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Compared with the major outer membrane proteins (MOMPs) of the other chlamydial species, the *Chlamydia pneumoniae* MOMP appears to be less antigenically complex, and as determined by immunoblot analysis, it does not appear to be the immunodominant antigen recognized during infection. Nucleotide sequence analysis of the *C. pneumoniae* MOMP gene (*ompA*) revealed that it consisted of a 1,167-base open reading frame with an inferred 39,344-dalton mature protein of 366 amino acids plus a 23-amino-acid leader sequence. A ribosomal-binding site was located in the 5' upstream region, and two stop codons followed by an 11-base dyad forming a stable stem-loop structure were identified. This sequence shares 68 and 71% DNA sequence homology to the *Chlamydia trachomatis* serovar L2 and *Chlamydia psittaci* ovine abortion agent MOMP genes, respectively. Interspecies alignment identified regions, corresponding to the variable domains, which share little sequence similarity with the other chlamydial MOMPs. All seven cysteines conserved in the *C. trachomatis* and *C. psittaci* MOMPs, which are involved in the formation of disulfide cross-linkages, are found in the *C. pneumoniae* MOMP.

Chlamydia pneumoniae has been established as an important cause of acute respiratory diseases in humans (7). Structural studies of this organism have shown that, as in the other Chlamydia spp. (12), the major outer membrane protein (MOMP) exists in disulfide-linked protein complexes within the outer membrane complex and functions in maintaining cell wall rigidity (3). Although the MOMP of C. pneumoniae plays a similar structural role, antigenic analysis has demonstrated that it has characteristics that differ from those of the other chlamydial MOMPs (3). In contrast to Chlamydia trachomatis and Chlamydia psittaci, in which the MOMP is the immunodominant antigen recognized during infection, immunoblot analysis of proteins recognized by human sera from C. pneumoniae patients has suggested that the MOMP is not the immunodominant antigen recognized during C. pneumoniae infection (4).

Monoclonal antibodies (MAbs) against the C. trachomatis MOMP have identified serovar-, subspecies-, and speciesspecific epitopes on the protein (5, 18, 22). The MOMP genes of several C. trachomatis and C. psittaci strains have been sequenced and were found to be highly conserved except in four variable domains (VDs) (16, 18, 22). Epitope mapping has shown that three of the VDs (VDI, VDII, and VDIV) contain the serovar-, subspecies-, and species-specific antigenic determinants (1, 5, 18, 20). Immunoblot analyses of C. pneumoniae with anti-C. pneumoniae rabbit sera or human sera from patients with C. pneumoniae infection have shown that the recognition of the MOMP is genus reactive (3, 4). To date, no other antigenic reactivities have been identified in the C. pneumoniae MOMP. The purpose of our studies was to define the coding sequence of the C. pneumoniae MOMP gene and to investigate its relationship to the other Chlamvdia MOMP genes.

The C. pneumoniae isolate AR-39, which had been adapted to grow in HeLa 229 cells, was harvested and purified in a linear gradient of meglumine diatrizoate (Hypaque-76; Winthrop-Breon Laboratories, New York, N.Y.)

(11). The final products usually contained 1.0×10^8 to 5.0×10^8 inclusion-forming units per ml of organisms.

A partial gene bank was constructed by digesting C. pneumoniae DNA with EcoRI, ligating it into similarly cut lambda gt11, and packaging it into phage particles (9). Using a 1.1-kb EcoRI fragment encoding a portion of the C. trachomatis serovar L2 MOMP as a probe (15), a 1.5-kb EcoRI fragment was isolated and subcloned into the plasmid pTZ18r (Pharmacia, Piscataway, N.J.). The Sanger dideoxychain termination method of DNA sequencing (13) was carried out on single-stranded fragments cloned into M13mp18 and M13mp19 by using the Sequenase kit (United States Biochemical Corp., Cleveland, Ohio). Nested deletions were generated by using the Erase-a-Base kit (Promega Biotec, Madison, Wis.). Sequence analyses were performed by using the Pustell Sequence Analysis Program (IBI) and the University of Wisconsin Genetics Computer Group programs. DNA sequence analysis of the 1.5-kb EcoRI fragment showed that it contained 0.5 kb of the 3' end of the translated sequence but lacked the 5' terminus of the gene.

The 5'-terminal sequences of the MOMP genes have been shown to be highly conserved in C. trachomatis and C. psittaci (16, 20, 21). Two different DNA probes that contain this area were used to identify MOMP gene sequences in C. pneumoniae by Southern blots. The probes used were a 280-bp XhoI-EcoRI fragment (AM-11), which contains the 5' terminus of the C. trachomatis serovar A MOMP gene, and GPM-4, which contains the entire coding region of the C. psittaci guinea pig inclusion conjunctivitis MOMP (21). Both were graciously provided by Y.-X. Zhang, NIH Rocky Mountain Laboratories, Hamilton, Mont. The 1.5-kb EcoRI fragment was recognized by the GPM-4 probe but not by the AM-11 probe, demonstrating that this fragment did not contain the 5' end of the MOMP gene. When AM-11 was used to probe C. pneumoniae chromosomal digests, it hybridized to 0.9-kb HindIII, 3.5-kb EcoRI, 3.8-kb BamHI, and 3.7-kb MspI fragments. By constructing two partial gene banks of C. pneumoniae DNA and using the ³²P-labeled AM-11 fragment as a probe, the 0.9-kb HindIII and 3.7-kb EcoRI fragments were isolated and subsequently sequenced.

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-316	AMATTATATATATATAATGAAAAGAATATACAAAAAAGCTATAGCT														
-271	TTOCTATABCTCATAACAGAAGTTCTTGGTTGAAATATGOGGCTAAAAACACTTAATCTTCTTATOGTCTTTACTATAATAAGAAAAGTTT														
-180	GATATGTTTTCCACTAATGAGCTGTATGTTCATATTTAAGGCCCGTTTTTCAATGATAAGAGCTTCCTAAATTTGCCTGCAGGATATCTTGT														
-89															
1 -23	ATG AMA AMA CTC TTA AMG TOG GOG TTA TTA TOC GOC GCA TTT GCT GGT TCT GTC GGC TOC TTA CAA GOC Met Lys Lys Leu Leu Lys Ser Ala Leu Leu Ser Ala Ala Phe Ala Gly Ser Val Gly Ser Leu Gin Ala														
70 1	TTG CCT GTA GGG AAC CCT TCT GAT CCA AGC TTA TTA ATT GAT GGT ACA ATA TGG GAA GGT GCT GCA GGA Leu Pro Val Gly Asn Pro Ser Asp Pro Ser Leu Leu Ile Asp Gly Thr Ile Trp Glu Gly Ala Ala Gly 26 29 33														
139 24	GAT CET TEC GAT CET TEC GET ACT TEG TEC GAC GET ATT AGE TTA CET GET GEA TIT TAC GEA GAC TAT Asp Pro Cys Asp Pro Cys Ala Thr Trp Cys Asp Ala Ile Ser Leu Arg Ala Gly Phe Tyr Gly Asp Tyr														
	59														
208	GTT TTC GAC OGT ATC TTA AMA GTA GAT GCA OCT AMA ACA TTT TCT ATG GGA GOC AMG OCT ACT GGA TOC														
47	Val Phe Asp Arg Ile Leu Lys Val Asp Ala Pro Lys Thr Phe Ser Met Gly Ala Lys Pro Thr Gly Ser														
	81														
277	GCT GCT GCA MAC TAT ACT ACT GCC GTA GAT AGA CCT AAC COG GCC TAC AAT ANG CAT ITA CAC GAT GCA														
70	Ala Ala Ala Asn Tyr Thr Thr Ala Val Asp Arg Pro Asn Pro Ala Tyr Asn Lys His Leu His Asp Ala														
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346	113 GAG TGE TTC ACT AAT GCA GGC TTC ATT GCC TTA AAC ATT TGG GAT CGC TTT GAT GTT TTC TGT ACT TTA														
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346 93 415 116 484 139 553 162	113 GAG TGC TTC ACT AAT GCA GGC TTC ATT GCC TTA AAC ATT TGG GAT CGC TTT GAT GTT TTC TGT ACT TTA Glu Trp Phe Thr Asn Ala Gly Phe Ile Ala Leu Asn Ile Trp Asp Arg Phe Asp Val Phe Cys Thr Leu 137 GGA GCT TCT AAT GGT TAC ATT AGA GGA AAC TCT ACA GCG TTC AAT CTC GTT GGT TTA TTC GGA GTT AAA Gly Ala Ser Asn Gly Tyr Ile Arg Gly Asn Ser Thr Ala Phe Asn Leu Val Gly Leu Phe Gly Val Lys VD II														

FIG. 1. Nucleotide sequence and inferred amino acid sequence of the gene encoding the *C. pneumoniae* AR-39 MOMP. The gene contains an ATG start codon followed by a 1,167-nucleotide open reading frame and ends with two in-frame TAA and TAG stop codons. The 1 by the gene sequence marks the transitional initiation codon ATG, and the 1 by the peptide sequence marks the N terminus of the mature MOMP. The VDs and the conserved cysteines are boxed. A putative Shine-Dalgarno sequence is underlined. The 11-base transcription terminator dyad is indicated by inverted arrows from nucleotide 1202 to 1229.

The complete sequence of the *C. pneumoniae* MOMP structural gene and its predicted amino acid (AA) sequence is shown in Fig. 1. This gene is analogous to the *C. trachomatis* and *C. psittaci omp1*. Based on the recent recommendations by Yuan et al. (19), the *C. pneumoniae* MOMP gene has been designated *ompA*.

The *C. pneumoniae* MOMP gene open reading frame consists of 1,167 bp which encode a 389-AA pre-MOMP sequence. By comparison with known MOMP sequences, in

which the mature N terminus is a leucine preceded by a 22-AA leader sequence (16, 20, 21), we deduced that the *C. pneumoniae* MOMP leader sequence is composed of 23 AA. The leader peptide has the conserved basic amino terminus and the hydrophobic core found in the other chlamydia MOMPs, while the rest of the sequence shows significant heterogeneity. The first six residues of the mature MOMP sequences of *C. trachomatis* (serovars A, B, C, and L2), *C. psittaci*, guinea pig inclusion conjunctivitis, meningopneu-

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208	Phe	Ser	Val	Asn	i vs	Pm	l ve	61v	Tvr	lvs	Giv	Val	Ala	Phe	Pro	Leu	Pro	The	Aso	A1a	614	Val	41a
		233																		•			
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760	ACA	C T	ACT	GGA	ACA	AAG	TCT	GOG	ACC	ATC	AAT	TAT	CAT	GAŅ	TGG	CAA	GTA	GGA	θÔC	TCT	CTA	TCT	TAC
231	Thr	Ala	Thr	Gly	Thr	Lys	Ser	Ala	Thr	Пe	Asn	Tyr	His	61u	Trp	Gln	Va1	Gly	Ala	Ser	Leu	Ser	Tyr
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254	Ana	Lau	Asn	Ser	Leu	Val	Pro	Tvr	Ile.	61v	Val	Gln	Tro	Ser	Ano	Ala	Thr	Phe	Aso	41a	Aso	Asn	TIe
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898	CGC	ATT	GCT	CAG	CCA	***	CTA	CCT	ACA	GCT	GTT	TTA	AAC	TTA	ACT	GCA	TGG	AAC	ССТ	TCT	TTA	CTA	GGA
277	Arg	110	Ala	Gln	Pro	Lys	Leu	Pro	Thr	Ala	Va]	Leu	Asn	Leu	Thr	Ala	Trp	Asn	Pro	Ser	Leu	Leu	61y
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300	Asn	Ala	Thr	Ala	Leu	Ser	Thr	Thr	Asp	Ser	Phe	Ser	Asp	Phe	Met	Gln	Ile	Va'l	Ser	Cys	Gln	Ile	Asn
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1036	AAG	π		тст	AGA		ect	TGT	GGA	GTT	ACT	GTA	GGA	GCT	ACT	TTA	ள	GAT	GCT	GAT		TGG	TCA
323	Lys	Phe	Lvs	Ser	Ana	Lvs	Ala	Cvs	Giv	Val	Thr	Val	Glv	Ala	Thr	Leu	Val	Aso	Ala	Aso	Lvs	Tro	Ser
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1105	СТТ	ACT	GCA	GAA	GCT	CGT	TTA	ATT	AAC	GAG	AGA	GCT	GCT	CAC	GTA	TCT	GGT	CAG	TTC	AGA	πс	TAA	AGA
346	Leu	Thr	Ala	Glu	Ala	Arg	Leu	Ile	Asn	Glu	Arg	Ala	Ala	His	Val	Ser	Gly	Gln	Phe	Arg	Phe	End	
1174	π	стт	AGAA	тпс	TOCT	CAOC	пат	TATC	IGAG	TCTA	CATG	TTAG	GCTC	TGAT	TTAT	стс	IGAG	me		пс	GAC	-	TT
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1447	TCG	ATTC	IGAT	CGGG	UGCT	TCT	TAC	NCAN	UCAN	GACT	6444	ITCT	GGCT	ITTA	ICIC	NGAA'	IGCO	i					

FIG. 1-Continued

monitis (22), ovine abortion (OA), and *C. pneumoniae* are identical. The mature *C. pneumoniae* MOMP contains 366 AA with a predicted molecular weight of 39,344, which is consistent with the observed mobility of the *C. pneumoniae* MOMP on sodium dodecyl sulfate-polyacrylamide gels (3). Comparison of the inferred AA sequence of the *C. pneumoniae* MOMP with those of the *C. trachomatis* L2 MOMP and the *C. psittaci* OA MOMP demonstrated 73 and 80% similarity, respectively (Fig. 2). The *C. pneumoniae* MOMP contains 33 basic and 31 acidic AA residues and has a weakly acidic estimated pI, as for the MOMPs of *C. psittaci* meningopneumitis and guinea pig inclusion conjunctivitis (21). In the *C. trachomatis* MOMPs the number of acidic residues is significantly higher, 39 to 42, resulting in a lower pI (2, 21).

Comparison of the C. trachomatis L2, C. psittaci OA, and C. pneumoniae AR-39 MOMP AA sequences (Fig. 2) indicate that, as observed for other chlamydial MOMPs, the

proteins are interspersed with four VDs (VDI to VDIV) and that all insertions and deletions occurred in these VDs (16, 20, 21). The seven cysteines observed in the MOMPs of the other chlamydial species (21) are in exactly the same positions in the C. pneumoniae MOMP. Three of them are located in the N-terminus region before VDI, one is located between VDI and VDII, two are located between VDII and VDIII, and the last one is located in the C-terminus region after the VDIV domain (Fig. 2). One cysteine (in position 203) which is conserved in all known C. trachomatis MO-MPs but not in the C. psittaci MOMP appears to be conserved in the C. pneumoniae MOMP (Fig. 2). The cysteine residues allow the formation of intra- and interdisulfide bonds (12, 21). These play an important role in the maintenance of the structural integrity of chlamydia, in the formation of diffusion channels, and in the regulation of chlamydial differentiation (8, 12).



FIG. 2. AA comparison of *C. pneumoniae* AR-39 MOMP with *C. trachomatis* L2 and *C. psittaci* OA MOMPs. A dot indicates the same AA as in the *C. pneumoniae* MOMP and a dash represents a gap in the sequence. The four VDs and the conserved cysteines are boxed. Numbers above the sequences denote AA residues of the *C. pneumoniae* MOMP.

The overall interspecies conservation of the C. pneumoniae MOMP gene with the C. trachomatis and C. psittaci MOMP genes is 68 and 71%, respectively. This degree of similarity is comparable to that observed between the C. trachomatis and C. psittaci MOMP genes (21). In the C. pneumoniae MOMP, the translation initiator ATG is preceded by a Shine-Dalgarno-like ribosomal-binding site, as has been shown for other MOMP genes (Fig. 1) (16, 21). While putative promoter and terminator regulatory regions have been identified in other chlamydial genes (14), no chlamydial consensus promoter sequences have been identified. Within the C. trachomatis and C. psittaci MOMP genes, multiple tandem promoters have been mapped (17, 19). The 5' flanking area of the C. pneumoniae MOMP gene shows 75.5 and 86.7% similarity with the corresponding regions of the C. trachomatis L2 and C. psittaci OA MOMP genes. The terminator sequences reported for other chlamydial genes demonstrate typical rho-independent stemloop structures (6, 16). In contrast, the region downstream of the translational stop signals in the C. pneumoniae MOMP gene revealed an 11-base dyad which forms a stem-loop structure followed by a second stem-loop structure, but there is no string of thymidine residues characteristic of the rho-independent terminator (Fig. 1). Transcription of the 11-base dyad would result in the folding of the mRNA into a stem-loop structure with a calculated least free energy of -14.0 kcal. If the transcription of the second stem-loop structure is included in this calculation, a lower calculated least free energy of -27.4 kcal would result. The terminator sequence for the *C. pneumoniae* 75-kDa gene also contains two stem-loop structures and lacks a poly(T) tail (10).

In the case of C. trachomatis and C. psittaci, the MOMP is a complex antigen that is immunodominant. Molecular analysis of the C. pneumoniae MOMP gene has shown that it has significant similarities in structure and sequence to MOMPs of the other chlamydial species, except in the VDs, where they share little sequence similarity. For C. trachomatis, the AA sequence variation within the VDs provides the molecular basis for serologic classification of C. trachomatis. To date, all monoclonal antibodies that we have prepared which recognize the C. pneumoniae MOMP are genus specific. Thus, we have been unable to identify any serological domains on the C. pneumoniae MOMP. Further sequence analysis and comparison of the MOMPs of other C. pneumoniae isolates will aid in elucidating the relationship of the VDs to the antigenic nature of the C. pneumoniae MOMP.

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