Antigenic Analysis of Avian *Chlamydia psittaci* using Monoclonal Antibodies to the Major Outer Membrane Protein

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ABSTRACT. Monoclonal antibodies to the major outer membrane protein (MOMP) of *Chlamydia psittaci* derived from a parrot were established for antigenic analysis of avian *C. psittaci*. With 17 monoclonal antibodies to MOMP, 19 reactivity patterns were identified on 112 strains of *C. psittaci*, *C. pneumoniae* and *C. trachomatis*, which were isolated from birds, mammals and humans in Japan, U.S.A., Canada and Taiwan, from 1938 to 1987. Immunological reactivity of budgerigar-derived strains to the monoclonal antibodies was different from that of pigeon-derived strains. Imported bird-derived strains were distinguishable from domestic bird-derived strains by the reactivity to the monoclonal antibodies. A close relationship between the subtypes and geographic origins was indicated on budgerigar-derived strains. On the contrary, various reactivity patterns were shown in pigeon-derived strains isolated in a narrow area. The monoclonal antibodies established in the present work may be useful probes for ecological study of avian *C. psittaci*.—KEY WORDS: *Chlamydia psittaci,* immunological subtyping, monoclonal antibody.

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Chlamydia psittaci is an important pathogen for birds, mammals and humans [18]. *C. psittaci* strains show diverse immunogenicity, and the immunological typing system has not been established. Several attempts at immunological typing of *C. psittaci* were reported for mammalian and limited number of avian strains [1, 15, 18–20].

We have reported immunological typing of *C. psittaci* strains derived from birds and mammals using hyperimmune antisera [2] and a panel of monoclonal antibodies, which reacted with antigens of lipopolysaccharide (LPS), a major outer membrane protein (MOMP) and a molecular weight of 60,000 antigen [5], and also genetic grouping and typing based on DNA-DNA homology and restriction endonuclease digestion patterns [3]. All of this immunological and genetic typing showed well agreement. *C. psittaci* was classified into two genetic groups; one consisted of avian, feline and guinea pig strains, and the other of cattle and sheep strains by DNA-DNA hybridization assay. The former group was further divided into four genetic types of Av1, Av2, Fel and Gpl by DNA restriction endonuclease analysis. Immunological typing of MOMP with the hyperimmune antisera indicated that the Av1 type corresponded to MOMP types 1 and 2, and that the Av2 and Fel types to MOMP type 3. The MOMP types 1 to 3 contained several subtypes determined with monoclonal antibodies. The immunological analyses indicated the importance of the MOMP in immunological typing of *C. psittaci* as well as in that of *C. trachomatis*, although only two monoclonal antibodies to the MOMP were used in our previous study. Also, the immunochemical structure of MOMP has not sufficiently been recognized on *C. psittaci*.

In the present report, we give an antigenic analysis of avian *C. psittaci* with monoclonal antibodies to the MOMP. The reactivity patterns showed antigenic variation of MOMP and relationships between the antigenicity and their host and geographic distribution.

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MATERIALS AND METHODS

Organisms: C. psittaci Prt/GCP-1 strain was used to prepare monoclonal antibodies. The strain was isolated from a parrot *Amazona aestiva* affected by systemic psittacosis in 1980 in this laboratory [21]. Ninety-six strains of *C. psittaci* were derived from parrot [8], parakeet [8], budgerigar [7, 12], cockatiel [8], pigeon [14], turkey, cat, muskrat, cattle, sheep and psittacosis patients [11], in Japan, U.S.A. and Canada, from 1938 to 1987. TW183 strain of *C. pneumoniae* and each strain of the 15 serotypes of *C. trachomatis* were also used (Table 1). Purified EB and chlamydial outer membrane complexes

Table 1. Strains used in the present study

Chlamydia	Original host	Isolated year	Isolated place	Clinical condition	Source[a)]
Prt/GCP-1	Parrot	1980	Imported	Systemic infection	a
Ckt/Okame	Cockatiel	1980	Imported	Systemic infection	a
Prk/Daruma	Parakeet	1980	Imported	Systemic infection	a
Prk/6BC	Parakeet	1941	U.S.A.	Psittacosis	g
Prk/46,48,49	Parakeet	1980	Imported	Systemic infection	a
Bd/4 and other 26 strains[b)]	Budgerigar	1986	Japan (Aichi)	Normal	a
Bd/207 and other 13 strains[c)]	Budgerigar	1987	Japan (Aichi)	Normal	a
Bd/542P, 602H	Budgerigar	1980	Japan (Gifu)	Normal	a
Bd/Izawa	Budgerigar	1984	Japan (Okayama)	Normal	b
Bd/NIH1, NIH2, NIH3	Budgerigar	1986	Japan (Tokyo)	Normal	d
Pgn/593	Pigeon	1982	Japan (Aichi)	Normal	a
Pgn/PCM8 and other 19 strains[d)]	Pigeon	1985	Japan (Aichi)	Normal	e
Pgn/P-1041	Pigeon	1984	Japan (Hokkaido)	Normal	c
Pgn/WWD	Dove	1961	U.S.A.	Normal	f
Tk/NJ	Turkey	1960	U.S.A.(New Jersey)	Ornithosis	g
Tk/HT	Turkey		U.S.A.(Minnesota)	Ornithosis	g
Tk/Ore	Turkey		U.S.A.(Oregon)	Ornithosis	g
Tk/Tex	Turkey		U.S.A.(Texas)	Ornithosis	g
Tk/Cal	Turkey		U.S.A.(California)	Ornithosis	g
Fe/145	Cat	1969	U.S.A.	Conjunctivitis	g
Fe/Pn-1	Cat	1944	U.S.A.	Pneumonia	i
Mu/M56	Muskrat	1966	Canada	Systemic infection	i
Bo/Yokohama	Cattle	1961	Japan (Kanagawa)	Diarrhea	h
Bo/Maeda	Cattle	1950	Japan (Kagoshima)	Pneumonia	j
Bo/E58	Cattle	1940	U.S.A.	Encephalomyelitis	i
Ov/IPA	Sheep	1968	U.S.A.	Polyarthritis	i
Ov/B577	Sheep	1966	U.S.A.	Abortion	i
Gp/Ic	Guinea pig	1964	U.S.A.	Inclusion conjunctivitis	i
Frt-Hu/Cal10	(Human)	1938	U.S.A.	Cold?	b
Hu/Itoh	Human	1962	Japan (Tokyo)	Psittacosis	h
Hu/Borg	Human	1944	U.S.A.	Psittacosis	h

a) Sources of the strains were as follows:
a. Hirai, K., Gifu University, Gifu, Japan.
b. Matsumoto, A., Kawasaki Medical School, Kurashiki, Japan.
c. Hashimoto, N., Hokkaido University, Sapporo, Japan.
d. Hagiwara, T., National Institute of Health of Japan, Murayama, Japan.
e. Miyake, T., Aichi Prefectural Institute of Public Health, Nagoya, Japan.
f. Grimes, J.E., Texas A&M University, Texas, U.S.A.
g. Schachter, J., University of California, San Francisco, U.S.A.
h. Inaba, Y., National Institute of Animal Health of Japan, Tsukuba, Japan.
i. American Type Culture Collection.
j. Nippon Institute of Biological Science, Japan.
b) Bd/34,36,38,39,42,43,47,49,64,72,74,75,75a,83,89,93,97,98,102,103,107,112,115,121,125 and 130 (26 strains).
c) Bd/210,214,224,227,245,247,266,267,267a,275,280,286, and 291 (13 strains).
d) Pgn/9,10,12,16,23,24,27,30,35,41,55,61,62,65,69,74,101,131 and 146 (19 strains).
Blanks indicate that no information was available.

(COMC) were prepared as described previously [4, 5].

Establishment of monoclonal antibody-producing cell lines and immunochemical analysis: Monoclonal antibody-producing cells were cloned and established as described previously [5]. Components of EB were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli [11]. Pocedures of immunoblotting analysis were described previously [5]. The principal ELISA procedure was described previously [6]. Complement fixation test was done with sodium deoxycholate-extracted antigen [4]. Indirect immunofluorescence antibody test was conducted as described previously [5].

RESULTS

In the present study, 28 clones were obtained and analyzed for their specificity and immunochemical properties for recognizing antigens. The monoclonal antibodies reacted with MOMP, 56–64K, or LPS

Table 2. Immunization and specificity of monoclonal antibodies established

Primary Immunization		Secondary Immunization		Number of monoclonal antibody		
				LPS	MOMP	56-64K
COMC	(i.m.)	COMC	(in vitro)	0	1	1
EB/Triton X[a)]	(i.m.)	Sarkocyl soluble	(i.p.)	3	1	0
EB/Triton X	(i.m.)	COMC	(i.p.)	0	1	0
EB/DOC[b)]	(i.v.)	COMC	(i.p.)	0	14	7

a) EB treated with 0.5% TritonX-100 at 37°C for 30 min.
b) EB treated with 0.5% sodium deoxycholate at 37°C for 30 min.

Table 3. Immunochemical properties of monoclonal antibodies against the MOMP

Monoclonal antibody	Triton Only	Antigen treated with				CF	IFA
		NaIO4	Proteinase K	Heat	2ME		
G/3B3[a)]	0.12[e)]	0.25	0.01	0.02	0.02	8>	51200
G/1B6[b)]	0.24	0.48	0.02	0.05	0.02	512	51200
G/1A1/10[c)]	0.54	0.54	0.02	0.02	0.02	8>	51200
G/1B3[d)]	0.20	0.20	0.01	0.02	0.02	512	51200

a)–d) Following antibodies showed the identical reactivity:
a) G/1A1/9.
b) G/2B5, G/2A3.
c) G/2D3, G/1D4 G/2B1 and G/3A2.
d) G/2C2, G/3C2, G/4B4, G/4D2 G/4B5 and G/B3.
e) Figures are OD_{240} values in ELISA.

antigens on immunoblotting assay (Table 2). Most of the monoclonal antibodies to MOMP were obtained using spleen cells of the mouse primed with deoxycholate-treated EB as the first immunization antigen. We mainly describe the MOMP below, because the MOMP is one of the most important antigens.

Immunochemical properties of the MOMP recognized by the monoclonal antibodies were analysed with proteinase K and sodium periodate. Reactivity of EB to five out of the 17 monoclonal antibodies (G/1B6, G/2A3, G/2B5, G/3B3 and 1A1/9) was enhanced two- to three-fold with sodium periodate oxidization of EB (Table 3). Proteinase K digestion, heated at 100°C for 15 min, and 2-ME reduction treatments inactivated immunological reactivity of this antigen (Table 3). Complement fixing antibodies accounted for 10 out of 17 antibodies (Table 3).

Previous studies indicated the MOMP was a type specific antigen. Therefore, immunological subtyping was examined with the monoclonal antibodies to MOMP by indirect immunofluorescence antibody assay. A panel of the monoclonal antibodies to MOMP showed 19 reaction patterns to the 112 strains of *Chlamydiae* (Table 4). One of the monoclonal antibodies, G/1A1/9, reacted with avian and human psittacosis strains only (Table 4). Five monoclonal antibodies, G/2A3, G/2B1, G/1B3, G/1D4 and G/3A2, reacted with budgerigar-derived strains but not with most of the other bird-derived strains (Table 4). Particularly, the monoclonal antibody, G/1B3, reacted only with Prt/GCP-1 and the budgerigar-derived strains.

Chlamydiae strains used were classified into 16 reactivity patterns of avian and human psittacosis strains and 3 reactivity patterns of mammalian and *C. trachomatis* strains (Table 4). Strains of budgerigar, pigeon, turkey and parakeet origins showed 4 (1, 2, 3 and 8), 8 (4 to 7, 9 to 11 and 16), 2 (6 and 15), and 3 (13, 14 and 16) reactivity patterns, respectively. Each of the reactivity patterns 2 to 5 and 7 to 15 was shown by a single avian species; reactivity patterns 1, 6 and 16 were shown by 2 to 3 bird species. Human psittacosis strains showed 2 reactivity patterns (reactivity patterns 1 and 12). Mammalian strains scarcely reacted with the monoclonal antibodies. *C. pneumoniae* and all *C. trachomatis* strains did not react with the monoclonal antibodies.

Table 4. Reactivity patterns of avian *Chlamydia psittaci* to a panel of monoclonal antibodies against the MOMP

Reactivity pattern	G/1A1/9	G/2B5	G/1B6	G/3B3	G/3C2	G/2C2	G/1A1/10	G/3B3	G/4B4	G/4D2	G/4B5	G/2D3	G/2A3	G/2B1	G/1B3	G/1D4	G/3A2	Host origin		Place		Year
1	+[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Parrot	(1/1)[b]	Imported	(1/1)[c]	1980
																		Budgerigar	(44/49)	Aichi	(43/43)	1986,87
																		Human	(1/4)	Tokyo	(1/1)	1962
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	Budgerigar	(4/49)	Tokyo	(3/3)	1984
3	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	Budgerigar	(1/49)	Gifu	(1/2)	1980
4	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	Pigeon	(1/23)	Aichi	(1/22)	1985
5	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	Pigeon	(1/23)	Gifu	(1/1)	1982
6	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	Pigeon	(15/23)	Aichi	(14/23)	1985
																		Pigeon	(1/23)	Hokkaido	(1/1)	1984
																		Turkey	(4/5)	U.S.A.	(4/5)	
7	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	Pigeon	(1/23)	Aichi	(1/22)	1985
8	+	+	+	−	+	+	+	−	−	−	−	−	−	+	+	−	−	Budgerigar	(1/49)	Gifu	(1/2)	1980
9	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	Pigeon	(1/23)	Aichi	(1/22)	1985
10	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	Pigeon	(1/23)	Aichi	(1/22)	1985
11	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−	−	−	Pigeon	(1/23)	Aichi	(1/22)	1985
12	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Ferret(Human)	(1/1)	U.S.A.	(1/1)	1938
13	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	Parakeet	(1/5)	Imported	(1/3)	1980
14	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Parakeet	(2/5)	Imported	(2/3)	1980
15	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	Turkey	(1/5)	U.S.A.	(1/5)	
16	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Parakeet	(1/5)	Imported	(1/1)	1980
																		Parakeet	(1/5)	U.S.A.	(1/1)	1941
																		Cockatiel	(1/1)	Imported	(1/1)	1980
																		Dove	(1/1)	U.S.A.	(1/1)	1961
17	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Cattle	(1/3)	Kagoshima	(1/1)	1960
																		Muskrat	(1/1)	Canada	(1/1)	1966
18	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Cattle	(1/3)	Imported	(1/1)	1961
																		Cattle	(1/3)	U.S.A.	(1/1)	1940

Following strains did not react with any monoclonal antibodies: Hu/Borg, Cat-derived strains, Sheep-derived strains, a guinea pig strain, *C. pneumoniae* and *C. trachomatis* strains.
a) Reactivity was evaluated by indirect immunofluorescence method as described in the previous paper[5].+ indicates reaction to the monoclonal antibody and − indicates no reaction to the monoclonal antibody.
b) Positive number/a total number of samples of the same host origin. c) Positive number/a total number of samples of the same geographic origin.

Most of budgerigar-derived strains of the same geographic origin showed a similar reactivity patterns (Table 4); *i.e.*, strains of Aichi and Okayama Prefectures showed the reactivity pattern 1, strains of Tokyo did the reactivity pattern 2, although strains of Gifu Prefecture showed reactivity patterns 3 and 4. On the other hand, pigeon-derived strains isoalted at several parks and temples in geographically limited areas of Nagoya City in 1985 [12] showed 6 reactivity patterns of 4, 6, 7, 9, 10 and 11.

DISCUSSION

A total of 27 hybridoma cell lines that secrete monoclonal antibodies to EB of *C. psittaci* GCP-1 were established and were used to characterize immunochemical properties of their recognizing antigens. The established monoclonal antibodies recognized MOMP, 56–64K antigens and LPS, which would be principle antigens as suggested in our recent study of immunoblotting analysis of cats and cattle immune response [13].

A human psittacosis isolate of Hu/Itoh, which was isolated from a patient affected from his budgerigar [9], and a psittacosis-related budgerigar isolate of Bd/Izawa, which was isolated from a budgerigar kept by a psittacosis patient [12], showed the same reactivity pattern 1 (Table 4). It is suggested that some strains possessing a similar antigenicity of the MOMP show pathogenicity to humans. It is important that immunological analysis presented in this study is applied to strains isolated from psittacosis patients and budgerigars kept by psittacosis patients.

The clear distinction between the budgerigar- and pigeon-derived strains isolated in the identical prefectural area, Aichi Prefecture, may reflect a rare transmission of *C. psittaci* among populations of these birds. Immunological differences between budgerigar- and pigeon-derived strains were also described by Takahashi *et al.* [19]. The mode of geographic distribution of strains showing various reactivity patterns to the monoclonal antibodies differed between these two avian species. In budgerigar-derived strains, relatively close relationship was observed between geographic distribution and reactivity patterns shown with the monoclonal antibodies, whereas distribution of several reactivity patterns was shown in pigeon-derived strains isolated in Nagoya City [14]. These results may be reasonable because of ecological insulation between caged budgerigars and free-living feral pigeons and also of differences of their behavioral areas. Thus, transmission of *C. psittaci* among the same and varied avian species would depend on the ecological characteristics of the host birds.

Immunological analysis using monoclonal antibodies revealed a striking variety of epitopes on chlamydial EB, especially on the MOMP, and ecological state of *C. psittaci* in birds. Several monoclonal antibodies should be selected to clarify and establish immunological subtypes for applicable subtyping systems, because the analysis presented in this study is too complicated to make the routine immunological identification. Establishment of immunological typing for avian *C. psittaci* is the essential step to analyse and control the chlamydial infection in birds and humans.

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