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# Monoclonal Antibody Typing of *Chlamydia psittaci* Strains Derived from Avian and Mammalian Species

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**A total of 77 *Chlamydia psittaci* strains of avian, human, and mammalian origin were grouped into four serovars with 11 monoclonal antibodies recognizing the lipopolysaccharide and the major outer membrane protein antigens. The avian and human strains, which were closely related to each other, were distinct from the mammalian strains. Immunological typing of *C. psittaci* with monoclonal antibodies seems practical.**

The genus *Chlamydia* includes two species, *Chlamydia trachomatis* and *Chlamydia psittaci* (20). The two species are differentiated on the basis of susceptibility to sulfadiazine, production of glycogen, and genomic homology (18, 20). *C. trachomatis* is classified into three biovars and 15 immunotypes (18). *C. psittaci*, on the other hand, is still not classified into distinct biovars or immunotypes (18). In mammalian *C. psittaci* strains, Schachter et al. (23, 24) found two serotypes of ovine and bovine strains related to pathogenicity by using a plaque reduction test. Storz and coworkers (21, 28) reported eight biotypes and nine immunotypes in mammalian *C. psittaci* strains. However, avian strains remain to be classified, despite their importance as reservoirs of *C. psittaci* (16).

Polypeptide and antigen composition analyses in our laboratory (H. Fukushi, M. Furui, and K. Hirai, submitted for publication) showed multiple antigens with various immunological specificities on elementary bodies (EBs). In the present report, we show that avian and mammalian *C. psittaci* strains may be grouped into four serovars by using monoclonal antibodies against antigens with molecular masses of 3 to 5, 40, and 61 kilodaltons (kDa).

## MATERIALS AND METHODS

**Chlamydiae.** The original hosts of 77 strains of *C. psittaci* and 3 strains of *C. trachomatis* are shown in Table 1. The 80 strains were grown in HeLa 229 cells for 48 to 78 h. The infected cell cultures were harvested and precipitated by centrifugation. The precipitate was suspended in a one-half to one-third volume of phosphate-buffered saline (PBS) and dotted on Multitest slides (Flow Laboratories, Inc.). The slides were fixed in cold acetone for 15 min before use in the indirect immunofluorescence test.

Strain Prt/GCP-1, EBs of which were used as immunizing antigens for preparing monoclonal antibodies, was grown in an L-cell suspension (29). EBs of the strain were purified as described by Tamura and Higashi (29).

**Monoclonal antibodies.** The monoclonal antibodies used in this study are shown in Table 2. Monoclonal antibodies against strain Prt/GCP-1 were prepared by the modified method (19) of Köhler and Milstein (10, 11). The detailed protocol for establishing the monoclonal antibodies will be described elsewhere (H. Fukushi, A. Kikuta, T. Yoshida, and K. Hirai, submitted for publication). Monoclonal antibodies were used as ascitic fluids diluted 1:100 in 1.0% bovine serum albumin in PBS.

**ELISA with chemical pretreatments.** An enzyme-linked immunosorbent assay (ELISA) was performed as described previously (5). Purified EBs were solubilized with 0.5% Triton X-100–5% 2-mercaptoethanol for 30 min at 37°C. For periodate oxidation of the antigen, the antigen-coated plates were incubated with 0.05 M $NaIO_4$–0.01 M sodium acetate (pH 4.4) for 24 h at 4°C (0.1 ml per well), followed by incubation with 0.25 M sucrose–0.01 M Tris hydrochloride (pH 7.2). For protein digestion of the antigen, the antigen-coated plates were treated with 50 μg of proteinase K per ml of PBS for 4 h at 37°C (0.1 ml per well) before incubation with bovine serum albumin-PBS.

**Immunoblotting analysis.** Monoclonal antibodies recognizing antigens were detected by immunoblotting. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Laemmli (12), a sodium dodecyl sulfate-polyacrylamide gel was layered on a nitrocellulose sheet and electrophoretically transferred as described by Towbin et al. (30), with the minor modification that the transfer buffer did not contain methanol. The nitrocellulose sheet was incubated with monoclonal antibodies diluted 1:20 with PBS containing 0.1% bovine serum albumin. Antigen-antibody complexes were detected with horseradish peroxidase-conjugated *Staphylococcus aureus* protein A by using 0.2 mg of 3,3′-diaminobenzidine per ml–0.5% $H_2O_2$–0.05 M Tris hydrochloride (pH 7.6) as the substrate solution.

**Indirect immunofluorescence test.** The antigen dots were covered with diluted monoclonal antibody solution for 1 h at room temperature. After vigorous washing, an optimum dilution of fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin G serum was mounted on the slides. Fluorescence was observed with an Olympus fluorescence microscope.

## RESULTS

**Establishment of monoclonal antibodies.** We established 11 monoclonal antibodies which reacted with three antigens of EBs (Table 1). The antigens recognized by the monoclonal antibodies were characterized by ELISA and immunoblotting. Eight monoclonal antibodies recognized a periodate-sensitive and proteinase K-resistant antigen with a molecular mass of 3 to 5 kDa; this is lipopolysaccharide (LPS) antigen. Monoclonal antibodies G-B3P and G-2D3P reacted with a

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TABLE 1. Strains used in this study[a]

| Strain | Original host | Yr isolated | Place isolated | Clinical condition | Source or reference |
|---|---|---|---|---|---|
| *Chlamydia psittaci* | | | | | |
| Prt/GCP-1 | *Amazona aestiva* | 1980 | Unknown (imported) | Systemic infection | 32 |
| Prk/Okame | *Nymphicus hollandicus* | 1981 | Unknown (imported) | Systemic infection | 8 |
| Prk/Daruma | *Psittacula alexandri fasciata* | 1980 | Unknown (imported) | Systemic infection | 32 |
| Prk/46, 48, 49, 58, 60, and 64 | *Psittacula krameri manillensis* | 1980 | Unknown (imported) | Systemic infection | 32 |
| Bd/4, 31, 36, 37, 38, 39, 43, 44, 47, 49, 64, 74, 83, 89, 93, 98, 99, 102, 103, 115, 116, 121, 125, 130, 133, 134, and 135 | Budgerigar | 1986 | Aichi | Normal | This laboratory |
| Bd/542P and 602H | Budgerigar | 1980 | Gifu | Normal | 33 |
| Bd/Izawa | Budgerigar | | Okayama | Normal | 15 |
| Bd/F11, F16, M1F, M2F, and 5697 | Budgerigar | | Hokkaido | | |
| Bd/NIH2 and NIH3 | Budgerigar | | Tokyo | | |
| Pg/593 | Pigeon | 1982 | Aichi | Normal | 6 |
| PCM8, 9, 10, 12, 16, 23, 24, 27, 30, 35, 41, 55, 61, 62, 65, 69, 74, 101, 131, and 146 | Pigeon | 1985 | Aichi | Normal | 17 |
| P-1041 | Pigeon | 1984 | Hokkaido | Normal | 2 |
| Tk/NJ | Turkey | | New Jersey | | |
| Tk/Cal | Turkey | | California | | |
| Tk/HT | Turkey | | Huttering | | |
| Tk/Ore | Turkey | | Oregon | | |
| Tk/Tex | Turkey | | Texas | | |
| FP145 | Cat | | | | 27 |
| Ov789 | Sheep | | Japan | Normal | |
| Cal10 | Ferret (human) | 1935 | United States | Cold? | 4 |
| Itoh | Human | | Tokyo | Psittacosis | 9 |
| *Chlamydia trachomatis* | | | | | |
| L1 | Human | 1967 | San Francisco | LGV[b] | 26 |
| L2 | Human | 1967 | San Francisco | LGV | 26 |
| L3 | Human | 1967 | San Francisco | LGV | 26 |

[a] Blank spaces indicate that no information was available.
[b] LGV, Lymphogranuloma venereum.

periodate-resistant and proteinase K-sensitive antigen with a molecular mass of 40 kDa; this is a major outer membrane protein (MOMP) antigen. Monoclonal antibody G-5D3P reacted with a periodate-resistant and proteinase K-sensitive antigen with a molecular mass of 61 kDa.

**Immunological grouping of *C. psittaci*.** The *C. psittaci* and *C. trachomatis* strains were grouped with the panel of monoclonal antibodies in an indirect immunofluorescence test. The reactivity patterns of 80 strains against the monoclonal antibodies are shown in Tables 3 and 4. The reactivity patterns were designated A1, A2, and A3 for monoclonal antibodies recognizing the LPS antigen and Pa, Pb, and Pc for monoclonal antibodies recognizing the MOMP antigen. Through combinations of these reactivity patterns, the 80 strains were classified into five serovars (Table 5). A monoclonal antibody against a 61-kDa antigen reacted with all strains examined. Therefore, this monoclonal antibody was eliminated from further analysis.

TABLE 2. Monoclonal antibodies used in this study[a]

| Monoclonal antibody | Mol wt of recognizing antigen | Titer in ELISA | Titer in ELISA with: | |
|---|---|---|---|---|
| | | | $NaIO_4$ | Proteinase K |
| G/C4L | 3,000–5,000 | 1.55 | 0.21 (13.5) | 1.29 (83.2) |
| G/B5L | 3,000–5,000 | 1.50 | 0.20 (13.3) | 1.18 (78.7) |
| G/D11L | 3,000–5,000 | 1.13 | 0.22 (19.5) | 0.76 (67.3) |
| G/A6L | 3,000–5,000 | 0.85 | 0.04 (4.7) | 0.47 (55.3) |
| G/D5L | 3,000–5,000 | 1.56 | 0.76 (48.7) | 1.38 (88.5) |
| G/B1L | 3,000–5,000 | 1.59 | 1.11 (69.8) | 1.46 (91.8) |
| G/A7L | 3,000–5,000 | 1.59 | 1.26 (79.2) | 1.38 (86.8) |
| G/A9L | 3,000–5,000 | 1.61 | 1.53 (95.0) | 1.40 (87.0) |
| G/B3P | 40,000 | 0.46 | 0.52 (113.0) | 0.00 (0) |
| G/2D3P | 40,000 | 0.19 | 0.26 (136.8) | 0.01 (5.3) |
| G/5D3P | 61,000 | 0.17 | 0.08 (47.1) | 0.06 (35.3) |

[a] Titers are expressed as the $A_{492}$. Numbers in parentheses indicate percentages of titers with chemical treatments relative to titers with no chemical treatments (taken to be 100%).

All psittacine strains were classified as serovar 1. On the other hand, budgerigar strains were classified as serovars 1 and 2. The budgerigar isolates of Aichi Prefecture and Hokkaido Prefecture were divided into both serovars, although most of the Hokkaido isolates and Tokyo isolates were serovar 2. Pigeon and human strains were grouped into serovars 1 and 3. Four of five turkey strains were serovar 3, and the other strain was serovar 4 (this group included the two mammalian strains). The *C. trachomatis* strains were classified as serovar 5.

## DISCUSSION

A total of 77 strains of avian, human, and mammalian *C. psittaci* were classified into four serovars with 11 monoclonal antibodies recognizing the LPS and MOMP antigens. The results indicate that *C. psittaci* strains may be classified into several serovars with monoclonal antibodies. We determined serovars by examining the reactivity patterns against a panel of monoclonal antibodies prepared against strain Prt/GCP-1. Therefore, the serovars described in this study reveal immunological similarities between strain Prt/GCP-1 and other strains. For precise immunological classification of *C. psittaci*, one must use monoclonal antibodies against several strains which are immunologically different from each other. Furthermore, only 4 of 11 monoclonal antibodies were useful in distinguishing each serovar, because most of the monoclonal antibodies used recognized the LPS antigen, which is known to be a genus-specific antigen (25).

Monoclonal antibodies revealed an unexpected degree of antigenic heterogeneity of the LPS. Brade et al. (1) described some differences between the LPSs extracted from a ewe enzootic abortion strain of *C. psittaci* and a strain of *C. trachomatis*. Their analysis revealed an additional hexosamine, D-galactosamine, which had not been found in the LPS of *C. trachomatis*, in the LPS of *C. psittaci*, and a slower migration rate of *C. psittaci* LPS than *C. trachomatis* LPS in sodium dodecyl sulfate-polyacrylamide gel electrophoresis with silver stain. Furthermore, they proposed that *C. psittaci* possesses, in addition to the genus-specific epitope, a second, species-specific determinant located in the carbohydrate moiety of the LPS. However, it is not known whether the differences observed in our study are identical to those reported by Brade et al. (1), because different *C. psittaci* strains were used in both studies. Chemical and immunochemical analyses with various strains and monoclonal antibodies would reveal the precise nature of the antigenic heterogeneity of chlamydial LPS and would reveal the immunochemical basis of the immunological classification of chlamydiae.

The avian and human strains, except for a turkey strain, were distinct from mammalian strains. The immunological similarities among avian and human strains agreed with our results on the polypeptide composition and antigenic specificities of nine chlamydial strains determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting analysis (Fukushi, Furui, and Hirai, submitted) and early observations of other researchers (14, 22–24). The results support the epidemiological implications of close relationships between psittacosis and human chlamydiosis (13).

Budgerigar and pigeon strains were divided into two serovars, in contrast to the single serovar of psittacine strains. Toyofuku et al. (31) classified 16 strains from budgerigars into four groups and 9 *C. psittaci* strains of pigeon origin into three serological groups with monoclonal antibodies, although they did not mention the geographical origins of those strains. Most of the budgerigar strains originated from several small breeding aviaries in Aichi Prefecture, and most of the pigeon strains were isolated from feral pigeons in Nagoya City. The results indicate a mixed prevalence of immunologically distinct *C. psittaci* strains in a population of birds in a geographically limited area. Furthermore, the geographic differences in prevailing *C. psittaci* strains suggest that the majority of budgerigar strains from Aichi Prefecture were serovar 1 and that most of the strains from Hokkaido Prefecture and two strains from Tokyo Metropolitan Prefecture were serovar 2. The distribution of *C. trachomatis* immunotypes differs geographically (7). Therefore, monoclonal antibodies may be useful tools for revealing the ecology and epidemiology of *C. psittaci*.

In this work, relationships between the immunological specificity and pathogenicity of each of the strains were not found. Biotyping and immunological typing of mammalian strains suggested some relationships among biotypes, immunotypes, and pathogenicities (21, 28). DeLong and Magee (3) reported a monoclonal antibody which was specific for ovine abortion strains of *C. psittaci* and nonreactive with isolates from the joints of lambs with polyarthritis. The use

TABLE 3. Reactivity of 80 strains with eight monoclonal antibodies recognizing the LPS antigen

| Reactivity pattern | Reactivity with monoclonal antibody: | | | No. of strains reactive |
|---|---|---|---|---|
| | G-A6L[a] | G-B1L | G-D11L | |
| A1 | + | + | + | 63 |
| A2 | + | + | − | 14 |
| A3 | + | − | + | 3 |

[a] Five other monoclonal antibodies had the same reactivity patterns.

TABLE 4. Reactivity of 80 strains with two monoclonal antibodies recognizing the MOMP antigen

| Reactivity pattern | Reactivity with monoclonal antibody: | | No. of strains reactive |
|---|---|---|---|
| | G-2D3P | G-B3P | |
| Pa | + | + | 64 |
| Pb | + | − | 10 |
| Pc | − | − | 6 |

TABLE 5. Immunological grouping of 80 strains of *C. psittaci* and *C. trachomatis* by their reactivity with monoclonal antibodies

| Serovar | Reactivity pattern | Strains |
|---|---|---|
| 1 | A1:Pa | Prt/GCP-1; Prk/46, 48, 49, 58, 60, and 64; Prk/Daruma; Prk/Okame; Bd/31, 36, 37, 39, 43, 44, 47, 49, 89, 98, 99, 102, 103, 115, 116, 121, 130, 134, and 135; Bd/542P and 602H; Bd/Izawa; Bd/F11; Pg/593; P-1041; PCM8, 9, 12, 16, 23, 24, 30, 41, 55, 61, 62, 65, 69, 131, and 146; Itoh |
| 2 | A2:Pa | Bd/4, 38, 64, 74, 83, 93, 125, and 133; Bd/F16, M1F, M2F, and 5697; Bd/NIH2 and NIH3 |
| 3 | A1:Pb | PCM10, 27, 35, 74, and 101; Tk/Tex; Tk/Ore; Tk/HT; Tk/Cal; Cal10 |
| 4 | A1:Pc | Tk/NJ; Ov789; FP145 |
| 5 | A3:Pc | L1; L2; L3 |

of more monoclonal antibodies and *C. psittaci* strains derived from various birds and animals would reveal the relationships between immunological specificity and pathogenicity. The establishment of an immunological typing system for *C. psittaci* that uses monoclonal antibodies would provide a better understanding of the immunological and molecular bases of the epidemiology and pathogenicity of chlamydiae.

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