1) [13], Izawa-1 (budgerigar origin) [12], Yokohama (bovine origin) [9], SPV-789 (ovine origin) [8] and *C. trachomatis* strains L-2/434/Bu (L-2) [17] and J/Uw-36/CX (J/Uw-36) [22]. All strains had been passed five times in L-929 cells prior to use in the experiments.

Preparation of monoclonal antibodies: Monoclonal antibodies were prepared against *C. psittaci* P-1041 and Izawa strains. Chlamydiae were propagated on an L-929 cell monolayer, inactivated with β-propiolactone (BPL) [4] and used for immunization of BALB/c mice. The mice were immunized by intraperitoneal injections with 0.5 m*l* of BPL-antigens of the P-1041 or Izawa strain. A booster immunization was performed with the same antigen after 4 to 6 weeks; three or four days thereafter, the spleen was obtained for fusion. The procedure for fusion was essentially the same as that described previously [21]. Briefly, 10^8 immune spleen cells were fused with 10^7 myeloma cells (SP2/o-Ag14) with the aid of polyethylene glycol 1,000 (Wako Pure Chemicals, Osaka) at a final concentration of 50%. The cell mixture was added to hypoxanthine-aminopterine-thymidine medium and seeded into a microculture plate with 96 wells. The culture fluid was tested for the antibody by enzyme-linked immunosorbent assay (ELISA). The hybridoma cell producing antibody was cloned in soft agar to obtain a line cell. Briefly, the cultured cells (50 to 100μ*l*) were seeded on the soft agar medium (5m*l* in a petri dish) consisting of Dulbeco's modified Eagle's medium supplemented with 0.35% of Bacto agar, 10% fetal calf serum, 2 mM of glutamine and 40 μg/m*l* of gentamycin. After 7 to 12 days of incubation, colony was picked up under a microscope and was transferred into Dulbeco's modified Eagle's medium for the cell growth. The monoclonal antibody was obtained as ascitic fluid by inoculating hybridoma cells into BALB/c mice primed with pristane (2, 6, 10, 14-tetramethylpentadecane, Aldrich Chemical Co., U.S.A.). Immunoglobulin-class of monoclonal antibodies was identified by immunodiffusion with antiserum (Miles Research Laboratory, U.S.A.) against each subclass of mouse Ig.

ELISA: As described previously [21], a polystyrene microplate (96 wells) was coated with 8 units of BPL-antigen and incubated with antibody, and then anti-mouse Ig conjugated with horseradish peroxidase and substrate solution (0.05 M citrate buffer containing 0.2mM azino-di-3-ethylbenzthiazoline sulfonic acid and 0.004% H_2O_2, pH7.4) were added. Between steps, the plates were washed with phosphate buffered saline (PBS, pH7.4) containing 0.05% Tween 20. After addition of the substrate, coloring reaction was measured in a microplate spectrophotometer (Corona MTP-12, Corona Electric, Japan) at a wave length of 405 nm.

Indirect micro-immunofluorescent test (MIFT): MIFT was performed as described by Wang *et al* [23]. The BPL-antigen was then spotted on a multitest slide, which was dried and fixed with ice-cold acetone for 10 min. Antibody was applied and the slides were incubated at 37°C for 30 min. After the wells were washed, anti-mouse Ig conjugated with fluorescein isothiocyanate was added and the plate was incubated at 37°C for 30 min. After washing with PBS again, the plate was observed under a fluorescence microscope.

Complement fixation (CF) test: CF test was carried out according to the 50% hemolysis method described by Inoue [6].

KIO_4, pronase and heat treatments of chlamydial antigen: KIO_4-treatment of the antigen was done as described previously [21]. BPL-antigen (1.8 m*l*) was mixed with 0.05 M KIO_4 (0.2 m*l*), the mixture was incubated at 37°C for 30 min, and 0.1m*l* of 5% glucose was added to stop the reaction. This antigen was used as a KIO_4-treated antigen. For pronase treatment, a microplate for ELISA was coated with BPL-antigen followed by 50μl of pronase (100 μg/m*l*), and then incubated at 37°C for 2 hr. The plate was washed and used as pronase-treated antigen. Heat-treated antigen was prepared by treating the antigen at 100°C for 150 min.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE was followed by the method described by Laemmli [10]. The separating gel (10% gel) contained 10% acrylamide, 0.12% N, N-methylene-bis-acrylamide (Bis), 0.1% SDS in 0.357M Tris-HCl buffer (Tris) (pH8.9). The stacking gel (4.5% gel) contained 4.5% acrylamide, 0.12% Bis, and 0.1% SDS in 0.07M Tris (pH6.8). Samples were mixed with 0.125 M Tris (pH6.8) containing 4% SDS, 10% 2-mercaptoethanol (2ME), 20% glycerol and 0.002% bromophenol blue, and the mixture was boiled for 3 min. Electrophoresis was carried out at 10mA for 4.5% gel and 20mA for 10% gel. The marker-proteins used for determining molecular weight were as follows; lysozyme (molecular weight: 14,400), soybean trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), bovine serum albumin (66,200) and phosphorylase B (92,500) (Bio-Rad Laboratories, Japan).

Procedure for western blotting: After SDS-PAGE, the gel and nitrocellulose-filter paper (Bio-Rad Laboratories, Japan) were stuck together. Then, the electrophoretic transfer of proteins was performed at 15V for 20 hr in a chamber filled with a blotting buffer composed of 25mM Tris base, 194mM glycine and 20% methanol in distilled water. Marker proteins transferred were stained with 0.05% Coomassie brilliant blue in 50% methanol and 8% acetic acid. The filter paper with sample antigen was immersed in 3% bovine serum albumin in PBS to reduce the nonspecific reaction at 37°C for 40 min. After five successive washings with PBS containing 0.5% Tween 20, the filter paper was treated with monoclonal antibodies for 45 min at 37°C. After washing, the filter paper was treated with horseradish peroxidase-conjugated anti-mouse Ig goat serum (Cappel, U.S.A.) and incubated for 45 min at 37°C. After washing and drying, the filter paper was placed in substrate solution composed of 10mg 3,3'diaminobenzidine and 10μl of 30% H_2O_2 in 100m*l* of 10mM Tris-HCl buffer (pH7.4). After incubation for 5 min at room temperature, the filter paper was washed with distilled water to stop the reaction. The molecular weight of the band was estimated by comparison with the marker proteins.

RESULTS

Fifteen hybridoma cell lines secreting antibody against P-1041 strain were established. Classes and subclasses of the monoclonal antibodies are listed in Table 1. These included one clone each of IgG1, IgG2a, IgG2b, five clones of IgG3 and seven clones of IgM. ELISA titers of the antibodies were determined against BPL-antigens of six *C. psittaci* strains: P-1041, P-1313, Bud-1, Izawa, Yokohama and SPV-789, and two *C. trachomatis* strains: L-2 and J/UW-36 (Table 1). A2 antibody showed equally high titers ($10^{5.5}$–$10^{6.5}$) to all *C. psittaci* and *C. trachomatis* strains. 4G5 and D2 antibodies also reacted with all the chlamydial strains. 1G5, I21, 1F8, 3B5, 4F5, 2E6, 2C5 and 4B11 antibodies reacted to the homologous P-1041 strain at relatively high titers ($10^{4.4}$–$10^{5.5}$) and also to some heterologous

Table 1. ELISA titers of anti-P-1041 monoclonal antibodies against several chlamydial strains

Monoclonal antibodiy		*C. psittaci*						*C. trachomatis*	
No.	Ig Isotype	P-1041	P-1313	Bud-1	Izawa	Yokohama	SPV-789	L-2	J/UM-36
A2	IgG1	6.1[a)]	6.4	6.1	5.8	6.4	5.5	6.1	6.5
4G5	IgG2b	4.6	4.1	3.2	4.7	3.2	3.2	4.2	4.5
D2	IgM	4.1	3.2	3.5	3.2	3.5	2.9	3.2	4.1
1G5	IgG2a	4.6	5.1	4.1	5.1	<2.3	<2.3	3.6	2.6
I21	IgM	4.4	3.2	3.8	3.8	<2.3	<2.3	4.1	2.6
1FB	IgG3	4.9	2.6	4.7	<2.3	<2.3	<2.3	<2.3	<2.3
3B5	IgG3	5.2	3.2	3.2	<2.3	<2.3	<2.3	<2.3	<2.3
4F5	IgM	4.9	2.6	3.8	<2.3	<2.3	<2.3	<2.3	<2.3
2E6	IgM	5.5	2.9	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
2C5	IgM	4.9	2.6	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
4B11	IgG3	4.6	3.2	<2.3	<2.3	3.5	3.5	<2.3	<2.3
2B5	IgM	6.4	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
4E5	IgM	4.6	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
F2	IgG3	3.2	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3
H9	IgG3	3.2	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3	<2.3

a) Log 10 of reciprocals of the highest dilution producing a positive reaction. The cut off point was 2.3 (1: 200)

Table 2. ELISA titers of anti-Izawa monoclonal antibodies against several chlamydial strains

Monoclonal antibodiy		*C. psittaci*						*C. trachomatis*	
No.	Ig Isotype	P-1041	P-1313	Bud-1	Izawa	Yokohama	SPV-789	L-2	J/UM-36
4D5	IgG2a	4.7[a)]	4.7	4.5	5.1	4.7	2.9	5.9	5.7
4D11	IgG3	3.5	3.5	3.8	3.9	3.5	2.9	3.5	3.5
4F11	IgM	4.1	<2.3	<2.3	6.4	<2.3	<2.3	5.7	5.7
2D2	IgM	2.9	<2.3	<2.3	3.9	<2.3	<2.3	3.8	3.8
4F8	IgG2a	4.7	<2.3	6.1	6.1	<2.3	<2.3	<2.3	<2.3
4E11	IgG2a	<2.3	4.7	5.2	5.2	<2.3	<2.3	<2.3	<2.3
4E7	IgG2a	<2.3	<2.3	6.1	6.1	<2.3	<2.3	<2.3	<2.3
4F7	IgG2a	<2.3	<2.3	5.4	5.8	<2.3	<2.3	<2.3	<2.3
2F4	IgG1	<2.3	<2.3	5.1	5.5	<2.3	<2.3	<2.3	<2.3
1D4	IgG3	<2.3	<2.3	4.2	4.5	<2.3	<2.3	<2.3	<2.3
3F9	IgG2	<2.3	4.7	<2.3	5.4	<2.3	<2.3	<2.3	<2.3
3E9	IgG2	<2.3	4.7	<2.3	5.1	<2.3	<2.3	<2.3	<2.3

a) Log 10 of reciprocals of the highest dilution producing a positive reaction. The cut off point was 2.3 (1: 200)

strains. The titers of I21, 1F8, 3B5, 4F5, 2E6, 2C5 and 4B11 antibodies to the homologous strains were higher than those to the heterologous strains. 2B5, 4E5, F2 and H9 antibodies reacted only with the homologous P-1041 strain at $10^{3.2}$ to $10^{6.4}$ titers.

Twelve hybridoma cell lines secreting antibody against Izawa strain were established. Classes and subclasses of the monoclonal antibodies are listed in Table 2. These included one clone of IgG1, two clones each of IgG3 and IgM and seven clones of IgG2a. ELISA titers of the antibodies were determined against eight chlamydial strains (Table 2). 4D5 antibody reacted with all chlamydial strains at high titers ($10^{4.5}$–$10^{5.9}$) except SPV-789 ($10^{2.9}$). 4D11 antibody also reacted to all chlamydial strains. The other

Table 3. ELISA, MIFT and CF reactions of anti-P-1041 monoclonal antibodies against several chlamydial strains

Antibody		*C. psittaci*																			*C. trachomatis*				
Group	No.	P-1041			P-1313			Bud-1			Izawa			Yokohama			SPV-789			L-2			J/UM-36		
		E[a]	M[b]	C[c]	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C
1	A2	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	−	⧺	⧺	⧺
	4G5	⧺	+	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	⧺	−	⧺	⧺	−	⧺	+	⧺
	D2	⧺	+	⧺	⧺	−	+	⧺	−	+	⧺	−	+	⧺	−	+	+	−	−	⧺	−	−	⧺	−	+
2a	1G5	⧺	⧺	−	⧺	⧺	−	⧺	⧺	−	⧺	⧺	−	−	−	−	−	−	−	⧺	⧺	−	+	−	−
	I21	⧺	⧺	−	⧺	+	−	⧺	+	−	⧺	+	−	−	−	−	−	−	−	⧺	⧺	−	+	+	−
b	1F8	⧺	⧺	⧺	+	−	−	⧺	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	3B5	⧺	⧺	−	⧺	−	−	⧺	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	4F5	⧺	⧺	⧺	+	−	−	⧺	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
c	2E6	⧺	⧺	⧺	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	2C5	⧺	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
d	4B11	⧺	+	⧺	⧺	−	−	−	−	−	−	−	−	⧺	−	−	⧺	+	−	−	−	−	−	−	−
3	2B5	⧺	⧺	⧺	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	4E5	⧺	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	F2	⧺	+	⧺	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	H9	⧺	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

a) ELISA: (−); <1:200, (+); 1:200 to <1:1,000, (⧺); 1:1,000 to <1:10,000 and (⧺); ≥1:10,000.
b) MIFT: (−); <1:100, (+); 1:100 to <1:1,000, (⧺); 1:1,000 to <1:10,000 and (⧺); ≥1:10,000.
c) CF: (−); <1:16, (+); 1:16 to <1:100, (⧺); 1:100 to <1:1,000 and (⧺); ≥1:1,000.

antibodies (4F11, 2D2, 4F8, 4E11, 4E7, 4F7, 2F4, 1D4, 3F9 and 3E9) reacted to the homologous Izawa strain and some of the heterologous strains.

MIFT and CF titers of anti-P-1041 monoclonal antibodies were also determined against eight chlamydial strains. The antibody titers including those in ELISA were expressed as the degree of each reaction and are listed in Table 3. According to the reaction patterns, 15 monoclonal antibodies were divided into three groups. Group 1 antibodies (A2, 4G5, D2) showed a wide range of reaction patterns to all strains of *C. psittaci* and *C. trachomatis*. Group 2 antibodies (1G5, I21, 1F8, 3B5, 4F5, 2E6, 2C5 and 4B11) reacted to two strains of feral pigeon origin and to some of the other strains. Group 3 antibodies (2B5, 4E5, F2 and H9) reacted only to homologous P-1041. Eight clones of group 2 were divided further into four subgroups (a, b, c and d) according to the reaction patterns to heterologous strains. Subgroup 'a' antibodies (1G5 and I21) reacted to *C. psittaci* strains of bird origin and *C. trachomatis* strains but not to *C. psittaci* strains of mammalian origin. Subgroup 'b' antibodies (1F8, 3B5 and 4F5) reacted to two pigeon strains and one budgerigar strain but not to mammalian strains of *C. psittaci* or *C. trachomatis* strains. Subgroup 'c' antibodies (2E6 and 2C5) reacted only to pigeon strains. Subgroup 'd' antibody (4B11) reacted to pigeon strains and mammalian strains of *C. psittaci*.

Anti-Izawa monoclonal antibodies were also tested in MIFT and CF test, and the results are shown in Table 4 together with those of ELISA. According to the reaction patterns, 12 monoclonal antibodies were divided into two groups. Group 1 antibodies (4D5 and 4D11) reacted to all eight chlamy-

Table 4. ELISA, MIFT and CF reactions of anti-Izawa monoclonal antibodies against several chlamydial strains

Antibody		*C. psittaci*																							
Group	No.	P-1041			P-1313			Bud-1			Izawa			Yokohama			SPV-789			L-2			J/UM-36		
		E[a]	M[b]	C[c]	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C
1	4D5	++++	+++	++++	++++	+++	++++	++++	+++	++++	++++	++++	++++	++++	+++	++++	+	+++	−	++++	+++	−	++++	+++	++++
	4D11	+++	+	+++	+++	+	+++	+++	+	+++	+++	+	+++	+++	+	+++	+	+	+	+++	+	−	+++	−	+++
2a	4F11	++++	+++	−	−	−	−	−	−	−	++++	++++	++++	−	−	−	−	−	−	++++	+	−	++++	+++	++++
	2D2	+	−	−	−	−	−	−	−	−	+++	+++	+	−	−	−	−	−	−	+	−	−	+++	+	−
b	4F8	++++	++++	−	−	−	−	++++	++++	+	++++	++++	+	−	−	−	−	−	−	−	−	−	−	−	−
	4E11	−	−	−	++++	+++	−	++++	+++	+	++++	+++	+	−	−	−	−	−	−	−	−	−	−	−	−
c	4E7	−	−	−	−	−	−	++++	++++	+++	++++	++++	+++	−	−	−	−	−	−	−	−	−	−−	−	−
	4F7	−	−	−	−	−	−	++++	+++	+++	++++	++++	+	−	−	−	−	−	−	−	−	−	−	−	−
	2F4	−	−	−	−	−	−	++++	+++	−	++++	++++	−	−	−	−	−	−	−	−	−	−	−	−	−
	1D4	−	−	−	−	−	−	++++	+	−	++++	+++	−	−	−	−	−	−	−	−	−	−	−	−	−
d	3F9	−	−	−	++++	+++	−	−	−	−	++++	++++	−	−	−	−	−	−	−	−	−	−	−	−	−
	3E9	−	−	−	++++	+++	−	−	−	−	++++	+++	−	−	−	−	−	−	−	−	−	−	−	−	−

a) ELISA: (−); <1:200, (+); 1:200 to <1:1,000, (++); 1:1,000 to <1:10,000 and (+++); ≥1:10,000.
b) MIFT: (−); <1:100, (+); 1:100 to <1:1,000, (++); 1:1,000 to <1:10,000 and (+++); ≥1:10,000.
c) CF: (−); <1:16, (+); 1:16 to <1:100, (++); 1:100 to <1:1,000 and (+++); ≥1:1,000.

dial strains. The other clones were classified into group 2, which reacted to homologous Izawa strain and to some of the heterologous strains. Group 2 antibodies were subdivided into four subgroups (a, b, c and d). Subgroup 'a' antibodies (4F11 and 2D2) reacted to homologous Izawa and P-1041 strains of *C. psittaci* and to two strains of *C. trachomatis*. Subgroup 'b' antibodies (4F8 and 4E11) reacted to two strains of budgerigar origin and P-1041 of pigeon origin. Subgroup 'c' antibodies (4E7, 4F7, 2F4 and 1D4) reacted to only two strains of budgerigar origin. Subgroup 'd' antibodies (3F9 and 3E9) reacted to homologous Izawa and one of the pigeon strains (P-1313).

Among anti-P-1041 and anti-Izawa monoclonal antibodies, some showed discrepancies in reactions in the three tests, ELISA, MIFT and CF. The antibodies occasionally lacked CF titers even in the presence of high titers in ELISA and MIFT. In comparison with anti-P-1041 monoclonal antibodies, including a strain-specific one, none of the anti-Izawa monoclonal antibodies showed any strain-specific reaction. None of the anti-Izawa subspecies-specific antibodies reacted to mammalian strains of *C. psittaci*. Similar results were observed with anti-P-1041 antibodies among which reactions to mammalian strains of *C. psittaci*. Similar results were observed with anti-P-1041 antibodies among which reactions to mammalian strains of *C. psittaci* were limited. These results suggest that antigenic components of *C. psittaci* beside genus-specific ones were quite different between avian and mammalian strains. Antigenic variations were also recognized among avian strains of the same and different species.

BPL-antigens of P-1041 and Izawa strains were treated with KIO_4, pronase and heat, and reactivities of the treated antigens to homologous monoclonal antibody were tested by ELISA to determine the effect of these treatments. Antibody titers before and after treatment of antigens were ex-

Table 5. Effect of KIO_4, pronase- and heat-treatments of chlamydial antigens on ELISA titers of monoclonal antibodies

Antibody	KIO_4		Pronase		Heat	
	Before	After	Before	After	Before	After
Anti-P-1041						
A2	6.1[a)]	<2.0	6.1	6.1	6.1	6.1
I21	4.4	4.1	4.4	<2.0	4.0	<2.0
2B5	6.4	2.6	6.4	<2.0	6.4	<2.0
Anti-Izawa						
4D5	5.1	<2.0	5.1	5.1	5.1	4.8
4F11	6.4	6.4	6.4	<2.0	6.4	<2.0
4E7	6.1	6.1	6.1	<2.0	6.1	<2.0

a) Log 10 of reciprocals of the highest dilution of the preparation with a positive reaction.

pressed in log 10, and the results with representative antibodies are shown in Table 5. Titers of A2 and 4D5 were reduced after KIO_4 treatment of antigen but not affected by pronase or heat treatment, which suggests that the antigens recognized by these antibodies are polysaccharide. The antigens recognized by I21, 4F11 and 4E7 were resistant to KIO_4 and sensitive to pronase and heat; therefore, the antigenic determinants against these antibodies are assumed to be protein. The titers of 2B5 were reduced after KIO_4, pronase and heat treatments of the antigen, which suggest that glycoprotein might be involved in the antigenic components recognized by 2B5. The other monoclonal antibodies were also tested in ELISA before and after treatment of antigens and the results were expressed in terms of reduction in the ELISA titer (Table 6). Among genus-specific monoclonal antibodies (group 1), 4G5 and 4D11 showed the same patterns as A2 and 4D5, so these antibodies recognized genus-specific polysaccharide antigen. On the other hand, the titer of D2 was reduced slightly after KIO_4 treatment of antigen and moderately after pronase and heat treatments. The results suggest that protein, probably glycosilated, is responsible for the antigenic determinant recognized by D2. Among anti-P-1041 antibodies of group 2, titers of 3B5, 4B11, 1F8 and 2E6 were affected by KIO_4, pronase and heat treatments of antigen; therefore, the glycoprotein might be involved in the antigenic components recognized by these antibodies. Titers of 4F5, 2C5, 1G5 and I21 were reduced by pronase and heat treatments of antigen, which suggest that these antibodies recognize protein antigens. 2B5 and 4E5 antibodies of group 3 seemed to react to glycoprotein antigen, and F2 and H9 antibodies to protein antigen. All of the group 2 antibodies to Izawa strain seemed to recognize protein antigens.

SDS-PAGE and immuno-blotting were applied to determine the molecular weights of antigenic components recognized by monoclonal antibodies. Chlamydial components were separated in SDS-PAGE, transferred to a nitrocellulose membrane, and applied with monoclonal antibodies in enzyme immunoassay. Results with representative anti-P-1041 monoclonal antibodies are shown in Fig. 1. A2 reacted to an about 10,000-daltons, I21 to an about 40,000-daltons and 2B5 to an about 90,000-daltons component. The remaining monoclonal antibodies were also examined in immunoblotting analysis. All of genus-specific anti-

Table 6. The degree of reduction of ELISA titers of monoclonal antibodies after KIO_4-, pronase- and heat-treatments of chlamydial antigen

Antibody			Effect of the treatment with		
Against	Group	No.	KIO_4	Pronase	Heat
P-1041	1	A2	+++[a)]	−	−
		4G5	+++	−	−
		D2	+	++	++
	2	3B5	+++	+++	+++
		4B11	++	+++	+++
		1F8	+	+++	+++
		2E6	+	+++	+++
		4F5	−	+++	+++
		2C5	−	+++	+++
		1G5	−	+++	+
		I21	−	++	++
	3	2B5	+++	+++	+++
		4E5	+	+++	+++
		F2	−	+	+
		H9	−	+	+
Izawa	1	4D5	++	−	−
		4D11	+++	−	−
	2	4F11	−	+++	+++
		4E11	−	+++	+++
		4E7	−	+++	+++
		4F7	−	+++	+++
		2F4	−	+++	+++
		3F9	−	+++	+++
		3E9	−	+++	+++
		2D2	−	+++	++
		1D4	−	++	++
		4F8	−	+	+++

a) Degree of reduction of ELISA titer in log 10: (−); 0 to <0.5, (+); 0.5 to <1.5, (++); 1.5 to 2.5, and (+++); ≥2.5.

bodies (4G5, D2, 4D5 and 4D11) reacted to the about 10,000-daltons component. Thirteen clones (1G5, 1F8, 4F5, 2E6, 2C5, 4B11, 4F8, 4E11, F2, H9, 4F11, 2D2 and 2F4) responded to 40,000-daltons components. Some of these clones showed a faint band of 25,000-daltons beside that of 40,000-daltons, but no firm conclusions was drawn. Seven clones (4E5, 3B5, 4E7, 1D4, 3F9 and 3E9) recognized an about 90,000-daltons component, and some of the clones also showed a faint band at about 50,000-daltons.

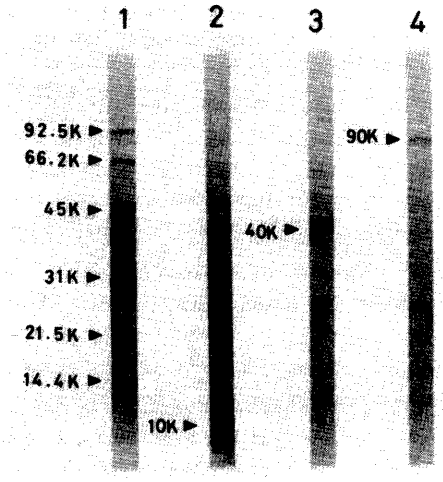

Fig. 1. Immunoblotting patterns of chlamydial antigens detected with monoclonal antibodies. Lane 1 was run with marker proteins and stained with Coomassie brilliant blue. Lane 2 was applied with A2 monoclonal antibody, lane 3 with I21, lane 4 with 2B5 after separation of chlamydial antigen in SDS-PAGE and transfer onto nitrocellulose paper.

DISCUSSION

In the present study, antigenic analysis of *C. psittaci* was further extended from our previous report [21] by increasing the number of monoclonal antibodies and physicochemically characterizing the antigenic components recognized by the antibodies. A total of 27 monoclonal antibodies were prepared against *C. psittaci* pigeon or budgerigar strain and the reaction patterns of these antibodies to various chlamydial strains were examined. Some of the monoclonal antibodies were genus-specific, while many of the antibodies were subspecies- or strain-specific. Four (2B5, 4E5, F2 and H9) of anti-P-1041 antibodies were strain-specific, whereas none of the anti-Izawa antibodies was so. This difference may be due to the lack of strain-specific antigenic determinants on Izawa strain or such a

circumstance that the number of anti-Izawa monoclonal antibodies was insufficient to cover the strain-specific determinants. Since most of the strain- or subspecies-specific antibodies did not react to Yokohama (bovine origin) or SPV-789 (ovine origin), the avian and mammalian strains of *C. psittaci* might share very few common antigenic determinants besides genus-specific ones.

Antigenic variations were also demonstrated among the pigeon and budgerigar strains, and within the strains from the same bird species. The monoclonal antibodies established in this study are useful tools for serological classification of *C. psittaci* strains and epidemiological investigation of human psittacosis. In our previous study, *C. psittaci* strains of pigeon and budgerigar origins were classified into several serological groups with monoclonal antibodies [21]. Furthermore, we applied the monoclonal antibody sets to identify the antigenic type of strains from psittacosis patients and from their pet birds suspected to be the source of infection [20].

The physicochemical nature of the antigenic components recognized by the monoclonal antibodies was revealed by KIO_4, pronase and heat treatments of chlamydial antigen and also by immunoblotting analysis. Among genus-specific monoclonal antibodies, A2, 4G5, 4D5 and 4D11 were revealed as reacting to the polysaccharide antigenic components sensitive to KIO_4 and resistant to pronase and heat treatments. The antigenic components had a molecular weight of about 10,000-daltons and were considered to correspond to genus-specific lipopolysaccharide of *C. trachomatis* [19]. An antigenic determinant recognized by D2 seemed to be present on glycoprotein which was pronase-sensitive and heat-labile and was affected slightly by KIO_4. It was not clear, however, whether the antigenic determinant was located on the carbohydrate or protein moiety of glycoprotein. Concerning the differentiation of the epitope recognized by each antibody, further study such as competitive binding assay is needed to obtain the information.

In *C. trachomatis*, the major outer membrane protein (MOMP) of 40,000-daltons carries the type-, subspecies- and species-specific antigenic determinants [19]. Some of the strain- or subspecies-specific monoclonal antibodies to *C. psittaci* in this study reacted to the pronase-sensitive and heat-labile protein of 40,000-daltons, which suggests the existence of MOMP in *C. psittaci*. The other strain- or subspecies-specific monoclonal antibodies responded to the protein of 90,000-daltons. Caldwell *et al.* [2] isolated high-molecular weight chlamydial protein of 90,000-daltons from SDS-treated antigen. Nano *et al.* [14] detected the subspecies-specific protein of 51,000 daltons from dithiothreitol-treated antigen. It is possible that the 90,000-dalton component is a dimer which is dissociated into 51,000-daltons after breakage of a disulfide bond by reduction with dithiothreitol in their studies. 2-mercaptoethanol was used as a reducing agent in the present study and this chemical has milder effect than dithiothreitol. Therefore, the 90,000-daltons component might mostly remain in a dimeric form under the reducing condition with 2-mercaptoethanol.

The reaction patterns of monoclonal antibodies in ELISA, MIFT and CF tests were the same to the given antigen in most instances but were different in some cases. Some of the antibodies with positive ELISA and MIFT titers lacked CF titers. When antibody titers were measured in the three tests, the titers correlated well between ELISA and MIFT but not between ELISA and CF tests, or MIFT and CF tests (Seki: unpublished data). In ELISA and MIFT, the secondary antibody reacts to the monoclonal antibody bound to the chlamydial antigen. In CF test, however, sequential

cascade reactions of complement components take place on the antigen-antibody complex. This difference may be the reason for the different reaction patterns of monoclonal antibodies in the three tests.

It was surprising that some monoclonal antibodies, other than genus-specific ones, failed to react to mammalian strains of *C. psittaci* but showed a positive reaction to *C. trachomatis* L-2 and J/UW-36 strains. These common reactions were also observed with L-1 and L-3 strains among 15 standard strains of *C. trachomatis* (Seki: unpublished data). When monoclonal antibodies were prepared against *C. trachomatis* L-2 strain and tested against several chlamydial strains, common antigenic components other than genus-specific ones were detected between L-2 and *C. psittaci* pigeon strains (Sato: unpublished data). These results indicate that common antigenic components other than genus-specific lipopolysaccharide were shared by *C. psittaci* avian strains and *C. trachomatis* LGV group.

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要　　約

単クローン性抗体を用いた *Chlamydia psittaci* の抗原解析：関智代子，高島郁夫，有川二郎，橋本信夫（北海道大学獣医学部獣医公衆衛生学教室）——*Chlamydia psittaci* P-1041株（ドバト由来）と Izawa 株（セキセイインコ由来）を免疫源として27種の単クローン性抗体を作出し，ELISA，蛍光抗体法および補体結合反応を用いて８種類のクラミジア株に対する反応パターンを比較した．15種の抗 P-1041 単クローン性抗体は，属特異的，亜種特異的および株特異的な３群にまた12種の抗 Igawa 単クローン性抗体は，属特異的および亜種特異的な２群に大別された．属特異的な抗体のほとんどは KIO_4 感受性，プロナーゼ抵抗性かつ耐熱性の分子量10,000の抗原を認識していた．亜種あるいは株特異的な抗体は，プロナーゼ感受性で易熱性の分子量40,000または90,000の抗原を認識していた．