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Characterization of *Chlamydia pneumoniae* Species-Specific Proteins Immunodominant in Humans

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Proteins of *Chlamydia pneumoniae* immunodominant in humans were characterized with the sera of 13 patients who were not likely to have been exposed to *C. trachomatis* or *C. psittaci*. The serological responses among these patients were similar on a qualitative basis, but some differences were found quantitatively. However, the serological responses of the patients who were infected with *C. pneumoniae* differed markedly from those of two patients who were infected with *C. trachomatis* and two who were infected with *C. psittaci* and those of mice that were transtracheally infected with *C. pneumoniae*. Among proteins immunodominant in the patients who were infected with *C. pneumoniae*, a 40-kDa major outer membrane protein was genus specific and 53-, 46-, and 43-kDa proteins were species specific in their reactions with the majority of the human sera used. A few sera reacted strongly with a 73-kDa protein genus specifically. Some proteins with weak immunogenicity exhibited species specificity. An antigenic analysis with human sera and murine monoclonal antibodies against the 53-kDa protein showed that the antigenicities were strictly conserved among the seven strains of *C. pneumoniae* tested. The genus-specific 73-kDa protein was solubilized with octylglucoside. All of the species-specific immunodominant proteins were solubilized with sodium dodecyl sulfate, but the genus-specific major outer membrane protein was not. These results suggest that a serological diagnosis of *C. pneumoniae* infection could be achieved species specifically by comparison of the serum responses to sodium dodecyl sulfate- and octylglucoside-soluble fractions.

Members of genus *Chlamydia* are gram-negative, obligate intracellular bacteria which cause diseases such as lymphogranuloma venereum, trachoma, and acute respiratory infections. The genus *Chlamydia* has a unique developmental cell cycle which alternates between the rigid elementary body (EB) and the relatively fragile large reticulate body (RB). The EB is infectious and nonvegetative, whereas the RB is noninfectious and vegetative (24, 32).

The genus *Chlamydia* is classified into three species, *Chlamydia trachomatis*, *C. psittaci*, and *C. pneumoniae*. Recently, Fukushi and Hirai (10) proposed the establishment of a new species, *C. pecorum*, which is derived from ruminants. *C. pneumoniae*, formerly designated *C. psittaci* TWAR, has been classified as a new species according to various analyses, including DNA homology, morphology, antigenicity, and biological characteristics (11). A pear-shaped EB has been proposed as a morphological criterion for the classification of *C. pneumoniae*. Since recent studies, however, have revealed some *C. pneumoniae* strains with round EBs (7, 26), immunological and genetic studies are becoming more important for the identification of *C. pneumoniae*. Kuo et al. (18) demonstrated *C. pneumoniae* strains sharing genus- and species-specific antigenicities and established a species-specific murine monoclonal antibody, RR-402. However, the protein recognized by RR-402 has not been identified (17a), probably because RR-402 does not recognize the denatured protein. Proteins which are species specifically recognized by infected hosts, especially humans, are not well characterized because of serological cross-reactions among members of the genus *Chlamydia*. In this report, we describe the characteristics of the immunodominant proteins of *C. pneumoniae* in natural infections because of their importance in the serological diagnosis of *C. pneumoniae* infections in humans as well as in the immunological classification of *C. pneumoniae*. Furthermore, we report a monoclonal antibody which specifically recognizes the 53-kDa protein of *C. pneumoniae*.

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MATERIALS AND METHODS

Strains. The *Chlamydia* strains used in this study were as follows: *C. pneumoniae* TW-183, AR-39, and AR-388 (12), IOL-207 (8), Kajaani-6 (26), YK-41 (16), and KKpn-1 (unpublished data), *C. psittaci* meningopneumonitis strain Cal 10 (9) and *C. trachomatis* L2/434/Bu. All of the *C. pneumoniae* strains were grown in HeLa 929 or HL cells. *C. psittaci* Cal 10 and *C. trachomatis* L2/434/Bu were grown in L929 suspension or monolayer cell cultures. The EBs of *C. psittaci* Cal 10 were purified by the methods of Tamura and Higashi (30). The EBs of the other strains were also purified, and the purity of each EB preparation was confirmed by electron microscopy as described previously (23). No host cell debris was found, and less than 0.5% of RBs were contaminated.

Sera from patients and mice. The background of the patients and healthy donors and their antibody titers are given in Table 1. The antibody titers were detected by the microplate immunofluorescent-antibody technique (1). The titers obtained with this technique were similar to those obtained with the microimmunofluorescence method (27, 31). The sera used were derived from patients A and B, from whom *C. pneumoniae* YK-41 and KKpn-1, respectively, were isolated, and from patients whose diseases were serologically diagnosed as *C. pneumoniae* infections. We selected 11 patients (1 to 11) whose sera exhibited antibody titers of 512 to 2,048 for

TABLE 1. Background of patients with chlamydial infections and healthy donors and their serum titers

Patient	Age (yr)	Sex	Days after onset of symptoms	Titer[a] of the following antibody against the indicated organism:								
				IgG			IgA			IgM		
				Pn	Ps	Tr	Pn	Ps	Tr	Pn	Ps	Tr
A	15	M	12	512	256	256	64	32	32	256	—[b]	64
			83	2,048	256	256	64	—	—	128	—	—
B	4	F	60	512	—	—	128	—	—	128	—	—
1	40	F	180	2,048	128	64	128	—	—	—	—	—
2	26	M	90	512	32	—	32	—	—	—	—	—
3	64	M	NC[c]	1,024	—	32	128	—	—	—	—	—
4	77	M	NC	2,048	128	128	256	—	—	—	—	—
5	15	F	20	1,024	—	—	128	—	—	—	—	—
6	16	M	16	512	—	—	64	—	—	—	—	—
7	43	M	10	1,024	32	—	32	—	—	—	—	—
8	75	M	32	1,024	32	64	—	—	—	—	—	—
9	79	M	30	512	64	32	64	—	—	—	—	—
10	46	M	31	512	32	64	32	—	—	—	—	—
11	62	M	71	1,024	32	—	64	—	—	—	—	—
12	36	F	NC	128	128	1,024	—	—	128	—	—	—
13	20	F	NC	128	512	2,048	32	—	128	—	—	—
14	68	M	NC	1,024	4,096	1,024	—	—	32	—	—	—
15	51	F	NC	128	256	64	—	64	—	—	—	—
16	21	F		128	64	—	—	—	—	—	—	
17	21	F		128	—	32	—	—	—	—	—	—

[a] Reciprocal of the serum dilution. Pn, *C. pneumoniae* TW-183; Ps, *C. psittaci* Cal 10; Tr, *C. trachomatis* L2/434/Bu.
[b] —, <16.
[c] NC, the time of the onset of symptoms was not certain.

immunoglobulin G (IgG) against *C. pneumoniae* TW-183 and titers of less than 128 for IgG against both *C. psittaci* Cal 10 and *C. trachomatis* L2/434/Bu. As controls, sera from two patients (12 and 13) with *C. trachomatis* infections, from two patients (14 and 15) with *C. psittaci* infections, and from healthy donors (16 and 17) were tested.

Specific-pathogen-free male MCH mice were intranasally immunized with *C. pneumoniae* TW-183 twice with an interval of 35 days. Sera were collected 14 days after the last immunization. The mice were kept under conditions free from other pathogens.

Preparation of detergent-soluble fractions. The purified EBs of *C. pneumoniae* were incubated with 0.125% sodium dodecyl sulfate (SDS) or 1% octylglucoside at 37°C for 30 min and centrifuged at 15,000 × *g* for 5 min. The supernatants were filtrated through 0.22-μm-pore-size filters (Millipore, Toyo Roshi Kaisha Ltd., Tokyo, Japan).

Iodination of membrane proteins. To label the exposed proteins on the EB surface of *C. pneumoniae* TW-183, purified EBs, which were confirmed to be morphologically intact by electron microscopy, were iodinated by the chloramine-T method (14). In brief, 20 μl of purified EBs in 250 mM Tris-HCl buffer (pH 7.3)–4.7 μl of ^{125}I (0.5 mCi; DuPont, Dreieich, Germany)–10 μl of chloramine-T (1 mg/ml in 25 mM Tris-HCl buffer [pH 7.3]) were mixed for 1 min by use of a pipette, and the reaction was stopped by the addition of sodium metabisulfite and bovine serum albumin. Iodinated EBs were washed with 250 mM Tris-HCl buffer five times. The proteins of the EBs were labeled at a radioactivity of 3.4×10^7 cpm/mg of protein.

SDS-PAGE. The proteins of chlamydial EBs were separated by discontinuous SDS-polyacrylamide gel electrophoresis (PAGE) with 10% acrylamide gels (20) to obtain a better differentiation of proteins larger than 40 kDa in size than could be obtained with the 12% gels used in previous reports (2, 6). The protein bands were then visualized by use of a silver staining kit (Wako Pure Chemicals, Osaka, Japan). Proteins labeled with ^{125}I (2.0×10^5 cpm) were analyzed by use of an image analyzer (model BAS2000 Bio-image analyzer; Fuji, Tokyo, Japan).

Immunoblotting analysis. Proteins separated by SDS-PAGE were electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with 1% ovalbumin in Tris-buffered saline (TBS; 20 mM Tris, 0.5 M NaCl [pH 7.5]) for 1 h, washed with TBS containing 0.1% Tween 20 (TTBS) for 3 min, and then incubated with sera diluted 100-fold with TTBS for 45 min. After three washes with TTBS for 3 min each time, the membranes were incubated with peroxidase-conjugated anti-human IgG or anti-mouse IgG (diluted 1:1,000; Bio-Rad Laboratories, Richmond, Calif.) for 45 min. After three washes with TTBS and one wash with TBS, the membranes were incubated with a color-developing substrate solution. The substrate used was 4-methoxy-1-naphthol.

Production of anti-*C. pneumoniae* monoclonal antibodies. Male BALB/c mice were intraperitoneally immunized with a mixture of the SDS-soluble fraction of strain KKpn-1 and Freund's complete adjuvant once, with a mixture of the SDS-soluble fraction and incomplete adjuvant twice, and with the SDS-soluble fraction without adjuvant once. The intervals between immunizations were 8 to 14 days. On the third day following the last immunization, spleen cells and NS-1 mouse myeloma cells were fused with 37% polyethylene glycol. Hybridoma-producing antibodies against *C. pneumoniae* were screened by an enzyme-linked immunosorbent assay (ELISA) with KKpn-1 EBs as the capture antigen.

RESULTS

Protein profiles. Figure 1 shows the protein profiles of the purified EBs of all strains used. The protein profiles of the seven *C. pneumoniae* strains were similar to each other and obviously different in the molecular sizes of proteins and band

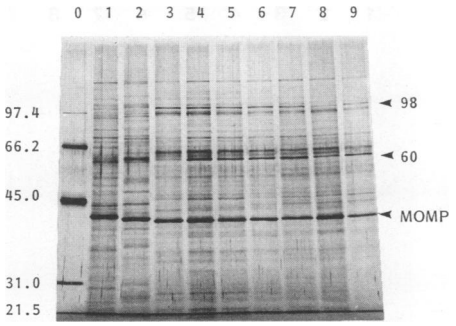

FIG. 1. Protein profiles of the purified EBs of *Chlamydia* strains. The purified EBs (0.12 μg of protein) were electrophoresed by SDS-PAGE with 10% acrylamide, and the proteins were visualized by the silver staining method. Lanes: 0, molecular weight markers (in thousands); 1, *C. psittaci* Cal 10; 2, *C. trachomatis* L2/434/Bu; 3 to 9, *C. pneumoniae* TW-183, AR-39, AR-388, IOL-207, Kajaani-6, YK-41, and KKpn-1, respectively.

patterns from those of *C. psittaci* Cal 10 and *C. trachomatis* L2/434/Bu. The molecular weight of the major outer membrane protein (MOMP) of *C. psittaci* Cal 10 was the highest among these nine strains. All the MOMPs of the seven *C. pneumoniae* strains migrated at approximately 40 kDa, and their deviations were less than 100 Da. The MOMP of *C. trachomatis* L2/434/Bu, the molecular weight of which is 40,607, as calculated from the DNA sequence (28), migrated between those of *C. psittaci* and *C. pneumoniae*. The protein profiles of the seven *C. pneumoniae* strains were similar, but the proteins varied quantitatively among the strains; the quantities of the 60-kDa proteins were smaller in strains TW-183 and YK-41 than in the other five *C. pneumoniae* strains, and the quantity of the 98-kDa protein in strain YK-41, which was reported to be species specific (see below), was markedly small.

Immunodominant protein analysis. The immunodominant proteins of *C. pneumoniae* were analyzed by the immunoblotting method with sera from 2 patients from whom strains YK-41 and KKpn-1 were isolated, sera from 11 patients whose diseases were serologically diagnosed as *C. pneumoniae* infections, and sera from two immunized mice.

Immunoblots with convalescent-phase serum (83 days after onset) from patient A, infected with *C. pneumoniae* YK-41, are shown in Fig. 2a. The serum reacted strongly with the 73-, 60-, and 53-kDa proteins and weakly with several proteins, including the 51-, 46-, and 43-kDa proteins and the 40-kDa MOMP, of all seven *C. pneumoniae* strains tested (Fig. 2a, lanes 3 to 9). No strain-specific recognition was seen at the antigen concentration used (lane 8). Acute-phase serum (12 days after onset) from this patient reacted with only the 73-kDa protein (data not shown). These results suggested that antibodies against the 60-, 53-, 51-, 46-, and 43-kDa proteins and the 40-kDa MOMP were produced by strain YK-41 infection and that the antigenicities of the *C. pneumoniae* strains were strictly conserved. As for recognition of the antigens of *C. psittaci* and *C. trachomatis* (Fig. 2a, lanes 1 and 2), the 73-kDa proteins of both *C. psittaci* and *C. trachomatis* were recognized by the convalescent-phase serum, and the MOMP of *C. trachomatis* was very weakly recognized by it. The 73-kDa protein and the MOMP have been reported to have common antigenicities for this genus (17, 21, 29). These results suggested that patient A had not been exposed to *C. psittaci* or *C. trachomatis* or that antibodies had decreased to undetectable levels, even if the patient had been previously exposed to *C. psittaci* and/or *C. trachomatis*. These results also indicated that the recognition of the 73-kDa proteins of *C. psittaci* and *C. trachomatis* and the MOMP of *C. trachomatis* was due to the serological cross-reaction.

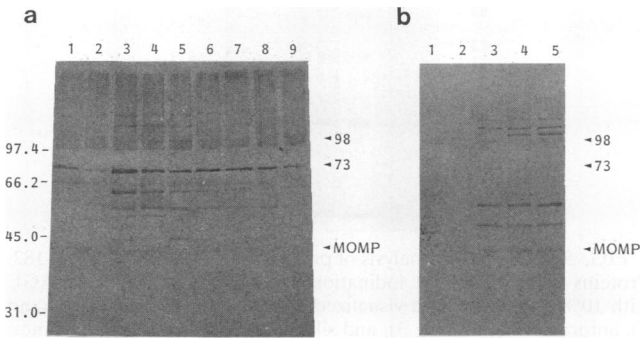

FIG. 2. Immunoblots demonstrating immunodominant proteins. The purified EBs of *Chlamydia* strains (0.4 μg of protein) were electrophoresed and electroblotted. The sera used were derived from patients infected with YK-41 (a) and KKpn-1 (b). The lane numbers are identical to those in Fig. 1.

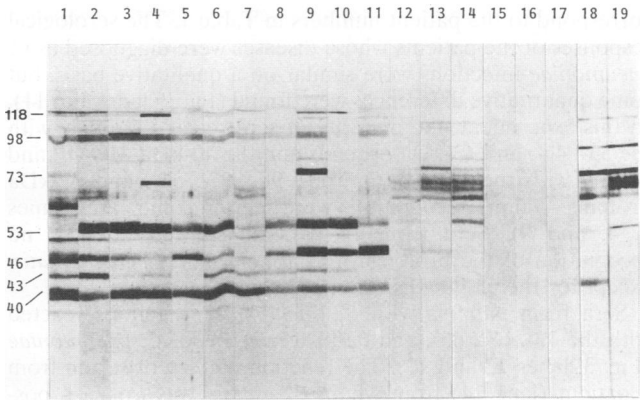

FIG. 3. Immunoblot of the EBs of *C. pneumoniae* TW-183 with sera from humans and mice. Lanes 1 to 11, patients with *C. pneumoniae* infections; 12 and 13, patients with *C. trachomatis* infections; 14 and 15, patients with *C. psittaci* infections; 16 and 17, healthy adults; 18 and 19, mice immunized intranasally with *C. pneumoniae* TW-183. Each lane contained 2 μg of chlamydial protein. Numbers at left are in kilodaltons.

The serum from patient B, who was infected with strain KKpn-1, reacted strongly with the 53-, 46-, and 43-kDa proteins and the 40-kDa MOMP, moderately with the 116- and 117-kDa proteins, and weakly with several proteins, including the 135- and 51-kDa proteins, of the *C. pneumoniae* strains (Fig. 2b, lanes 3 to 5). The proteins recognized by the serum were conserved among the three *C. pneumoniae* strains. A similar recognition was seen for the other *C. pneumoniae* strains, including autologous KKpn-1 (data not shown). No proteins of *C. psittaci* and *C. trachomatis* were recognized by the serum, except for weak recognition of the MOMPs (Fig. 2b, lanes 1 and 2). These results suggested that patient B had not been exposed to *C. psittaci* or *C. trachomatis* or that antibodies had decreased to undetectable levels and that the weak reaction with the MOMPs of *C. psittaci* and *C. trachomatis* was due to the cross-reaction.

Figure 3 shows the serological responses of patients (lanes 1 to 17) and mice (lanes 18 and 19). The lane numbers in Fig. 3

correspond to the patient numbers in Table 1. The serological responses of the patients whose diseases were diagnosed as *C. pneumoniae* infections were similar on a qualitative basis, but some quantitative differences were found (Fig. 3, lanes 1 to 11). In this experiment, the majority of sera reacted strongly with the 53-, 46-, and 43-kDa proteins and the 40-kDa MOMP and weakly with the 118-, 116-, 100-, 98-, 97-, 51-, and 37-kDa proteins. Unique recognitions were seen in some cases (lanes 1, 4, and 9). Sera from 11 patients showed little or no recognition of the proteins of *C. psittaci* and *C. trachomatis*, except for their MOMPs (data not shown).

Sera from patients with *C. trachomatis* infections reacted with the 73-, 68-, 65-, and 62-kDa proteins of *C. pneumoniae* (Fig. 3, lanes 12 and 13). The reaction pattern of serum from a patient (lane 14) from whom *C. psittaci* was isolated, possessing high titers against three *Chlamydia* species, was similar to those of sera from patients (lanes 12 and 13) with *C. trachomatis* infections. Although *C. psittaci* was isolated from patient 14, it was not evident whether he was infected with *C. psittaci* alone or with both *C. psittaci* and *C. trachomatis* or *C. pneumoniae*. Serum from another patient (lane 15) with a *C. psittaci* infection and sera from two healthy donors (lanes 16 and 17) showed little recognition of *C. pneumoniae* proteins. Very weak recognition of the 53- and 46-kDa proteins and the 40-kDa MOMP was observed in for sera from two persons (lanes 12 and 13). Serum obtained from a *C. psittaci* patient (lane 14) and showing high titers against three *Chlamydia* species reacted very weakly with the 46-kDa protein and the 40-kDa MOMP but strongly with the 53-kDa protein. There is no denying the exposure of the serum donors to *C. pneumoniae*. Therefore, it seems that the reaction occurred because of the presence of antibody to *C. pneumoniae*.

The reaction patterns of sera from immunized mice (Fig. 3, lanes 18 and 19) differed markedly from those of sera from patients with *C. pneumoniae* infections (lanes 1 to 11). Although a distinct difference in the reaction strength was seen between the sera from the two mice (lane 18 and 19), the reaction patterns were similar to each other. These responses were relatively similar to those of sera from patients with *C. trachomatis* infections (lanes 12 and 13). The 40-kDa MOMP and the 51-kDa protein were weakly recognized by both sera from humans with *C. pneumoniae* infections and sera from mice.

On the basis of the results mentioned above, the following conclusions were drawn. (i) The antigenicities of *C. pneumoniae* were strictly conserved. (ii) The serological responses of humans against *C. pneumoniae* were similar on a qualitative basis, although some quantitative differences were seen. (iii) The serological responses of humans who were infected with *C. pneumoniae* differed markedly from those of humans who were infected with *C. trachomatis* or *C. psittaci*. (iv) *C. pneumoniae* antigens, such as the 53-, 46-, and 43-kDa proteins and the 40-kDa MOMP, were immunodominant in humans. (v) Antibodies against the 73-kDa proteins and the 40-kDa MOMPs of *C. pneumoniae* strains caused the serological cross-reactivities with *C. psittaci* and *C. trachomatis*. (vi) The serological responses of humans and mice differed markedly.

Specificities of monoclonal antibodies. We established some species-specific monoclonal antibodies. These monoclonal antibodies reacted specifically with the seven strains of *C. pneumoniae* tested, as measured by an ELISA (data not shown), indicating that the antigenicities of the *C. pneumoniae* strains were well conserved. One of the monoclonal antibodies, SCP-53, reacted with the 53-kDa protein (Fig. 4, lanes 1 to 6). This monoclonal antibody also recognized the 53-kDa protein of *C. pneumoniae* AR-388 (data not shown). No reaction of this monoclonal antibody was seen for the proteins of *C. trachomatis* and *C. psittaci* (Fig. 4, lanes 7 and 8). These results may indicate that the 53-kDa protein is one of the immunodominant proteins in humans, as described above.

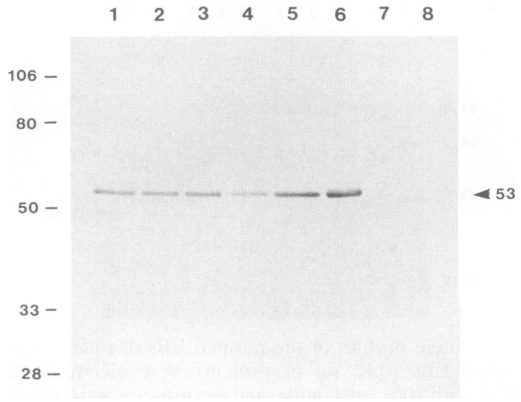

FIG. 4. Immunoblotting of chlamydial EBs with monoclonal antibody SCP-53. The purified EBs were electrophoresed and electroblotted. The blotted membrane was incubated with purified monoclonal antibody SCP-53 at a concentration of 10 μg/ml. Lanes: 1 to 6, *C. pneumoniae* TW-183, AR-39, IOL-207, Kajaani-6, YK-41, and KKpn-1, respectively; 7, *C. psittaci* Cal 10; 8, *C. trachomatis* L2/434/Bu. Numbers at left are in kilodaltons.

Antigenicities of proteins solubilized with detergents. Proteins were solubilized with SDS or octylglucoside (Fig. 5), and their immunoreactivities are shown in Fig. 6. The effect of octylglucoside on EBs appeared to be milder than that of SDS, since fewer proteins were extracted by octylglucoside than by SDS (Fig. 5, lanes 5 and 6). Octylglucoside solubilized the genus-specific 73-kDa protein (Fig. 5, lane 6, and Fig. 6, lane 5). SDS solubilized all the immunodominant proteins, except for the genus-specific 40-kDa MOMP (Fig. 6, lanes 3 and 4).

Membrane protein analysis by labeling. Detergents have been used to prepare the outer membrane of EBs (4, 22). Since detergents solubilize membrane proteins, we labeled the proteins of intact EBs with ^{125}I. The proteins intensively iodinated

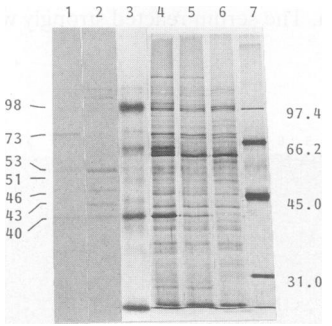

FIG. 5. SDS-PAGE analysis of proteins of *C. pneumoniae* TW-183. Proteins with or without iodination were separated by SDS-PAGE with 10% acrylamide and visualized by immunoblotting (lanes 1 and 2), autoradiography (lane 3), and silver staining (lanes 4 to 7). Lanes: 1 and 2, the proteins of the EBs of strain TW-183 were immunoblotted with sera from human patients infected with YK-41 (lane 1) and KKpn-1 (lane 2); 3, iodinated EBs were electrophoresed; 4, proteins of whole EBs; 5, proteins of EBs were solubilized with 0.125% SDS; 6, proteins of EBs were solubilized with 1% octylglucoside; 7, molecular weight markers (in thousands). Numbers at left are in kilodaltons.

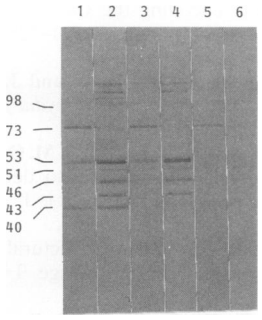

FIG. 6. Immunoblots of immunodominant proteins solubilized with detergents. The materials used in SDS-PAGE were a whole-EB lysate of strain TW-183 (lanes 1 and 2), proteins of EBs solubilized with SDS (lanes 3 and 4), and proteins of EBs solubilized with octylglucoside (lanes 5 and 6). Each lane contained 0.4 μg of protein as starting material. The electroblotted proteins were reacted with sera from patients infected with YK-41 (lanes 1, 3, and 5) and KKpn-1 (lanes 2, 4, and 6). Numbers at left are in kilodaltons.

were the 98-, 63-, and 40-kDa proteins (Fig. 5, lane 3). The 40-kDa MOMP was recognized by the sera from the patients, but the 98- and 63-kDa proteins showed little immunogenicity (Fig. 5, lanes 1 and 2). With the exception of the above-mentioned three proteins, the proteins which showed immunogenicity and were iodinated were the 135- and 53-kDa proteins, whereas iodinated proteins which might be localized on the EB surface numbered not less than ten.

DISCUSSION

C. pneumoniae is an important human respiratory pathogen that causes pharyngitis, bronchitis, and pneumonia (13). However, the serological diagnosis seems to be hampered for the following reasons. (i) Immunological cross-reactions occur among members of the genus *Chlamydia* (19, 21, 29). (ii) serological responses specific for natural *C. pneumoniae* infections in humans are not well known (2, 6, 19), because the prevalence of antibodies to members of the genus *Chlamydia* is over 50% (25). (iii) Analyses of species-specific antigens have not been improved, because the large-scale purification of the EBs of *C. pneumoniae* strains is difficult. (iv) Antigenic variations among the strains of *C. pneumoniae* have not been clarified (2).

We reported a *C. pneumoniae* infection in which the serum reacted with the 73-, 60-, 53-, 46-, 43-, and 40-kDa proteins of three *C. pneumoniae* strains (15). In the present study, we have shown that seven *C. pneumoniae* strains appear to have similar protein profiles and that the proteins of these seven strains share strictly species-specific antigenicities. The two patient sera used in the present study were derived from young (15- and 4-year-old) patients, who were very unlikely to have been exposed to *C. psittaci* or *C. trachomatis*. Thus, we could demonstrate clearly the *C. pneumoniae*-specific antigens. We also demonstrated immunodominant proteins of *C. pneumoniae* by using sera from 11 other patients with *C. pneumoniae* infections (Fig. 3). Ladany et al. (19) reported that 80- and 30-kDa proteins were recognized species specifically with rabbit antiserum and that a protein with a molecular mass of approximately 55 kDa reacted species specifically with human serum. This 55-kDa protein may be identical to the protein which we call the 53-kDa protein. Campbell et al. reported that a 98-kDa protein was recognized by both human serum (6) and rabbit serum (5) species specifically. On the basis of our results, the 98-kDa protein existing on the membrane (Fig. 5, lane 3) is probably species specific, but it is not immunodominant in humans (Fig. 3 and Fig. 5, lanes 1 and 2). Since the serological responses to each antigen of *C. pneumoniae* varied between humans and mice (19) (Fig. 5), the antigens immunodominant in humans should be characterized for a clinical diagnosis. Our results showed that the genus-specific 73-kDa protein, which may be a DnaK heat shock protein (17), was solubilized with octylglucoside and that the immunodominant proteins, with the exception of the genus-specific MOMP, were solubilized with SDS. These results suggested that the serodiagnosis of *C. pneumoniae* infections could be achieved species specifically by comparing the serum responses to an SDS-soluble fraction, including the 73-, 53-, 46-, and 43-kDa proteins, and an octylglucoside-soluble fraction, including the 73-kDa protein.

Ladany et al. (19) reported that the cross-reactivity of the antibody against the *C. pneumoniae* antigen with the *C. trachomatis* antigen was decreased by treatment of the *C. pneumoniae* antigen with sodium deoxycholate, which extracted common lipopolysaccharide antigens. However, the MOMP, containing a genus-specific antigen, was not extracted by either detergent used here, SDS or octylglucoside (Fig. 6, lanes 3 to 6). These results suggested that the EB antigens treated with sodium deoxycholate may contain the MOMP and still have cross-reactivity. Furthermore, little serological response to lipopolysaccharide was seen in the present study, since no immunoblot band was seen near the bottom, where lipopolysaccharide should have migrated in SDS-PAGE (3). The results of the present study have led us to consider that an ELISA system could be constructed with SDS-soluble proteins, including antigens immunodominant in humans.

Black et al. (2) reported an antigenic variation of *C. pneumoniae* by using strain CWL-011, which may possess an MOMP with a different molecular weight, and suggested that the serological diagnosis of *C. pneumoniae* infections may require the use of more than one strain. Further characterization of strain CWL-011 should be done, whether or not this strain is classified as *C. pneumoniae*, because the results obtained in the present study demonstrated very similar protein profiles (5) (Fig. 1) and the presence of species-specific antigenicities among seven strains of *C. pneumoniae* (Fig. 2).

In addition, we showed that the genus-specific 40-kDa MOMP and the species-specific 98-kDa protein were well iodinated, suggesting that these proteins are localized on the EB surface of *C. pneumoniae*. The species-specific 53-kDa protein, which had strong immunogenicity, was weakly iodinated. In addition, we established a *C. pneumoniae*-specific monoclonal antibody against the 53-kDa protein. This is the first report of a protein recognized by a *C. pneumoniae*-specific monoclonal antibody. The localization of the other immunodominant proteins, including the 46- and 43-kDa proteins, was not determined.

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REFERENCES

1. **Bessho, H., and A. Matsumoto.** 1984. Detection of antibody to *Chlamydia psittaci*: comparison of antibody titers in human sera assayed by enzyme-linked immunosorbent assay (ELISA) and

microplate immunofluorescence antibody technique. Kawasaki Med. J. **10:**217–224.
2. **Black, C. M., J. E. Johnson, C. E. Farshy, T. M. Brown, and B. P. Berdal.** 1991. Antigenic variation among strains of *Chlamydia pneumoniae*. J. Clin. Microbiol. **29:**1312–1316.
3. **Caldwell, H. D., and P. J. Hitchcock.** 1984. Monoclonal antibody against a genus-specific antigen of *Chlamydia* species: location of the epitope on chlamydial lipopolysaccharide. Infect. Immun. **44:**306–314.
4. **Caldwell, H. D., J. Kromhout, and J. Schachter.** 1981. Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. Infect. Immun. **31:**1161–1176.
5. **Campbell, L. A., C.-C. Kuo, and J. T. Grayston.** 1990. Structural and antigenic analysis of *Chlamydia pneumoniae*. Infect. Immun. **58:**93–97.
6. **Campbell, L. A., C.-C. Kuo, S.-P. Wang, and J. T. Grayston.** 1990. Serological response to *Chlamydia pneumoniae* infection. J. Clin. Microbiol. **28:**1261–1264.
7. **Carter, M. W., S. A. H. Al-Mahdawi, I. G. Giles, J. D. Treharne, M. E. Ward, and I. N. Clarke.** 1991. Nucleotide sequence and taxonomic value of the major outer membrane protein gene of *Chlamydia pneumoniae* IOL-207. J. Gen. Microbiol. **137:**465–475.
8. **Dwyer, R., St. C., J. D. Treharne, B. R. Jones, and J. Herring.** 1972. Chlamydial infection. Results of micro-immunofluorescence tests for the detection of type-specific antibody in certain chlamydial infections. Br. J. Vener. Dis. **48:**452–459.
9. **Francis, T. F., and T. P. Magill.** 1938. An unidentified virus producing acute meningitis and pneumonitis in experimental animals. J. Exp. Med. **68:**147–160.
10. **Fukushi, H., and K. Hirai.** 1992. Proposal of *Chlamydia pecorum* sp. nov. for *Chlamydia* strains derived from ruminants. Int. J. Syst. Bacteriol. **42:**306–308.
11. **Grayston, J. T., C.-C. Kuo, L. A. Campbell, and S.-P. Wang.** 1989. *Chlamydia pneumoniae* sp. nov. for *Chlamydia* sp. strain TWAR. Int. J. Syst. Bacteriol. **39:**88–90.
12. **Grayston, J. T., C.-C. Kuo, S.-P. Wang, and J. Altman.** 1986. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N. Engl. J. Med. **315:**161–168.
13. **Grayston, J. T., S.-P. Wang, C.-C. Kuo, and L. A. Campbell.** 1989. Current knowledge on *Chlamydia pneumoniae*, strain TWAR, an important cause of pneumonia and other acute respiratory diseases. Eur. J. Clin. Microbiol. Infect. Dis. **8:**191–202.
14. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194:**495–496.
15. **Kanamoto, Y., Y. Iijima, S. Miyashita, A. Matsumoto, and T. Sakano.** 1993. Antigenic characterization of *Chlamydia pneumoniae* isolated in Hiroshima, Japan. Microbiol. Immunol. **37:**495–498.
16. **Kanamoto, Y., and T. Sakano.** 1992. Isolation of *Chlamydia pneumoniae* from a patient with acute bronchitis. J. Jpn. Assoc. Infect. Dis. **66:**637–642. (In Japanese.)
17. **Kornak, J. M., C.-C. Kuo, and L. A. Campbell.** 1991. Sequence analysis of the gene encoding the *Chlamydia pneumoniae dnaK* protein homolog. Infect. Immun. **59:**721–725.
17a. **Kuo, C.-C.** Personal communication.
18. **Kuo, C.-C., H.-H. Chen, S.-P. Wang, and J. T. Grayston.** 1986. Identification of a new group of *Chlamydia psittaci* strains called TWAR. Infect. Immun. **24:**1034–1037.
19. **Ladany, S., C. M. Black, C. E. Farshy, J. M. Ossewaarde, and R. C. Barnes.** 1989. Enzyme immunoassay to determine exposure to *Chlamydia pneumoniae* (strain TWAR). J. Clin. Microbiol. **27:**2778–2783.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
21. **Maclean, I. W., R. W. Peeling, and R. C. Brunham.** 1988. Characterization of *Chlamydia trachomatis* antigens with monoclonal and polyclonal antibodies. Can. J. Microbiol. **34:**141–147.
22. **Manire, G. P., and A. Tamura.** 1967. Purification and chemical composition of the cell walls of mature infectious dense forms of menigopneumonitis organisms. J. Bacteriol. **94:**1178–1183.
23. **Miyashita, M., and A. Matsumoto.** 1992. Establishment of a particle-counting method for purified elementary bodies of chlamydiae and evaluation of sensitivities of the IDEIA Chlamydia Kit and DNA Probe by using the purified elementary bodies. J. Clin. Microbiol. **30:**2911–2916.
24. **Moulder, J. W.** 1991. Interaction of chlamydiae and host cells in vitro. Microbiol. Rev. **55:**143–190.
25. **Osser, S., and K. Persson.** 1989. Immune response to genital chlamydial infection and influence of *Chlamydia pneumoniae* (TWAR) antibodies. Eur. J. Clin. Microbiol. Infect. Dis. **8:**532–535.
26. **Popov, V. L., A. A. Shatkin, V. N. Pankratova, N. S. Smirnoava, C.-H. von Bonsdroff, M.-R. Ekman, A. Morttinen, and P. Saikku.** 1991. Ultrastructure of *Chlamydia pneumoniae* in cell culture. FEMS Microbiol. Lett. **84:**129–134.
27. **Soejima, R.** 1991. Present state of *Chlamydia pneumoniae* infections. Clin. Microbiol. **18:**767–773. (In Japanese.)
28. **Stephens, R. S., R. Sanchez-Pescador, E. A. Wagar, C. Inouye, and M. S. Urdea.** 1987. Diversity of *Chlamydia trachomatis* major outer membrane protein genes. J. Bacteriol. **169:**3879–3885.
29. **Stephens, R. S., M. R. Tam, C.-C. Kuo, and R. C. Nowinski.** 1982. Monoclonal antibodies to *Chlamydia trachomatis*: antibody specificities and antigen characterization. J. Immunol. **128:**1083–1089.
30. **Tamura, A., and N. Higashi.** 1963. Purification and chemical composition of menigopneumonitis virus. Virology **20:**596–604.
31. **Wang, S.-P., and J. D. Grayston.** 1986. Microimmuno-fluorescence serological studies with TWAR organism, p. 329–662. *In* D. Oriel, G. Ridgway, J. Schachter, and D. Taylor-Robinson (ed.), Chlamydial infections. Proceedings of the Sixth International Symposium on Human Chlamydial Infections. Cambridge University Press, Cambridge.
32. **Ward, M. E.** 1988. The chlamydial developmental cycle, p. 71–95. *In* A. L. Barron (ed.), Microbiology of Chlamydia. CMC Press, Inc., Boca Raton, Fla.