



US 20030095973A1

(19) **United States**

(12) **Patent Application Publication**

Murdin et al.

(10) **Pub. No.: US 2003/0095973 A1**

(43) **Pub. Date: May 22, 2003**

(54) **CHLAMYDIA ANTIGENS AND
CORRESPONDING DNA FRAGMENTS AND
USES THEREOF**

(76) Inventors: **Andrew D. Murdin**, Newmarket (CA);
Raymond P. Oomen, Aurora (CA); **Joe
Wang**, Etobicoke (CA); **Pamela Dunn**,
Mississauga (CA)

Correspondence Address:

**Bernhard D Saxe
Foley & Lardner
3000 K Street N W
Suite 500
Washington, DC 20007-5109 (US)**

(*) Notice: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

(21) Appl. No.: **09/564,479**

(22) Filed: **May 3, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/132,270, filed on May 3, 1999. Provisional application No. 60/141,276, filed on Jun. 30, 1999.

Publication Classification

(51) **Int. Cl.⁷ A61K 39/40; A61K 39/00;
A61K 39/38**

(52) **U.S. Cl. 424/184.1**

ABSTRACT

The present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by a strain of Chlamydia, specifically *C. pneumoniae*, employing a vector containing a nucleotide sequence encoding full-length, 5'-truncated or 3'-truncated 76 kDa protein of a strain of *Chlamydia pneumoniae* and a promoter to effect expression of the 76 kDa protein gene in the host. Modifications are possible within the scope of this invention.

Figure 1: Full-length Sequence of *C. pneumoniae* 76kDa Gene.

```

ataaaatctt taaaaacagg ctcgcattaa ttattagtga gagctttttt tttattttt 60
ataataaaac taaaagattt ttattatttt ttgagttttt atg gtt aat cct att 115
Met Val Asn Pro Ile
1 5

ggc cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat ctt 163
Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp Leu
10 15 20

tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa gct 211
Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu Ala
25 30 35

caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc gat 259
Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr Asp
40 45 50

tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc atg 307
Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu Met
55 60 65

agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct tct 355
Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser
70 75 80 85

act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct acg 403
Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro Thr
90 95 100

cct cct cca ccc acg ttt gat gat tat aag act caa gcg caa aca gct 451
Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr Ala
105 110 115

tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct gct 499
Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala Ala
120 125 130

ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg gct 547
Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala Ala
135 140 145

act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat gcc 595
Thr Asp Glu Glu Thr Ala Ile Ala Glu Trp Glu Thr Lys Asn Ala
150 155 160 165

gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat gct 643
Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr Ala
170 175 180

tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc ttc 691
Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser Phe
185 190 195

```

Figure 1 (continued)

gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac aaa	739		
Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn Lys			
200	205	210	
gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca ggg	787		
Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly			
215	220	225	
aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct aca	835		
Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala Thr			
230	235	240	245
gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat ttt	883		
Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr Phe			
250	255	260	
gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat aac	931		
Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn Asn			
265	270	275	
agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct aag	979		
Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala Lys			
280	285	290	
aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att ctt	1027		
Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile Leu			
295	300	305	
caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa aat	1075		
Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys Asn			
310	315	320	325
atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca gtt	1123		
Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr Val			
330	335	340	
gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt tcc	1171		
Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val Ser			
345	350	355	
atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg tct	1219		
Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met Ser			
360	365	370	
ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat tct	1267		
Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp Ser			
375	380	385	
caa gct gcc caa cag gag ctc gca gca caa gct aga gca gcg aaa gcc	1315		
Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys Ala			
390	395	400	405
gct gga gat gac agt gct gct gca gcg ctg gca gat gct cag aaa gct	1363		
Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys Ala			
410	415	420	

Figure 1 (continued)

tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc aat	425	430	1411
Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gly Ile Leu Asn			
	435		
gct tta gga cag atc gct tct gct gtt gtg agc gca gga gtt cct	440	445	1459
Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val Pro			
	450		
ccc gct gca gca agt tct ata ggg tca tct gta aaa cag ctt tac aag	455	460	1507
Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val Lys Gln Leu Tyr Lys			
	465		
acc tca aaa tct aca ggt tct gat tat aaa aca cag ata tca gca ggt	470	475	1555
Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala Gly			
	480	485	
tat gat gct tac aaa tcc atc aat gat gcc tat ggt agg gca cga aat	490	495	1603
Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg Asn			
	500		
gat gcg act cgt gat gtg ata aac aat gta agt acc ccc gct ctc aca	505	510	1651
Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser Thr Pro Ala Leu Thr			
	515		
cga tcc gtt cct aga gca cga aca gaa gct cga gga cca gaa aaa aca	520	525	1699
Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys Thr			
	530		
gat caa gcc ctc gct agg gtg att tct ggc aat agc aga act ctt gga	535	540	1747
Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu Gly			
	545		
gat gtc tat agt caa gtt tcg gca cta caa tct gta atg cag atc atc	550	555	1795
Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile Ile			
	560	565	
cag tcg aat cct caa gcg aat aat gag gag atc aga caa aag ctt aca	570	575	1843
Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile Arg Gln Lys Leu Thr			
	580		
tcg gca gtg aca aag cct cca cag ttt ggc tat cct tat gtg caa ctt	585	590	1891
Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln Leu			
	595		
tct aat gac tct aca cag aag ttc ata gct aaa tta gaa agt ttg ttt	600	605	1939
Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys Leu Glu Ser Leu Phe			
	610		
gct gaa gga tct agg aca gca gct gaa ata aaa gca ctt tcc ttt gaa	615	620	1987
Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys Ala Leu Ser Phe Glu			
	625		
acg aac tcc ttg ttt att cag cag gtg ctg gtc aat atc ggc tct cta	630	635	2035
Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser Leu			
	640	645	

Figure 1 (continued)

tat tct ggt tat ctc caa taacaacacc taagtgttcg tttggagaga 2083
Tyr Ser Gly Tyr Leu Gln
650

ttattatgtg ctttggttaag gcctttgttg aggccttacc aacacactag aacgatcttc 2143
aataaaataaaa aga 2156

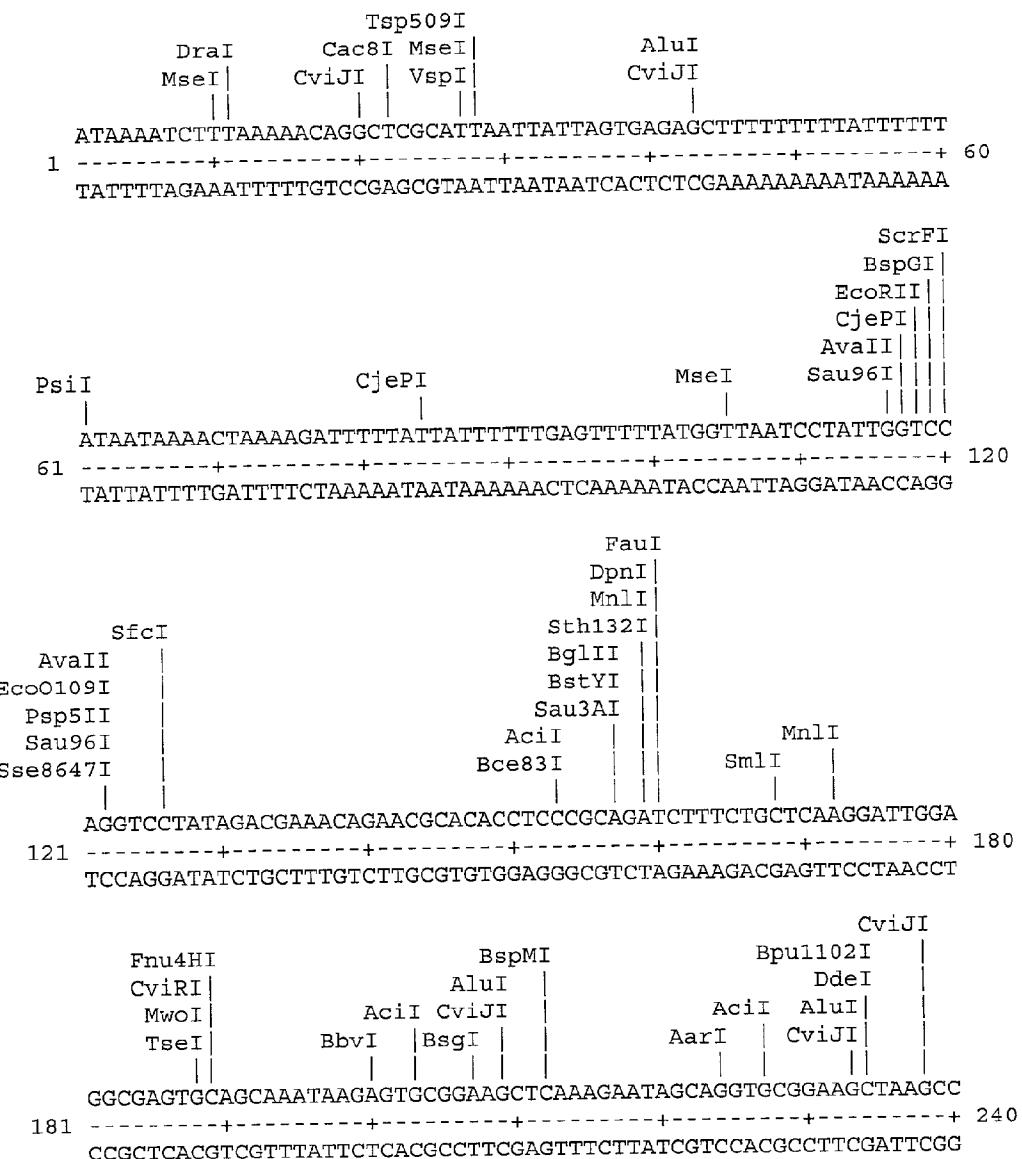
Figure 2: Restriction enzyme map of *C. pneumoniae* 76kDa gene.

Figure 2 (continued)

Figure 2 (continued)

HphI

AluI	BpmI	EcoRII	MaeIII
CviJI	CviJI	CviJI	Tsp45I
BbvI	TseI	SfaNI	MnII
			FokI

481 AGCTGACATACAGGCTGCTTGATGACCTCCAGGATGCTGTCAGTAAATATAAAGGATAC
TCGACTGTATGTCCGACGAAACCACTCGGAGGTCTACGACAGTGATTATTCCTATG 540

MnII

CviJI	AciI
Fnu4HI	Fnu4HI
TauI	BstAPI
AcII	MwoI
MspAII	BbvI
	AciI
	TseI

541 AGCGGCTACTGATGAGGAAACCGCAATCGCTCGGAGTGGAAACTAAGAATGCCATGC
TCGCCGATGACTACTCCTTGGCGTTAGCGACGCCCTACCCCTTGATTCTACGGCTACG 600

Tsp509I

HhaI				PleI
Cac8I	HhaI			HinfI
	ThaI			TfiI
MseI	BssHII	Tsp509I	MwoI	

601 AGTTAAAGTTGGCGCGCAAATTACAGAATTAGCGAAATATGCTTCGGATAACCAAGCGAT
TCAATTCAACCGCGCGTTAATGTCTTAATCGCTTATACGAAGCCTATTGGTCGCTA 660

Fnu4HI

CviJI	MnII			
	TseI			
		MboII		
HinfI		BbvI	BsII	EarI
Hpy178III		TaqI	ECONI	SapI

661 TCTTGACTCTTAGGTAAACTGACTTCCTCGACCTTACAGGCTGCTCTCCAATC
AGAACTGAGAAATCCATTGACTGAAGGAAGCTGGAGAATGTCCGACGAGAAGAGGTTAG 720

Figure 2 (continued)

MseI

BbvI

SfaNI

AluI

CviJI

Bpu1102I

DdeI

AluI

CviJI

MspAII

PvuII

Tth111II

Fnu4HI

BsmFI

BmrI

CviRI

BsrlI

BseMII TseI

721

TGTAGCAAACAATAACAAAGCAGCTGAGCTCTTAAAGAGATGCAAGATAACCCAGTAGT

ACATCGTTGTTATTGTTCGTCGACTCGAAGAATTCTACGTTCTATTGGGTACATCA 780

Hpy188IX

ScrFI

MunI

BsaJI

Tsp509I

BclI

BsaJI

CviRI

Sau3AI

EcoRII

Cac8I

SfaNI

SfcI

CCCAGGGAAACGCCCTGCAATTGCTCAATCTTAGTTAGTTGATCAGACAGATGCTACAGCGAC

781

GGGTCCCTTTGCGGACGTTAACGAGTTAGAAATCAACTAGTCTGTCTACGATGTCGCTG 840

CviRI

FokI

CjePI

BstAPI

NsI

BccI SfaNI

CviRI MwoI

AcelIII BfaI

ACAGATAGAGAAAGATGGAAATGCGATTAGGGATGCATATTTGCAGGACAGAACGCTAG

841

TGTCTATCTCTTACCTTACGCTAATCCCTACGTATAAACGTCCTGTCTTGCATC 900

SfcI

AluI

CviJI

MwoI

CjePI

TaaI

HinfI

AluI

MwoI

TfiI

CviJI

TseI

Fnu4HI

TGGAGCTGTAGAAAATGCTAAATCTAATAACAGTATAAGCAACATAGATTCAAGCTAAAGC

901

ACCTCGACATCTTACGATTAGATTGTACATATTGTTGATCTAAGTCGATTTCG 960

Figure 2 (continued)

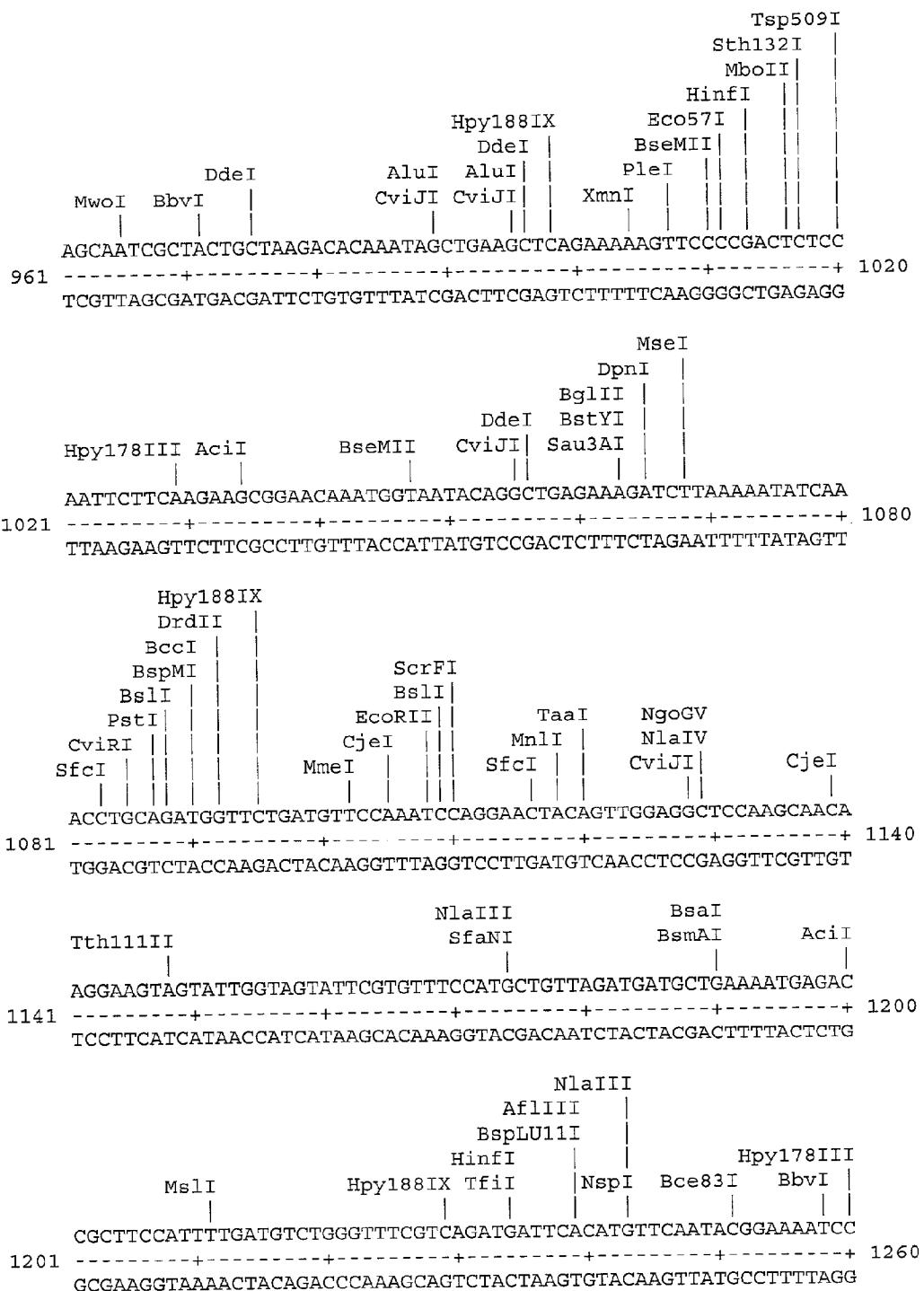


Figure 2 (continued)

Figure 2 (continued)

FauI
 PstI
 Fnu4HI
 MnI
 Sth132I
 CviRI
 TseI
 Fnu4HI
 SfcI
 MspAII
 BbvI TseI BbvI AluI
 HhaI AciI SfcI SimI CviJI
 1441 TGTGAGCGCAGGAGTTCCCTCCCGCTGCAGCAAGTTCTATAGGGTCATCTGTAAAACAGCT 1500
 ACACTCGCGTCTCAAGGAGGGCGACGTCGTTCAAGATATCCCAGTAGACATTTGTCGA

Hpy188IX SfaNI
 MnII EcoRV
 SfcI AlwNI PsII BspMI
 1501 TTACAAGACCTAAAACTACAGGTTCTGATTATAAAACACAGATATCAGCAGGTTATGA 1560
 AATGTTCTGGAGTTAGATGTCCAAGACTAATATTTGTCATAGTCGTCCAATACT

Bsp1286I Hpy178III
 BccI BsII BssSI
 SfaNI EcoNI PleI HinfI
 1561 TGCTTACAAATCCATCAATGATGCCATGGTAGGGCACGAAATGATGCGACTCGTGATGT 1620
 ACCAATGTTAGGTAGTTACTACGGATAACCATCCCGTGTACTACGCTGAGCACTACA

DpnI AvaI
 Sau3AI SmlI
 FauI XhoI
 AlwI AluI
 Sth132I CviJI
 BsrBI BsiHKAI MnI
 RsaI AciI Bsp1286I PpiI
 1621 GATAAACAAATGTAAGTACCCCCGCTCTCACACGATCCGTTCTAGAGCACGAACAGAAC 1680
 CTATTTGTTACATTCAATGGGGCGAGGTGTGCTAGGCAAGGATCTCGTGTCTTCG

Avall BsII
 Sau96I DpnI BfaI
 TaqI Sau3AI CviJI BsII MnI HphI
 1681 TCGAGGACCAGAAAAACAGATCAAGCCCTCGTAGGGTGATTTCTGGCAATAGCAGAAC 1740
 AGCTCCTGGTCTTTGTCTAGTTGGAGCGATCCACTAAAGACCGTTATCGTCTTG

Figure 2 (continued)

Ms1I
DdeI
BsbI

TGGTTATCTCCAATAACAAACACCTAACAGTTGGAGAGATTATTATGTGCTTGGT
2041 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2100
ACCAATAGAGGTTATTGTTGTGGATTCAAGCAAACCTCTCTAATAACACGAAACCA

MnlI
CviJI | CviJI | DpnI
HaeI | HaeI | Sau3AI |
HaeIII | HaeIII | MboII |
StuI | StuI | BsbI | BfaI |
AAGGCCTTGTTGAGGCCTAACCAACACACTAGAACGATCTTCAATAATAAAAGA
2101 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2156
TTCCGGAAACAACTCCGAATGGTTGTGATCTTGCTAGAAGTTATTTATTTCT

Figure 3: Sequence Containing Truncated Version of *C. pneumoniae* 76kDa Gene; (nucleotides 1 to 665 and 2122 to 2238 are unrelated to the 76kDa Gene).

```

atgacaaaaaa aacattatgc ttgggttgc gaagggattc tcaatcggtt gcctaaacag 60
tttttgcgtt aatgttagtgt tgcgtactgg aacacattcg ttccttcaga aacctccact 120
acagaaaaaaag ctgctacaaa cgctatgaaa tacaaatact gtgttggca gtggctcgcc 180
ggaaagcata gtcagggtcc ttggatcaat ggacagaaaaa agcctctata tctttatgga 240
gctttcttaa tgaacccttt agcaaaggct acgaagacta cgttaaatgg aaaagaaaaac 300
ctagcttggt ttattggagg aacttttaggg ggactcagaa aagctggaga ctggtctgcc 360
acagtacgtt atgagtagtgt cgaaggcttg tcgggtccag aaatagatgt ttcagggatt 420
ggccgtggta atttattaaa gtttggttc gccccagcaa ttgctgctaa ctatgatcct 480
aaagaggcta atggtttac aaattataaa ggattttccg ctctatataat gtatggcatc 540
acagattctc tatcattcag agcttatggg gcttactcca aaccagcaaa cgataaactc 600
ggcagtgatt ttactttccg aaagtttgcgat ctaggtataa tttcagcggtt ttaagtcaaa 660
ttttataaaa atctttaaaa acaggctcgcc attaattatt agtgagagct tttttttat 720
tttttataat aaaactaaaaa gatttttatt attttttgag ttttt atg gtt aat cct 777
Met Val Asn Pro
1

att ggt cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat 825
Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp
5 10 15 20
ctt tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa 873
Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu
25 30 35
gct caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc 921
Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr
40 45 50
gat tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc 969
Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu
55 60 65
atg agt ctg gca gat aag ctg ggt att gct tct agt aac agc tgc tct 1017
Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser
70 75 80
tct act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct 1065
Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro
85 90 95 100

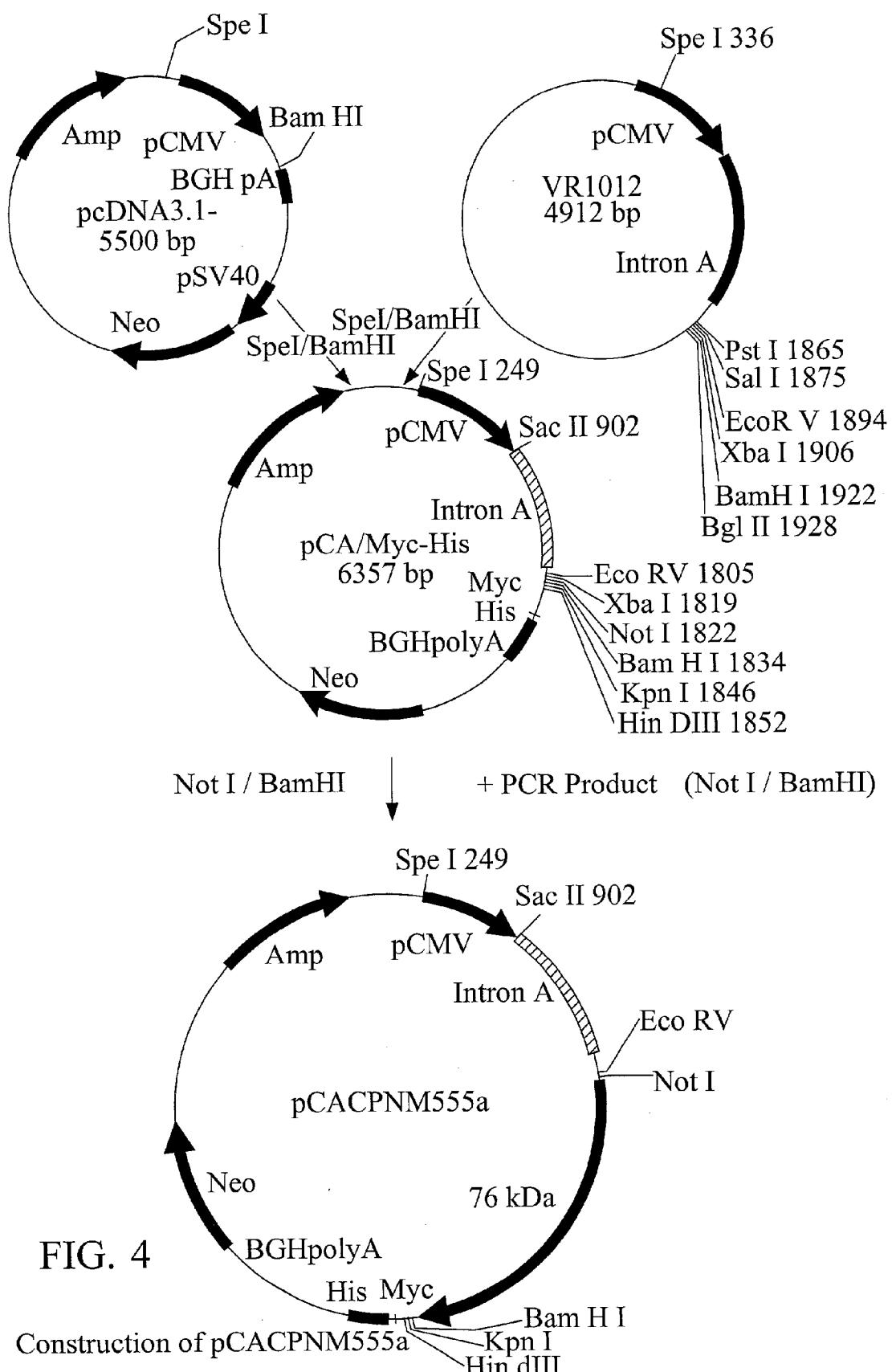
```

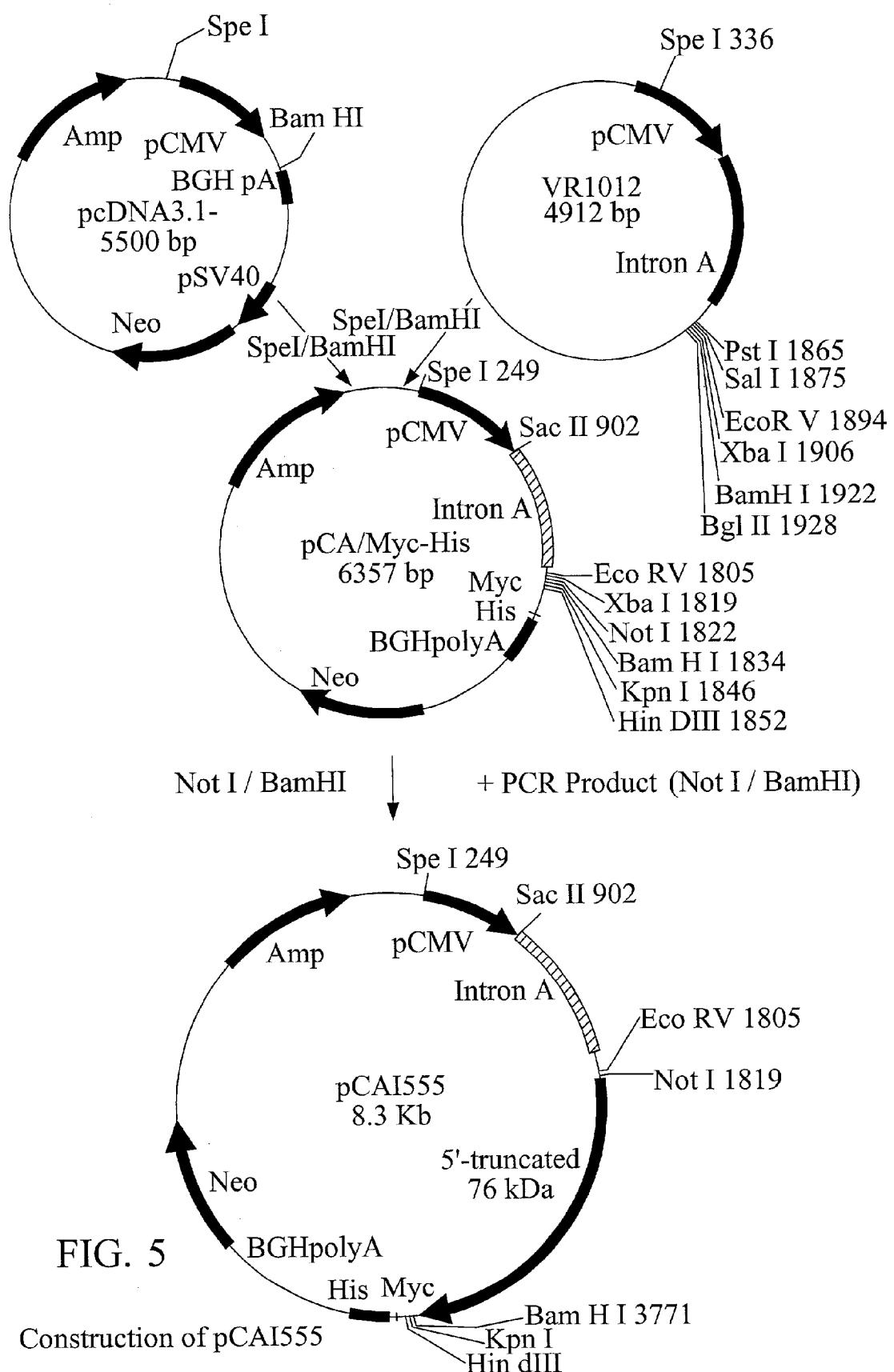
Figure 3 (continued)

acg cct cct cca ccc acg ttt gat gat tat aag act caa gcg caa aca	1113		
Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr			
105	110	115	
gct tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct	1161		
Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala			
120	125	130	
gct ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg	1209		
Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala			
135	140	145	
gct act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat	1257		
Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn			
150	155	160	
gcc gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat	1305		
Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr			
165	170	175	180
gct tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc	1353		
Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser			
185	190	195	
ttc gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac	1401		
Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn			
200	205	210	
aaa gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca	1449		
Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro			
215	220	225	
ggg aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct	1497		
Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala			
230	235	240	
aca gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat	1545		
Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr			
245	250	255	260
ttt gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat	1593		
Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn			
265	270	275	
aac agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct	1641		
Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala			
280	285	290	
aag aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att	1689		
Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile			
295	300	305	

Figure 3 (continued)

ctt caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa	310	315	320	1737
Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys				
aat atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca	325	330	335	1785
Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr				
gtt gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt	345	350	355	1833
Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val				
tcc atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg	360	365	370	1881
Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met				
tct ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat	375	380	385	1929
Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp				
tct caa gct gcc caa cag gag ctc gca gca caa gct aga gca gcg aaa	390	395	400	1977
Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys				
gcc gct gga gat gac agt gct gca gcg ctg gca gat gct cag aaa	405	410	415	2025
Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys				
gct tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc	425	430	435	2073
Ala Leu Glu Ala Ala Leu Gly Ala Gly Gln Gln Gly Ile Leu				
aat gct tta gga cag atc gct tct gct gct gtt gtg agc gca gga gta	440	445	450	2121
Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val				
ctc ccg ctg cag caa gtt cta tgg atc cga gct cggt tac caa gct tac	455	460	465	2169
Leu Pro Leu Gln Gln Val Leu Trp Ile Arg Ala Arg Tyr Gln Ala Tyr				
gta gaa caa aaa ctc atc tca gaa gag gat ctg aat agc gcc gtc gac	470	475	480	2217
Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp				
cat cat cat cat cat cat tga	485	490		2238
His His His His His His				





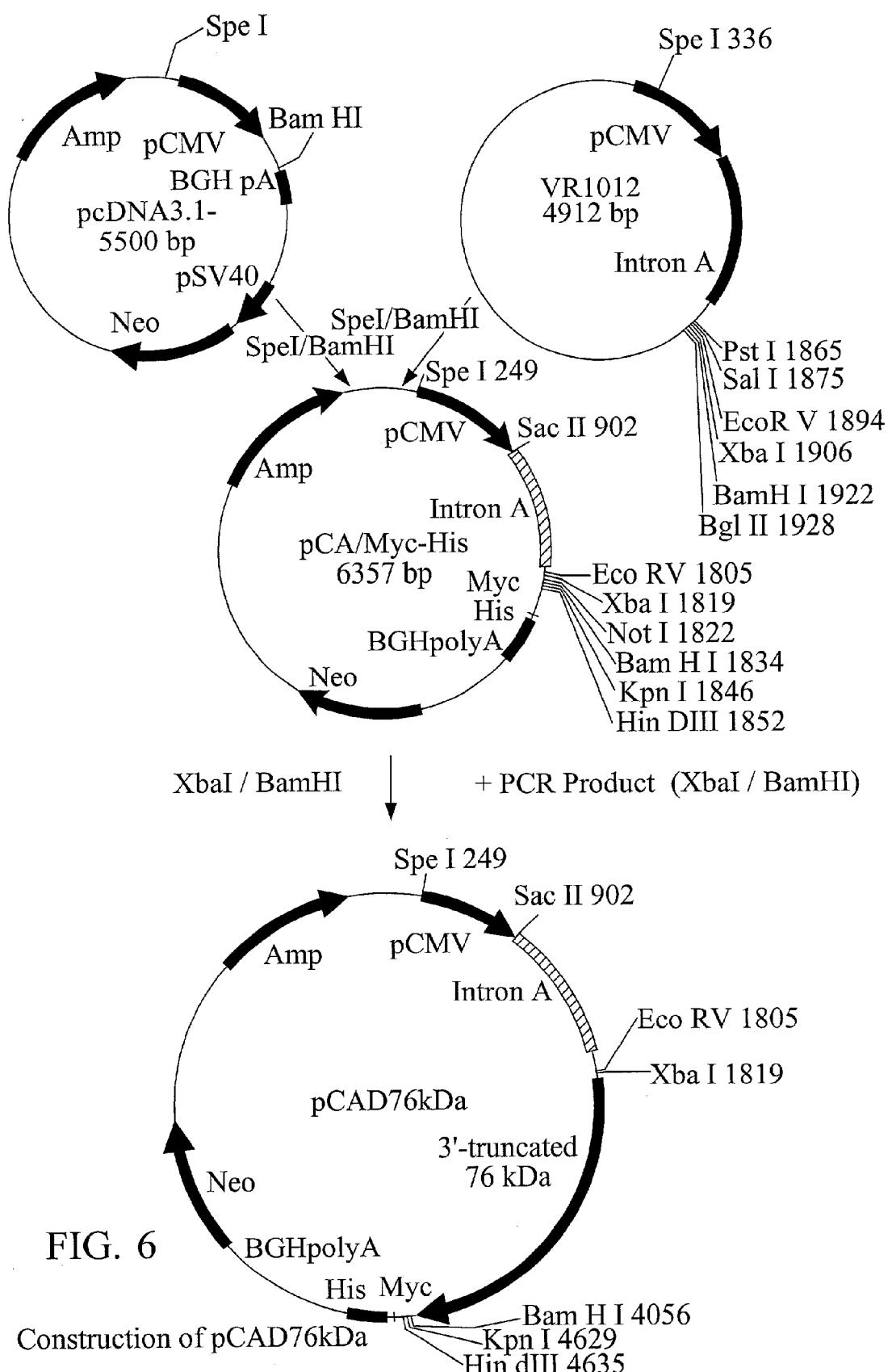


Figure 7: pCACPNM555a Confers Protection Against *C. pneumoniae* Infection .

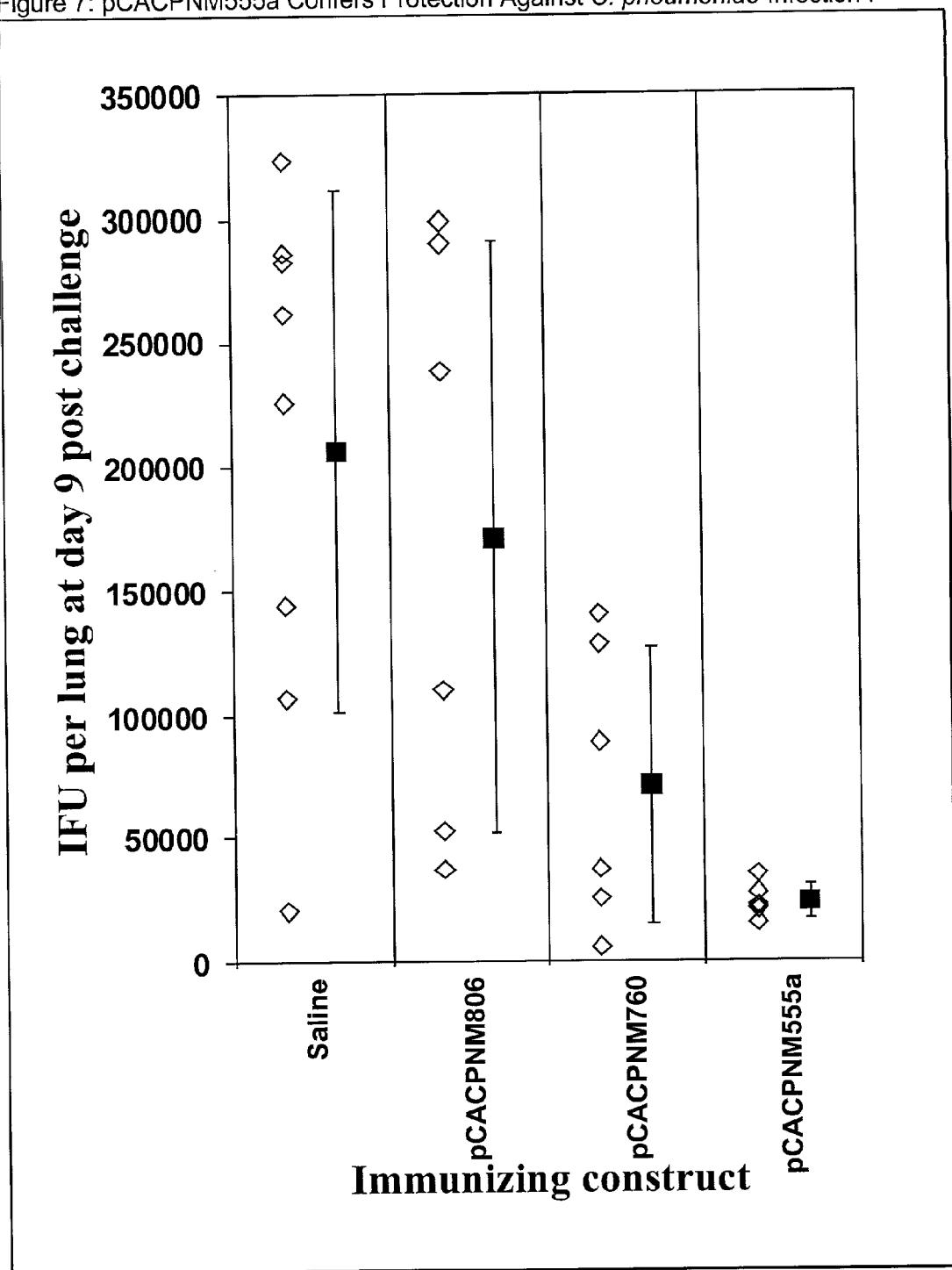


Figure 8: pCAI555 Confers Protection Against *C. pneumoniae* Infection.

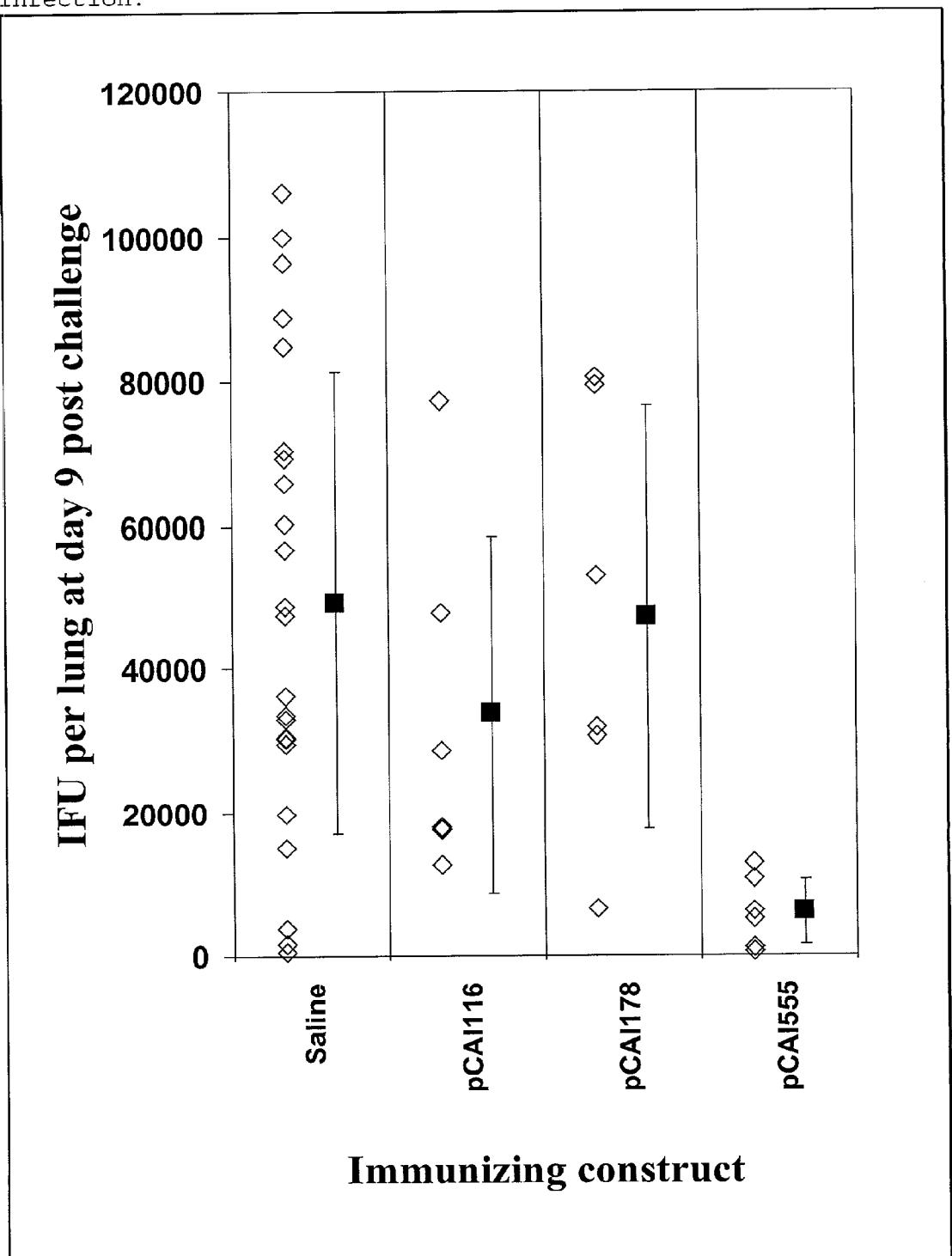
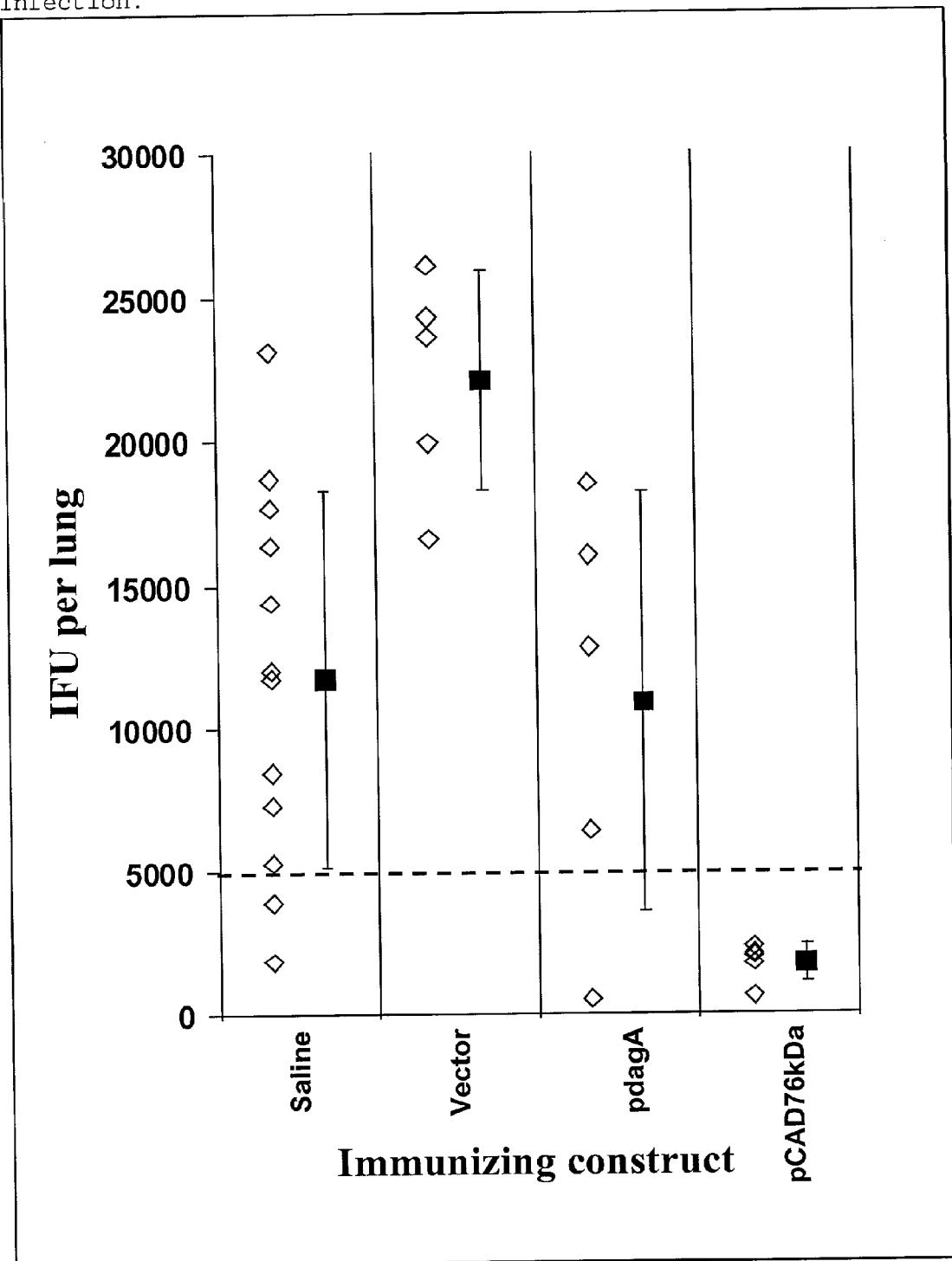


Figure 9: pCAD76kDa Confers Protection against *C. pneumoniae* Infection.



CHLAMYDIA ANTIGENS AND CORRESPONDING DNA FRAGMENTS AND USES THEREOF

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application Ser. No. 60/132,270, filed May 3, 1999, and U.S. provisional application Ser. No. 60/141,276 filed Jun. 30, 1999.

FIELD OF INVENTION

[0002] The present invention relates to the Chlamydia 76kDa protein and corresponding DNA molecules, which can be used to prevent and treat Chlamydia infection in mammals, such as humans.

BACKGROUND OF THE INVENTION

[0003] Chlamydiae are prokaryotes. They exhibit morphologic and structural similarities to gram-negative bacteria including a trilaminar outer membrane, which contains lipopolysaccharide and several membrane proteins that are structurally and functionally analogous to proteins found in *E. coli*. They are obligate intra-cellular parasites with a unique biphasic life cycle consisting of a metabolically inactive but infectious extracellular stage and a replicating but non-infectious intracellular stage. The replicative stage of the life-cycle takes place within a membrane-bound inclusion which sequesters the bacteria away from the cytoplasm of the infected host cell.

[0004] *C. pneumoniae* is a common human pathogen, originally described as the TWAR strain of *Chlamydia psittaci* but subsequently recognised to be a new species. *C. pneumoniae* is antigenically, genetically and morphologically distinct from other Chlamydia species (*C. trachomatis*, *C. pecorum* and *C. psittaci*). It shows 10% or less DNA sequence homology with either of *C. trachomatis* or *C. psittaci*.

[0005] *C. pneumoniae* is a common cause of community acquired pneumonia, only less frequent than *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Campos et al. (1995) Investigation of Ophthalmology and Visual Science 36:1477). It can also cause upper respiratory tract symptoms and disease, including bronchitis and sinusitis (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Grayston et al (1990) Journal of Infectious Diseases 161:618; Marrie (1993) Clinical Infectious Diseases. 18:501; Wang et al (1986) Chlamydial infections Cambridge University Press, Cambridge. p. 329. The great majority of the adult population (over 60%) has antibodies to *C. pneumoniae* (Wang et al (1986) Chlamydial infections. Cambridge University Press, Cambridge. p. 329), indicating past infection which was unrecognized or asymptomatic.

[0006] *C. pneumoniae* infection usually presents as an acute respiratory disease (i.e., cough, sore throat, hoarseness, and fever; abnormal chest sounds on auscultation). For most patients, the cough persists for 2 to 6 weeks, and recovery is slow. In approximately 10% of these cases, upper respiratory tract infection is followed by bronchitis or pneumonia. Furthermore, during a *C. pneumoniae* epidemic, subsequent co-infection with pneumococcus has been noted in about half of these pneumonia patients, particularly in the

infirm and the elderly. As noted above, there is more and more evidence that *C. pneumoniae* infection is also linked to diseases other than respiratory infections.

[0007] The reservoir for the organism is presumably people. In contrast to *C. psittaci* infections, there is no known bird or animal reservoir. Transmission has not been clearly defined. It may result from direct contact with secretions, from fomites, or from airborne spread. There is a long incubation period, which may last for many months. Based on analysis of epidemics, *C. pneumoniae* appears to spread slowly through a population (case-to-case interval averaging 30 days) because infected persons are inefficient transmitters of the organism. Susceptibility to *C. pneumoniae* is universal. Reinfections occur during adulthood, following the primary infection as a child. *C. pneumoniae* appears to be an endemic disease throughout the world, noteworthy for superimposed intervals of increased incidence (epidemics) that persist for 2 to 3 years. *C. trachomatis* infection does not confer cross-immunity to *C. pneumoniae*. Infections are easily treated with oral antibiotics, tetracycline or erythromycin (2 g/d, for at least 10 to 14 d). A recently developed drug, azithromycin, is highly effective as a single-dose therapy against Chlamydial infections.

[0008] In most instances, *C. pneumoniae* infection is often mild and without complications, and up to 90% of infections are subacute or unrecognized. Among children in industrialized countries, infections have been thought to be rare up to the age of 5 y, although a recent study (E Normann et al, *Chlamydia pneumoniae* in children with acute respiratory tract infections, *Acta Paediatrica*, 1998, Vol 87, Iss 1, pp 23-27) has reported that many children in this age group show PCR evidence of infection despite being seronegative, and estimates a prevalence of 17-19% in 2-4 y olds. In developing countries, the seroprevalence of *C. pneumoniae* antibodies among young children is elevated, and there are suspicions that *C. pneumoniae* may be an important cause of acute lower respiratory tract disease and mortality for infants and children in tropical regions of the world.

[0009] From seroprevalence studies and studies of local epidemics, the initial *C. pneumoniae* infection usually happens between the ages of 5 and 20 y. In the USA, for example, there are estimated to be 30,000 cases of childhood pneumonia each year caused by *C. pneumoniae*. Infections may cluster among groups of children or young adults (e.g., school pupils or military conscripts).

[0010] *C. pneumoniae* causes 10 to 25% of community-acquired lower respiratory tract infections (as reported from Sweden, Italy, Finland, and the USA). During an epidemic, *C. pneumoniae* infection may account for 50 to 60% of the cases of pneumonia. During these periods, also, more episodes of mixed infections with *S. pneumoniae* have been reported.

[0011] Reinfection during adulthood is common; the clinical presentation tends to be milder. Based on population seroprevalence studies, there tends to be increased exposure with age, which is particularly evident among men. Some investigators have speculated that a persistent, asymptomatic *C. pneumoniae* infection state is common.

[0012] In adults of middle age or older, *C. pneumoniae* infection may progress to chronic bronchitis and sinusitis. A study in the USA revealed that the incidence of pneumonia

caused by *C. pneumoniae* in persons younger than 60 years is 1 case per 1,000 persons per year; but in the elderly, the disease incidence rose three-fold. *C. pneumoniae* infection rarely leads to hospitalization, except in patients with an underlying illness.

[0013] Of considerable importance is the association of atherosclerosis and *C. pneumoniae* infection. There are several epidemiological studies showing a correlation of previous infections with *C. pneumoniae* and heart attacks, coronary artery and carotid artery disease (Saikku et al. (1988) Lancet;ii:983; Thom et al. (1992) JAMA 268:68; Linnanmaki et al. (1993), Circulation 87:1030; Saikku et al. (1992) Annals Internal Medicine 116:273; Melnick et al (1993) American Journal of Medicine 95:499). Moreover, the organisms has been detected in atherosomas and fatty streaks of the coronary, carotid, peripheral arteries and aorta (Shor et al. (1992) South African Medical Journal 82:158; Kuo et al. (1993) Journal of Infectious Diseases 167:841; Kuo et al. (1993) Arteriosclerosis and Thrombosis 13:1500; Campbell et al (1995) Journal of Infectious Diseases 172:585; Chiu et al. Circulation, 1997 (In Press)). Viable *C. pneumoniae* has been recovered from the coronary and carotid artery (Ramirez et al (1996) Annals of Internal Medicine 125:979; Jackson et al. Abst. K121, p272, 36th ICAAC, 15-18 Sept. 1996, New Orleans). Furthermore, it has been shown that *C. pneumoniae* can induce changes of atherosclerosis in a rabbit model (Fong et al (1997) Journal of Clinical Microbiology 35:48). Taken together, these results indicate that it is highly probable that *C. pneumoniae* can cause atherosclerosis in humans, though the epidemiological importance of Chlamydial atherosclerosis remains to be demonstrated.

[0014] A number of recent studies have also indicated an association between *C. pneumoniae* infection and asthma. Infection has been linked to wheezing, asthmatic bronchitis, adult-onset asthma and acute exacerbations of asthma in adults, and small-scale studies have shown that prolonged antibiotic treatment was effective at greatly reducing the severity of the disease in some individuals (Hahn DL, et al. Evidence for Chlamydia pneumoniae infection in steroid-dependent asthma. Ann Allergy Asthma Immunol. 1998 Jan; 80(1): 45-49.; Hahn DL, et al. Association of Chlamydia pneumoniae IgA antibodies with recently symptomatic asthma. Epidemiol Infect. 1996 Dec; 117(3): 513-517; Bjornsson E, et al. Serology of Chlamydia in relation to asthma and bronchial hyperresponsiveness. Scand J Infect Dis. 1996; 28(1): 63-69.; Hahn DL. Treatment of Chlamydia pneumoniae infection in adult asthma: a before-after trial. J Fam Pract. 1995 Oct.; 41(4): 345-351.; Allegro L, et al. Acute exacerbations of asthma in adults: role of Chlamydia pneumoniae infection. Eur Respir J. 1994 Dec.; 7(12): 2165-2168.; Hahn DL, et al. Association of Chlamydia pneumoniae (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. JAMA. Jul. 10, 1991; 266(2): 225-230).

[0015] In light of these results a protective vaccine against *C. pneumoniae* infection would be of considerable importance. There is not yet an effective vaccine for any human Chlamydial infection. It is conceivable that an effective vaccine can be developed using physically or chemically inactivated Chlamydiae. However, such a vaccine does not have a high margin of safety. In general, safer vaccines are made by genetically manipulating the organism by attenu-

ation or by recombinant means. Accordingly, a major obstacle in creating an effective and safe vaccine against human Chlamydial infection has been the paucity of genetic information regarding Chlamydia, specifically *C. pneumoniae*.

[0016] Studies with *C. trachomatis* and *C. psittaci* indicate that safe and effective vaccine against Chlamydia is an attainable goal. For example, mice which have recovered from a lung infection with *C. trachomatis* are protected from infertility induced by a subsequent vaginal challenge (Pal et al. (1996) Infection and Immunity 64:5341). Similarly, sheep immunized with inactivated *C. psittaci* were protected from subsequent Chlamydial-induced abortions and still-births (Jones et al. (1995) Vaccine 13:715). Protection from Chlamydial infections has been associated with Th1 immune responses, particularly the induction of INF γ —producing CD4+T-cells (Igietseme et al. (1993) Immunology 5:317). The adoptive transfer of CD4+ cell lines or clones to nude or SCID mice conferred protection from challenge or cleared chronic disease (Igietseme et al (1993) Regional Immunology 5:317; Magee et al (1993) Regional Immunology 5: 305), and in vivo depletion of CD4+ T cells exacerbated disease post-challenge (Landers et al (1991) Infection & Immunity 59:3774; Magee et al (1995) Infection & Immunity 63:516). However, the presence of sufficiently high titres of neutralising antibody at mucosal surfaces can also exert a protective effect (Cotter et al. (1995) Infection and Immunity 63:4704).

[0017] Antigenic variation within the species *C. pneumoniae* is not well documented due to insufficient genetic information, though variation is expected to exist based on *C. trachomatis*. Serovars of *C. trachomatis* are defined on the basis of antigenic variation in the major outer membrane protein (MOMP), but published *C. pneumoniae* MOMP gene sequences show no variation between several diverse isolates of the organism (Campbell et al (1990) Infection and Immunity 58:93; McCafferty et al (1995) Infection and Immunity 63:2387-9; Knudsen et al (1996) Third Meeting of the European Society for Chlamydia Research, Vienna). Melgosa et al. (Infect. Immun. 1994. 62:880) claimed to have cloned the gene encoding a 76 kDa antigen from a single strain of *C. pneumoniae*. An operon encoding the 9 kDa and 9 kDa cyteine-rich outer membrane protein genes has been described (Watson et al., Nucleic Acids Res (1990) 18:5299; Watson et al., Microbiology (1995) 141:2489). Many antigens recognized by immune sera to *C. pneumoniae* are conserved across all Chlamydiae, but 98 kDa, 76 kDa and several other proteins may be *C. pneumoniae*-specific (Perez Melgosa et al., Infect. Immun. 1994. 62:880; Melgosa et al., FEMS Microbiol Lett (1993) 112 :199.; Campbell et al., J Clin Microbiol (1990) 28 :1261; Iijima et al., J Clin Microbiol (1994) 32:583). An assessment of the number and relative frequency of any *C. pneumoniae* serotypes, and the defining antigens, is not yet possible. The entire genome sequence of *C. pneumoniae* strain CWL-029 is now known (<http://chlamydia-www.berkeley.edu:4231/>) and as further sequences become available a better understanding of antigenic variation may be gained.

[0018] Many antigens recognised by immune sera to *C. pneumoniae* are conserved across all Chlamydiae, but 98 kDa, 76 kDa and 54 kDa proteins appear to be *C. pneumoniae*-specific (Campos et al. (1995) Investigation of Ophthalmology and Visual Science 36:1477; Marrie (1993)

Clinical Infectious Diseases. 18:501; Wiedmann-Al-Ahmad M, et al. Reactions of polyclonal and neutralizing anti-p54 monoclonal antibodies with an isolated, species-specific 54-kilodalton protein of *Chlamydia pneumoniae*. Clin Diagn Lab Immunol. 1997 Nov; 4(6): 700-704).

[0019] Immunoblotting of isolates with sera from patients does show variation of blotting patterns between isolates, indicating that serotypes *C. pneumoniae* may exist (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Ramirez et al (1996) Annals of Internal Medicine 125:979). However, the results are potentially confounded by the infection status of the patients, since immunoblot profiles of a patient's sera change with time post-infection. An assessment of the number and relative frequency of any serotypes, and the defining antigens, is not yet possible.

[0020] Accordingly, a need exists for identifying and isolating polynucleotide sequences of *C. pneumoniae* for use in preventing and treating Chlamydia infection.

SUMMARY OF THE INVENTION

[0021] The present invention provides purified and isolated polynucleotide molecules that encode the Chlamydia polypeptide designated 76 kDa protein (SEQ ID No: 1) which can be used in methods to prevent, treat, and diagnose Chlamydia infection. In one form of the invention, the polynucleotide molecules are DNA that encode the polypeptide of SEQ ID No: 2.

[0022] Another form of the invention provides polypeptides corresponding to the isolated DNA molecules. The amino acid sequence of the corresponding encoded polypeptide is shown as SEQ ID No: 2.

[0023] Another form of the invention provides truncated polypeptides corresponding to truncated DNA molecules. In one embodiment, the truncated nucleotide and amino acid sequences are shown as SEQ ID Nos: 3 and 4 respectively. In another embodiment, the truncated nucleotide and amino acid sequences are shown as SEQ ID Nos: 5 and 6 respectively.

[0024] Although Melgosa et al. has reported cloning a 76 kDa protein from *C. pneumoniae*, comparison of the gene sequence as reported by Melgosa et al. to the published genome sequence of *C. pneumoniae* (<http://chlamydia-www.berkeley.edu:4231/>) reveals that, in fact, the genomic sequence in this region contains at least two open reading frames (ORFs), one in the 5' portion and one in the 3' portion. The sequence reported in Melgosa et al. is an in-frame fusion of the 5' end of the 5' ORF. Thus, Melgosa's deduced protein is merely a 76 kDa fusion protein and not the 76 kDa protein observed by immunoblotting from various *C. pneumoniae* isolates. By contrast, the 76 kDa protein of the present invention is the full-length protein encoded by the 3' ORF in this region of the genome. Notably, further analysis of the genome sequence (<http://chlamydia-www.berkeley.edu:4231/>) reveals at least one in-frame ATG upstream of the start codon of the 5' ORF, suggesting that the 5' ORF may form part of one or more larger ORFs.

[0025] Those skilled in the art will readily understand that the invention, having provided the polynucleotide sequences encoding the Chlamydia 76 kDa protein, also provides polynucleotides encoding fragments derived from such a polypeptide. Moreover, the invention is understood to pro-

vide mutants and derivatives of such polypeptides and fragments derived therefrom, which result from the addition, deletion, or substitution of non-essential amino acids as described herein. Those skilled in the art would also readily understand that the invention, having provided the polynucleotide sequences encoding Chlamydia polypeptides, further provides monospecific antibodies that specifically bind to such polypeptides.

[0026] The present invention has wide application and includes expression cassettes, vectors, and cells transformed or transfected with the polynucleotides of the invention. Accordingly, the present invention further provides (i) a method for producing a polypeptide of the invention in a recombinant host system and related expression cassettes, vectors, and transformed or transfected cells; (ii) a vaccine, or a live vaccine vector such as a pox virus, *Salmonella typhimurium*, or *Vibrio cholerae* vector, containing a polynucleotide of the invention, such vaccines and vaccine vectors being useful for, e.g., preventing and treating Chlamydia infection, in combination with a diluent or carrier, and related pharmaceutical compositions and associated therapeutic and/or prophylactic methods; (iii) a therapeutic and/or prophylactic use of an RNA or DNA molecule of the invention, either in a naked form or formulated with a delivery vehicle, a polypeptide or combination of polypeptides, or a monospecific antibody of the invention, and related pharmaceutical compositions; (iv) a method for diagnosing the presence of Chlamydia in a biological sample, which can involve the use of a DNA or RNA molecule, a monospecific antibody, or a polypeptide of the invention; and (v) a method for purifying a polypeptide of the invention by antibody-based affinity chromatography.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The present invention will be further understood from the following description with reference to embodiments shown in the drawings, in which:

[0028] FIG. 1 shows the full-length nucleotide sequence of the 76 kDa protein gene (SEQ ID No: 1) and the deduced amino acid sequence of the 76 kDa protein from *Chlamydia pneumoniae* (SEQ ID No: 2).

[0029] FIG. 2 shows the restriction enzyme analysis of the *C. pneumoniae* 76 kDa protein gene.

[0030] FIG. 3 shows the nucleotide sequence containing a 3'-truncated 76 kDa protein gene and its corresponding deduced amino acid sequence from *Chlamydia pneumoniae*; (note that nucleotides 1 to 665 and 2122 to 2238 are unrelated to the 76 kDa protein gene).

[0031] FIG. 4 shows the construction and elements of plasmid pCACPNM555a, containing the full-length 76 kDa gene.

[0032] FIG. 5 shows the construction and elements of plasmid pCAI555, containing a 5'-truncated version of the 76 kDa gene.

[0033] FIG. 6 shows the construction and elements of plasmid pCAD76kDa, containing a 3'-truncated version of the 76 kDa gene from FIG. 3.

[0034] FIG. 7 illustrates protection against *C. pneumoniae* infection by pCACPNM555a following DNA immunization.

[0035] FIG. 8 illustrates protection against *C. pneumoniae* infection by pCAI555 following DNA immunization.

[0036] FIG. 9 illustrates protection against *C. pneumoniae* infection by pCAD76kDa following DNA immunization. For FIGS. 7 to 9, individual data points are shown for each animal (hollow diamonds) as well as mean and standard deviations for each group (solid squares).

DETAILED DESCRIPTION OF INVENTION

[0037] The invention is described with reference to the following sequences which are embodiments of the invention: SEQ ID NO: 1 is the full-length sequence of the 76 kDa protein gene.

[0038] SEQ ID NO: 2 is the deduced full-length amino acid sequence of the 76 kDa protein.

[0039] SEQ ID NO: 3 is the 5'-truncated nucleotide sequence of the 76 kDa protein gene.

[0040] SEQ ID NO: 4 is the 5'-truncated amino acid sequence of the 76 kDa protein.

[0041] SEQ ID NO: 5 is the 3'-truncated nucleotide sequence of the 76 kDa protein gene.

[0042] SEQ ID NO: 6 is the 3'-truncated amino acid sequence of the 76 kDa protein, which forms the basis for immunoprotection by pCAD76kDa in FIG. 9.

[0043] SEQ ID NO: 7 is the sequence encoding a polypeptide containing a truncated 76 kDa protein. Using this sequence as a template, a fragment was amplified by PCR to form part of construct pCAD76kDa.

[0044] SEQ ID NO: 8 is the deduced amino acid sequence of a truncated 76 kDa protein, as expressed from pCAD76kDa.

[0045] SEQ ID NO: 9 is the 5' primer used to clone the full-length 76 kDa protein gene and to amplify the full-length 76 kDa protein gene for pCACPNM55a.

[0046] SEQ ID NO: 10 is the 3' primer used to clone the full-length 76 kDa protein gene and to amplify the full-length 76 kDa protein gene for pCACPNM55a.

[0047] SEQ ID NO: 11 is the 5' primer used to amplify the 5'-truncated 76 kDa protein gene fragment for pCAI555.

[0048] SEQ ID NO: 12 is the 3' primer used to amplify the 5'-truncated 76 kDa protein gene fragment for pCAI555.

[0049] SEQ ID NO: 13 is the 5' primer used to amplify the 3'-truncated 76 kDa protein gene fragment for pCAD76kDa.

[0050] SEQ ID NO: 14 is the 3' primer used to amplify the truncated 76 kDa protein gene fragment for pCAD76kDa.

[0051] An open reading frame (ORF) encoding the Chlamydial 76 kDa protein has been identified from the *C. pneumoniae* genome. The gene encoding this protein and its fragments have been inserted into expression plasmids and shown to confer immune protection against Chlamydial infection. Accordingly, this 76 kDa protein and related polypeptides can be used to prevent and treat Chlamydia infection.

[0052] According to a first aspect of the invention, isolated polynucleotides are provided which encode Chlamydia polypeptides, whose amino acid sequences are shown in SEQ ID Nos: 2, 4 and 6.

[0053] The term "isolated polynucleotide" is defined as a polynucleotide removed from the environment in which it naturally occurs. For example, a naturally-occurring DNA molecule present in the genome of a living bacteria or as part of a gene bank is not isolated, but the same molecule separated from the remaining part of the bacterial genome, as a result of, e.g., a cloning event (amplification), is isolated. Typically, an isolated DNA molecule is free from DNA regions (e.g., coding regions) with which it is immediately contiguous at the 5' or 3' end, in the naturally occurring genome. Such isolated polynucleotides may be part of a vector or a composition and still be defined as isolated in that such a vector or composition is not part of the natural environment of such polynucleotide.

[0054] The polynucleotide of the invention is either RNA or DNA (cDNA, genomic DNA, or synthetic DNA), or modifications, variants, homologs or fragments thereof. The DNA is either double-stranded or single-stranded, and, if single-stranded, is either the coding strand or the non-coding (anti-sense) strand. Any one of the sequences that encode the polypeptides of the invention as shown in SEQ ID No: 1, 3 or 5 is (a) a coding sequence, (b) a ribonucleotide sequence derived from transcription of (a), or (c) a coding sequence which uses the redundancy or degeneracy of the genetic code to encode the same polypeptides. By "polypeptide" or "protein" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). Both terms are used interchangeably in the present application.

[0055] Consistent with the first aspect of the invention, amino acid sequences are provided which are homologous to SEQ ID No: 2, 4 or 6. As used herein, "homologous amino acid sequence" is any polypeptide which is encoded, in whole or in part, by a nucleic acid sequence which hybridizes at 25-35° C. below critical melting temperature (Tm), to any portion of the nucleic acid sequence of SEQ ID No: 1, 3 or 5. A homologous amino acid sequence is one that differs from an amino acid sequence shown in SEQ ID No: 2, 4 or 6 by one or more conservative amino acid substitutions. Such a sequence also encompasses serotypic variants (defined below) as well as sequences containing deletions or insertions which retain inherent characteristics of the polypeptide such as immunogenicity. Preferably, such a sequence is at least 75%, more preferably 80%, and most preferably 90% identical to SEQ ID No: 2, 4 or 6.

[0056] Homologous amino acid sequences include sequences that are identical or substantially identical to SEQ ID No: 2, 4 or 6. By "amino acid sequence substantially identical" is meant a sequence that is at least 90%, preferably 95%, more preferably 97%, and most preferably 99% identical to an amino acid sequence of reference and that preferably differs from the sequence of reference by a majority of conservative amino acid substitutions.

[0057] Conservative amino acid substitutions are substitutions among amino acids of the same class. These classes include, for example, amino acids having uncharged polar side chains, such as asparagine, glutamine, serine, threonine, and tyrosine; amino acids having basic side chains, such as lysine, arginine, and histidine; amino acids having acidic side chains, such as aspartic acid and glutamic acid; and amino acids having nonpolar side chains, such as glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and cysteine.

[0058] Homology is measured using sequence analysis software such as Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705. Amino acid sequences are aligned to maximize identity. Gaps may be artificially introduced into the sequence to attain proper alignment. Once the optimal alignment has been set up, the degree of homology is established by recording all of the positions in which the amino acids of both sequences are identical, relative to the total number of positions.

[0059] Homologous polynucleotide sequences are defined in a similar way. Preferably, a homologous sequence is one that is at least 45%, more preferably 60%, and most preferably 85% identical to the coding sequence of SEQ ID No: 1, 3 or 5.

[0060] Consistent with the first aspect of the invention, polypeptides having a sequence homologous to SEQ ID No: 2, 4 or 6 include naturally-occurring allelic variants, as well as mutants or any other non-naturally occurring variants that retain the inherent characteristics of the polypeptide of SEQ ID No: 2, 4 or 6.

[0061] As is known in the art, an allelic variant is an alternate form of a polypeptide that is characterized as having a substitution, deletion, or addition of one or more amino acids that does not alter the biological function of the polypeptide. By "biological function" is meant the function of the polypeptide in the cells in which it naturally occurs, even if the function is not necessary for the growth or survival of the cells. For example, the biological function of a porin is to allow the entry into cells of compounds present in the extracellular medium. Biological function is distinct from antigenic property. A polypeptide can have more than one biological function.

[0062] Allelic variants are very common in nature. For example, a bacterial species such as *C. pneumoniae*, is usually represented by a variety of strains that differ from each other by minor allelic variations. Indeed, a polypeptide that fulfills the same biological function in different strains can have an amino acid sequence (and polynucleotide sequence) that is not identical in each of the strains. Despite this variation, an immune response directed generally against many allelic variants has been demonstrated. In studies of the Chlamydial MOMP antigen, cross-strain antibody binding plus neutralization of infectivity occurs despite amino acid sequence variation of MOMP from strain to strain, indicating that the MOMP, when used as an immunogen, is tolerant of amino acid variations.

[0063] Polynucleotides encoding homologous polypeptides or allelic variants are retrieved by polymerase chain reaction (PCR) amplification of genomic bacterial DNA extracted by conventional methods. This involves the use of synthetic oligonucleotide primers matching upstream and downstream of the 5' and 3' ends of the encoding domain. Suitable primers are designed according to the nucleotide sequence information provided in SEQ ID No:1, 3 or 5. The procedure is as follows: a primer is selected which consists of 10 to 40, preferably 15 to 25 nucleotides. It is advantageous to select primers containing C and G nucleotides in a proportion sufficient to ensure efficient hybridization; i.e., an amount of C and G nucleotides of at least 40%, preferably 50% of the total nucleotide content. A standard PCR reaction

contains typically 0.5 to 5 Units of Taq DNA polymerase per 100 μ L, 20 to 200 μ M deoxynucleotide each, preferably at equivalent concentrations, 0.5 to 2.5 mM magnesium over the total deoxynucleotide concentration, 10^5 to 10^6 target molecules, and about 20 pmol of each primer. About 25 to 50 PCR cycles are performed, with an annealing temperature 15° C. to 5° C. below the true Tm of the primers. A more stringent annealing temperature improves discrimination against incorrectly annealed primers and reduces incorporation of incorrect nucleotides at the 3' end of primers. A denaturation temperature of 95° C. to 97° C. is typical, although higher temperatures may be appropriate for denaturation of G+C-rich targets. The number of cycles performed depends on the starting concentration of target molecules, though typically more than 40 cycles is not recommended as non-specific background products tend to accumulate.

[0064] An alternative method for retrieving polynucleotides encoding homologous polypeptides or allelic variants is by hybridization screening of a DNA or RNA library. Hybridization procedures are well-known in the art and are described in Ausubel et al., (Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994), Silhavy et al. (Silhavy et al. Experiments with Gene Fusions, Cold Spring Harbor Laboratory Press, 1984), and Davis et al. (Davis et al. A Manual for Genetic Engineering: Advanced Bacterial Genetics, Cold Spring Harbor Laboratory Press, 1980)). Important parameters for optimizing hybridization conditions are reflected in a formula used to obtain the critical melting temperature above which two complementary DNA strands separate from each other (Casey & Davidson, Nucl. Acid Res. (1977) 4:1539). For polynucleotides of about 600 nucleotides or larger, this formula is as follows: $T_m=81.5+0.41\times(\% G+C)+16.6 \log(cation\ ion\ concentration)-0.63\times(\% formamide)-600/base\ number$. Under appropriate stringency conditions, hybridization temperature (Th) is approximately 20 to 40° C., 20 to 25° C., or, preferably 30 to 40° C. below the calculated Tm. Those skilled in the art will understand that optimal temperature and salt conditions can be readily determined.

[0065] For the polynucleotides of the invention, stringent conditions are achieved for both pre-hybridizing and hybridizing incubations (i) within 4-16 hours at 42° C., in 6×SSC containing 50% formamide, or (ii) within 4-16 hours at 65° C. in an aqueous 6×SSC solution (1 M NaCl, 0.1 M sodium citrate (pH 7.0)). Typically, hybridization experiments are performed at a temperature from 60 to 68° C., e.g. 65° C. At such a temperature, stringent hybridization conditions can be achieved in 6×SSC, preferably in 2×SSC or 1×SSC, more preferably in 0.5×SSC, 0.3×SSC or 0.1×SSC (in the absence of formamide). 1×SSC contains 0.15 M NaCl and 0.015 M sodium citrate.

[0066] Useful homologs and fragments thereof that do not occur naturally are designed using known methods for identifying regions of an antigen that are likely to tolerate amino acid sequence changes and/or deletions. As an example, homologous polypeptides from different species are compared; conserved sequences are identified. The more divergent sequences are the most likely to tolerate sequence changes. Homology among sequences may be analyzed using, as an example, the BLAST homology searching algorithm of Altschul et al., Nucleic Acids Res.; 25:3389-3402 (1997). Alternatively, sequences are modified such that

they become more reactive to T- and/or B-cells, based on computer-assisted analysis of probable T- or B-cell epitopes. Yet another alternative is to mutate a particular amino acid residue or sequence within the polypeptide *in vitro*, then screen the mutant polypeptides for their ability to prevent or treat Chlamydia infection according to the method outlined below.

[0067] A person skilled in the art will readily understand that by following the screening process of this invention, it will be determined without undue experimentation whether a particular homolog of SEQ ID No: 2, 4 or 6 may be useful in the prevention or treatment of Chlamydia infection. The screening procedure comprises the steps:

[0068] (i) immunizing an animal, preferably mouse, with the test homolog or fragment;

[0069] (ii) inoculating the immunized animal with Chlamydia;

[0070] and

[0071] (iii) selecting those homologs or fragments which confer protection against Chlamydia.

[0072] By "conferring protection" is meant that there is a reduction in severity of any of the effects of Chlamydia infection, in comparison with a control animal which was not immunized with the test homolog or fragment.

[0073] Consistent with the first aspect of the invention, polypeptide derivatives are provided that are partial sequences of SEQ ID No: 2, 4 or 6, partial sequences of polypeptide sequences homologous to SEQ ID No: 2, 4 or 6, polypeptides derived from full-length polypeptides by internal deletion, and fusion proteins.

[0074] It is an accepted practice in the field of immunology to use fragments and variants of protein immunogens as vaccines, as all that is required to induce an immune response to a protein is a small (e.g., 8 to 10 amino acid) immunogenic region of the protein. Various short synthetic peptides corresponding to surface-exposed antigens of pathogens other than Chlamydia have been shown to be effective vaccine antigens against their respective pathogens, e.g. an 11 residue peptide of murine mammary tumor virus (Casey & Davidson, *Nucl. Acid Res.* (1977) 4:1539), a 16-residue peptide of Semliki Forest virus (Snijders et al., 1991. *J. Gen. Virol.* 72:557-565), and two overlapping peptides of 15 residues each from canine parvovirus (Langeveld et al., *Vaccine* 12(15):1473-1480, 1994).

[0075] Accordingly, it will be readily apparent to one skilled in the art, having read the present description, that partial sequences of SEQ ID No: 2, 4 or 6 or their homologous amino acid sequences are inherent to the full-length sequences and are taught by the present invention. Such polypeptide fragments preferably are at least 12 amino acids in length. Advantageously, they are at least 20 amino acids, preferably at least 50 amino acids, and more preferably at least 75 amino acids and most preferably at least 100 amino acids in length.

[0076] Polynucleotides of 30 to 600 nucleotides encoding partial sequences of sequences homologous to SEQ ID No: 2, 4 or 6 are retrieved by PCR amplification using the parameters outlined above and using primers matching the sequences upstream and downstream of the 5' and 3' ends of

the fragment to be amplified. The template polynucleotide for such amplification is either the full length polynucleotide homologous to SEQ ID No: 1, 3 or 5, or a polynucleotide contained in a mixture of polynucleotides such as a DNA or RNA library. As an alternative method for retrieving the partial sequences, screening hybridization is carried out under conditions described above and using the formula for calculating Tm. Where fragments of 30 to 600 nucleotides are to be retrieved, the calculated Tm is corrected by subtracting (600/polynucleotide size in base pairs) and the stringency conditions are defined by a hybridization temperature that is 5 to 10° C. below Tm. Where oligonucleotides shorter than 20-30 bases are to be obtained, the formula for calculating the Tm is as follows: $Tm=4x(G+C)+2(A+T)$. For example, an 18 nucleotide fragment of 50% G+C would have an approximate Tm of 54° C. Short peptides that are fragments of SEQ ID No: 2, 4 or 6 or its homologous sequences, are obtained directly by chemical synthesis (E. Gross and H. J. Meinhofer, 4 *The Peptides: Analysis, Synthesis, Biology; Modern Techniques of Peptide Synthesis*, John Wiley & Sons (1981), and M. Bodanzki, *Principles of Peptide Synthesis*, Springer-Verlag (1984)).

[0077] Useful polypeptide derivatives, e.g., polypeptide fragments, are designed using computer-assisted analysis of amino acid sequences. This would identify probable surface-exposed, antigenic regions (Hughes et al., 1992. *Infect. Immun.* 60(9):3497). Analysis of 6 amino acid sequences contained in SEQ ID No: 2, 4 or 6, based on the product of flexibility and hydrophobicity propensities using the program SEQSEE (Wishart DS, et al. "SEQSEE: a comprehensive program suite for protein sequence analysis." *Comput Appl Biosci.* 1994 Apr;10(2):121-32), can reveal potential B- and T-cell epitopes which may be used as a basis for selecting useful immunogenic fragments and variants. This analysis uses a reasonable combination of external surface features that is likely to be recognized by antibodies. Probable T-cell epitopes for HLA-A0201 MHC subclass may be revealed by an algorithms that emulate an approach developed at the NIH (Parker KC, et al. "Peptide binding to MHC class I molecules: implications for antigenic peptide prediction." *Immunol Res* 1995;14(1):34-57).

[0078] Epitopes which induce a protective T cell-dependent immune response are present throughout the length of the polypeptide. However, some epitopes may be masked by secondary and tertiary structures of the polypeptide. To reveal such masked epitopes large internal deletions are created which remove much of the original protein structure and exposes the masked epitopes. Such internal deletions sometimes effect the additional advantage of removing immunodominant regions of high variability among strains.

[0079] Polynucleotides encoding polypeptide fragments and polypeptides having large internal deletions are constructed using standard methods (Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons Inc., 1994). Such methods include standard PCR, inverse PCR, restriction enzyme treatment of cloned DNA molecules, or the method of Kunkel et al. (Kunkel et al. *Proc. Natl. Acad. Sci. USA* (1985) 82:448). Components for these methods and instructions for their use are readily available from various commercial sources such as Stratagene. Once the deletion mutants have been constructed, they are tested for their ability to prevent or treat Chlamydia infection as described above.

[0080] As used herein, a fusion polypeptide is one that contains a polypeptide or a polypeptide derivative of the invention fused at the N- or C-terminal end to any other polypeptide (hereinafter referred to as a peptide tail). A simple way to obtain such a fusion polypeptide is by translation of an in-frame fusion of the polynucleotide sequences, i.e., a hybrid gene. The hybrid gene encoding the fusion polypeptide is inserted into an expression vector which is used to transform or transfect a host cell. Alternatively, the polynucleotide sequence encoding the polypeptide or polypeptide derivative is inserted into an expression vector in which the polynucleotide encoding the peptide tail is already present. Such vectors and instructions for their use are commercially available, e.g. the pMal-c2 or pMal-p2 system from New England Biolabs, in which the peptide tail is a maltose binding protein, the glutathione-S-transferase system of Pharmacia, or the His-Tag system available from Novagen. These and other expression systems provide convenient means for further purification of polypeptides and derivatives of the invention.

[0081] An advantageous example of a fusion polypeptide is one where the polypeptide or homolog or fragment of the invention is fused to a polypeptide having adjuvant activity, such as subunit B of either cholera toxin or *E. coli* heat-labile toxin. Another advantageous fusion is one where the polypeptide, homolog or fragment is fused to a strong T-cell epitope or B-cell epitope. Such an epitope may be one known in the art (e.g. the Hepatitis B virus core antigen, D. R. Millich et al., "Antibody production to the nucleocapsid and envelope of the Hepatitis B virus primed by a single synthetic T cell site", *Nature*. 1987. 329:547-549), or one which has been identified in another polypeptide of the invention based on computer-assisted analysis of probable T- or B-cell epitopes. Consistent with this aspect of the invention is a fusion polypeptide comprising T- or B-cell epitopes from SEQ ID No: 2, 4 or 6 or its homolog or fragment, wherein the epitopes are derived from multiple variants of said polypeptide or homolog or fragment, each variant differing from another in the location and sequence of its epitope within the polypeptide. Such a fusion is effective in the prevention and treatment of Chlamydia infection since it optimizes the T- and B-cell response to the overall polypeptide, homolog or fragment.

[0082] To effect fusion, the polypeptide of the invention is fused to the N-, or preferably, to the C-terminal end of the polypeptide having adjuvant activity or T- or B-cell epitope. Alternatively, a polypeptide fragment of the invention is inserted internally within the amino acid sequence of the polypeptide having adjuvant activity. The T- or B-cell epitope may also be inserted internally within the amino acid sequence of the polypeptide of the invention.

[0083] Consistent with the first aspect, the polynucleotides of the invention also encode hybrid precursor polypeptides containing heterologous signal peptides, which mature into polypeptides of the invention. By "heterologous signal peptide" is meant a signal peptide that is not found in naturally-occurring precursors of polypeptides of the invention.

[0084] Polynucleotide molecules according to the invention, including RNA, DNA, or modifications or combinations thereof, have various applications. A DNA molecule is used, for example, (i) in a process for producing the encoded polypeptide in a recombinant host system, (ii) in the con-

struction of vaccine vectors such as poxviruses, which are further used in methods and compositions for preventing and/or treating Chlamydia infection, (iii) as a vaccine agent (as well as an RNA molecule), in a naked form or formulated with a delivery vehicle and, (iv) in the construction of attenuated Chlamydia strains that can over-express a polynucleotide of the invention or express it in a non-toxic, mutated form.

[0085] Accordingly, a second aspect of the invention encompasses (i) an expression cassette containing a DNA molecule of the invention placed under the control of the elements required for expression, in particular under the control of an appropriate promoter; (ii) an expression vector containing an expression cassette of the invention; (iii) a prokaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, as well as (iv) a process for producing a polypeptide or polypeptide derivative encoded by a polynucleotide of the invention, which involves culturing a prokaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, under conditions that allow expression of the DNA molecule of the invention and, recovering the encoded polypeptide or polypeptide derivative from the cell culture.

[0086] A recombinant expression system is selected from prokaryotic and eucaryotic hosts. Eucaryotic hosts include yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris*), mammalian cells (e.g., COS1, NIH3T3, or JEG3 cells), arthropods cells (e.g., *Spodoptera frugiperda* (SF9) cells), and plant cells. A preferred expression system is a prokaryotic host such as *E. coli*. Bacterial and eucaryotic cells are available from a number of different sources including commercial sources to those skilled in the art, e.g., the American Type Culture Collection (ATCC; Rockville, Md.). Commercial sources of cells used for recombinant protein expression also provide instructions for usage of the cells.

[0087] The choice of the expression system depends on the features desired for the expressed polypeptide. For example, it may be useful to produce a polypeptide of the invention in a particular lipidated form or any other form.

[0088] One skilled in the art would readily understand that not all vectors and expression control sequences and hosts would be expected to express equally well the polynucleotides of this invention. With the guidelines described below, however, a selection of vectors, expression control sequences and hosts may be made without undue experimentation and without departing from the scope of this invention.

[0089] In selecting a vector, the host must be chosen that is compatible with the vector which is to exist and possibly replicate in it. Considerations are made with respect to the vector copy number, the ability to control the copy number, expression of other proteins such as antibiotic resistance. In selecting an expression control sequence, a number of variables are considered. Among the important variable are the relative strength of the sequence (e.g. the ability to drive expression under various conditions), the ability to control the sequence's function, compatibility between the polynucleotide to be expressed and the control sequence (e.g. secondary structures are considered to avoid hairpin structures which prevent efficient transcription). In selecting the

host, unicellular hosts are selected which are compatible with the selected vector, tolerant of any possible toxic effects of the expressed product, able to secrete the expressed product efficiently if such is desired, to be able to express the product in the desired conformation, to be easily scaled up, and to which ease of purification of the final product.

[0090] The choice of the expression cassette depends on the host system selected as well as the features desired for the expressed polypeptide. Typically, an expression cassette includes a promoter that is functional in the selected host system and can be constitutive or inducible; a ribosome binding site; a start codon (ATG) if necessary; a region encoding a signal peptide, e.g., a lipidation signal peptide; a DNA molecule of the invention; a stop codon; and optionally a 3' terminal region (translation and/or transcription terminator). The signal peptide encoding region is adjacent to the polynucleotide of the invention and placed in proper reading frame. The signal peptide-encoding region is homologous or heterologous to the DNA molecule encoding the mature polypeptide and is compatible with the secretion apparatus of the host used for expression. The open reading frame constituted by the DNA molecule of the invention, solely or together with the signal peptide, is placed under the control of the promoter so that transcription and translation occur in the host system. Promoters and signal peptide encoding regions are widely known and available to those skilled in the art and include, for example, the promoter of *Salmonella typhimurium* (and derivatives) that is inducible by arabinose (promoter araB) and is functional in Gram-negative bacteria such as *E. coli* (as described in U.S. Pat. No. 5,028,530 and in Cagnon et al., (Cagnon et al., Protein Engineering (1991) 4(7):843)); the promoter of the gene of bacteriophage T7 encoding RNA polymerase, that is functional in a number of *E. coli* strains expressing T7 polymerase (described in U.S. Patent No. 4,952,496); OspA lipidation signal peptide; and RlpB lipidation signal peptide (Takase et al., J. Bact. (1987) 169:5692).

[0091] The expression cassette is typically part of an expression vector, which is selected for its ability to replicate in the chosen expression system. Expression vectors (e.g., plasmids or viral vectors) can be chosen, for example, from those described in Pouwels et al. (Cloning Vectors: A Laboratory Manual 1985, Supp. 1987). Suitable expression vectors can be purchased from various commercial sources.

[0092] Methods for transforming/transfecting host cells with expression vectors are well-known in the art and depend on the host system selected as described in Ausubel et al., (Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994).

[0093] Upon expression, a recombinant polypeptide of the invention (or a polypeptide derivative) is produced and remains in the intracellular compartment, is secreted/excreted in the extracellular medium or in the periplasmic space, or is embedded in the cellular membrane. The polypeptide is recovered in a substantially purified form from the cell extract or from the supernatant after centrifugation of the recombinant cell culture. Typically, the recombinant polypeptide is purified by antibody-based affinity purification or by other well-known methods that can be readily adapted by a person skilled in the art, such as fusion of the polynucleotide encoding the polypeptide or its derivative to a small affinity binding domain. Antibodies useful for

purifying by immunoaffinity the polypeptides of the invention are obtained as described below.

[0094] A polynucleotide of the invention can also be useful as a vaccine. There are two major routes, either using a viral or bacterial host as gene delivery vehicle (live vaccine vector) or administering the gene in a free form, e.g., inserted into a plasmid. Therapeutic or prophylactic efficacy of a polynucleotide of the invention is evaluated as described below.

[0095] Accordingly, a third aspect of the invention provides (i) a vaccine vector such as a poxvirus, containing a DNA molecule of the invention, placed under the control of elements required for expression; (ii) a composition of matter comprising a vaccine vector of the invention, together with a diluent or carrier; specifically (iii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a vaccine vector of the invention; (iv) a method for inducing an immune response against Chlamydia in a mammal (e.g., a human; alternatively, the method can be used in veterinary applications for treating or preventing Chlamydia infection of animals, e.g., cats or birds), which involves administering to the mammal an immunogenically effective amount of a vaccine vector of the invention to elicit a protective or therapeutic immune response to Chlamydia; and particularly, (v) a method for preventing and/or treating a Chlamydia (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumonia*, *C. pecorum*) infection, which involves administering a prophylactic or therapeutic amount of a vaccine vector of the invention to an infected individual. Additionally, the third aspect of the invention encompasses the use of a vaccine vector of the invention in the preparation of a medicament for preventing and/or treating Chlamydia infection.

[0096] As used herein, a vaccine vector expresses one or several polypeptides or derivatives of the invention. The vaccine vector may express additionally a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12), that enhances the immune response (adjuvant effect). It is understood that each of the components to be expressed is placed under the control of elements required for expression in a mammalian cell.

[0097] Consistent with the third aspect of the invention is a composition comprising several vaccine vectors, each of them capable of expressing a polypeptide or derivative of the invention. A composition may also comprise a vaccine vector capable of expressing an additional Chlamydia antigen, or a subunit, fragment, homolog, mutant, or derivative thereof; optionally together with or a cytokine such as IL-2 or IL-12.

[0098] Vaccination methods for treating or preventing infection in a mammal comprises use of a vaccine vector of the invention to be administered by any conventional route, particularly to a mucosal (e.g., ocular, intranasal, oral, gastric, pulmonary, intestinal, rectal, vaginal, or urinary tract) surface or via the parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. Preferred routes depend upon the choice of the vaccine vector. Treatment may be effected in a single dose or repeated at intervals. The appropriate dosage depends on various parameters understood by skilled artisans such as the vaccine vector itself, the route of administration or the condition of the mammal to be vaccinated (weight, age and the like).

[0099] Live vaccine vectors available in the art include viral vectors such as adenoviruses and poxviruses as well as bacterial vectors, e.g., *Shigella*, *Salmonella*, *Vibrio cholerae*, *Lactobacillus*, *Bacille bilié de Calmette-Guérin* (BCG), and *Streptococcus*.

[0100] An example of an adenovirus vector, as well as a method for constructing an adenovirus vector capable of expressing a DNA molecule of the invention, are described in U.S. Pat. No. 4,920,209. Poxvirus vectors include *vaccinia* and *canary pox* virus, described in U.S. Pat. No. 4,722,848 and U.S. Pat. No. 5,364,773, respectively. (Also see, e.g., Tartaglia et al., *Virology* (1992) 188:217) for a description of a *vaccinia* virus vector and Taylor et al, *Vaccine* (1995) 13:539 for a reference of a *canary pox*.) Poxvirus vectors capable of expressing a polynucleotide of the invention are obtained by homologous recombination as described in Kiény et al., *Nature* (1984) 312:163 so that the polynucleotide of the invention is inserted in the viral genome under appropriate conditions for expression in mammalian cells. Generally, the dose of vaccine viral vector, for therapeutic or prophylactic use, can be of from about 1×10^4 to about 1×10^{11} , advantageously from about 1×10^7 to about 1×10^{10} , preferably of from about 1×10^7 to about 1×10^9 plaque-forming units per kilogram. Preferably, viral vectors are administered parenterally; for example, in 3 doses, 4 weeks apart. It is preferable to avoid adding a chemical adjuvant to a composition containing a viral vector of the invention and thereby minimizing the immune response to the viral vector itself.

[0101] Non-toxicogenic *Vibrio cholerae* mutant strains that are useful as a live oral vaccine are known. Mekalanos et al., *Nature* (1983) 306:551 and U.S. Pat. No. 4,882,278 describe strains which have a substantial amount of the coding sequence of each of the two *ctxA* alleles deleted so that no functional *cholerae* toxin is produced. WO 92/11354 describes a strain in which the *irgA* locus is inactivated by mutation; this mutation can be combined in a single strain with *ctxA* mutations. WO 94/01533 describes a deletion mutant lacking functional *ctxA* and *attRS1* DNA sequences. These mutant strains are genetically engineered to express heterologous antigens, as described in WO 94/19482. An effective vaccine dose of a *Vibrio cholerae* strain capable of expressing a polypeptide or polypeptide derivative encoded by a DNA molecule of the invention contains about 1×10^5 to about 1×10^9 , preferably about 1×10^6 to about 1×10^8 , viable bacteria in a volume appropriate for the selected route of administration. Preferred routes of administration include all mucosal routes; most preferably, these vectors are administered intranasally or orally.

[0102] Attenuated *Salmonella typhimurium* strains, genetically engineered for recombinant expression of heterologous antigens or not, and their use as oral vaccines are described in Nakayama et al. (*Bio/Technology* (1988) 6:693) and WO 92/11361. Preferred routes of administration include all mucosal routes; most preferably, these vectors are administered intranasally or orally.

[0103] Other bacterial strains used as vaccine vectors in the context of the present invention are described for *Shigella flexneri* in High et al., *EMBO* (1992) 11:1991 and Sizemore et al., *Science* (1995) 270:299; for *Streptococcus gordonii* in Medaglini et al., *Proc. Natl. Acad. Sci. USA* (1995) 92:6868; and for *Bacille Calmette Guerin* in Flynn J.

L., *Cell. Mol. Biol.* (1994) 40 (suppl. I):31, WO 88/06626, WO 90/00594, WO 91/13157, WO 92/01796, and WO 92/21376.

[0104] In bacterial vectors, the polynucleotide of the invention is inserted into the bacterial genome or remains in a free state as part of a plasmid.

[0105] The composition comprising a vaccine bacterial vector of the present invention may further contain an adjuvant. A number of adjuvants are known to those skilled in the art. Preferred adjuvants are selected as provided below.

[0106] Accordingly, a fourth aspect of the invention provides (i) a composition of matter comprising a polynucleotide of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a polynucleotide of the invention; (iii) a method for inducing an immune response against Chlamydia in a mammal by administration of an immunogenically effective amount of a polynucleotide of the invention to elicit a protective immune response to Chlamydia; and particularly, (iv) a method for preventing and/or treating a Chlamydia (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, or *C. pecorum*) infection, by administering a prophylactic or therapeutic amount of a polynucleotide of the invention to an infected individual. Additionally, the fourth aspect of the invention encompasses the use of a polynucleotide of the invention in the preparation of a medicament for preventing and/or treating Chlamydia infection. A preferred use includes the use of a DNA molecule placed under conditions for expression in a mammalian cell, especially in a plasmid that is unable to replicate in mammalian cells and to substantially integrate in a mammalian genome.

[0107] Use of the polynucleotides of the invention include their administration to a mammal as a vaccine, for therapeutic or prophylactic purposes. Such polynucleotides are used in the form of DNA as part of a plasmid that is unable to replicate in a mammalian cell and unable to integrate into the mammalian genome. Typically, such a DNA molecule is placed under the control of a promoter suitable for expression in a mammalian cell. The promoter functions either ubiquitously or tissue-specifically. Examples of non-tissue specific promoters include the early Cytomegalovirus (CMV) promoter (described in U.S. Pat. No. 4,168,062) and the Rous Sarcoma Virus promoter (described in Norton & Coffin, *Molec. Cell Biol.* (1985) 5:281). An example of a tissue-specific promoter is the desmin promoter which drives expression in muscle cells (Li et al., *Gene* (1989) 78:243, Li & Paulin, *J. Biol. Chem.* (1991) 266:6562 and Li & Paulin, *J. Biol. Chem.* (1993) 268:10403). Use of promoters is well-known to those skilled in the art. Useful vectors are described in numerous publications, specifically WO 94/21797 and Hartikka et al., *Human Gene Therapy* (1996) 7:1205.

[0108] Polynucleotides of the invention which are used as vaccines encode either a precursor or a mature form of the corresponding polypeptide. In the precursor form, the signal peptide is either homologous or heterologous. In the latter case, a eucaryotic leader sequence such as the leader sequence of the tissue-type plasminogen factor (tPA) is preferred.

[0109] As used herein, a composition of the invention contains one or several polynucleotides with optionally at

least one additional polynucleotide encoding another Chlamydia antigen such as urease subunit A, B, or both, or a fragment, derivative, mutant, or analog thereof. The composition may also contain an additional polynucleotide encoding a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12) so that the immune response is enhanced. These additional polynucleotides are placed under appropriate control for expression. Advantageously, DNA molecules of the invention and/or additional DNA molecules to be included in the same composition, are present in the same plasmid.

[0110] Standard techniques of molecular biology for preparing and purifying polynucleotides are used in the preparation of polynucleotide therapeutics of the invention. For use as a vaccine, a polynucleotide of the invention is formulated according to various methods outlined below.

[0111] One method utilizes the polynucleotide in a naked form, free of any delivery vehicles. Such a polynucleotide is simply diluted in a physiologically acceptable solution such as sterile saline or sterile buffered saline, with or without a carrier. When present, the carrier preferably is isotonic, hypotonic, or weakly hypertonic, and has a relatively low ionic strength, such as provided by a sucrose solution, e.g., a solution containing 20% sucrose.

[0112] An alternative method utilizes the polynucleotide in association with agents that assist in cellular uptake. Examples of such agents are (i) chemicals that modify cellular permeability, such as bupivacaine (see, e.g., WO 94/16737), (ii) liposomes for encapsulation of the polynucleotide, or (iii) cationic lipids or silica, gold, or tungsten microparticles which associate themselves with the polynucleotides.

[0113] Anionic and neutral liposomes are well-known in the art (see, e.g., *Liposomes: A Practical Approach*, RPC New Ed, IRL press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including polynucleotides.

[0114] Cationic lipids are also known in the art and are commonly used for gene delivery. Such lipids include Lipofectin™ also known as DOTMA (N-[1- (2,3 -dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (1,2-bis (oleyloxy) -3-(trimethylammonio)propane), DDAB (dimethyldioctadecylammonium bromide), DOGS (dioctadecylamidologlycyl spermine) and cholesterol derivatives such as DC-Chol (3 beta-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702, WO 90/11092, U.S. Pat. No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Pat. No. 5,527,928. Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPE (dioleyl phosphatidylethanolamine), as described in WO 90/11092 as an example.

[0115] Formulation containing cationic liposomes may optionally contain other transfection-facilitating compounds. A number of them are described in WO 93/18759, WO 93/19768, WO 94/25608, and WO 95/02397. They include spermine derivatives useful for facilitating the transport of DNA through the nuclear membrane (see, for example, WO 93/18759) and membrane-permeabilizing compounds such as GALA, Gramicidine S, and cationic bile salts (see, for example, WO 93/19768).

[0116] Gold or tungsten microparticles are used for gene delivery, as described in WO 91/00359, WO 93/17706, and Tang et al. *Nature* (1992) 356:152. The microparticle-coated polynucleotide is injected via intradermal or intraepidermal routes using a needleless injection device ("gene gun"), such as those described in U.S. Pat. No. 4,945,050, U.S. Pat. No. 5,015,580, and WO 94/24263.

[0117] The amount of DNA to be used in a vaccine recipient depends, e.g., on the strength of the promoter used in the DNA construct, the immunogenicity of the expressed gene product, the condition of the mammal intended for administration (e.g., the weight, age, and general health of the mammal), the mode of administration, and the type of formulation. In general, a therapeutically or prophylactically effective dose from about 1 μ g to about 1 mg, preferably, from about 10 μ g to about 800 μ g and, more preferably, from about 25 μ g to about 250 μ g, can be administered to human adults. The administration can be achieved in a single dose or repeated at intervals.

[0118] The route of administration is any conventional route used in the vaccine field. As general guidance, a polynucleotide of the invention is administered via a mucosal surface, e.g., an ocular, intranasal, pulmonary, oral, intestinal, rectal, vaginal, and urinary tract surface; or via a parenteral route, e.g., by an intravenous, subcutaneous, intraperitoneal, intradermal, intraepidermal, or intramuscular route. The choice of administration route depends on the formulation that is selected. A polynucleotide formulated in association with bupivacaine is advantageously administered into muscles. When a neutral or anionic liposome or a cationic lipid, such as DOTMA or DC-Chol, is used, the formulation can be advantageously injected via intravenous, intranasal (aerosolization), intramuscular, intradermal, and subcutaneous routes. A polynucleotide in a naked form can advantageously be administered via the intramuscular, intradermal, or subcutaneous routes.

[0119] Although not absolutely required, such a composition can also contain an adjuvant. If so, a systemic adjuvant that does not require concomitant administration in order to exhibit an adjuvant effect is preferable such as, e.g., QS21, which is described in U.S. Pat. No. 5,057,546.

[0120] The sequence information provided in the present application enables the design of specific nucleotide probes and primers that are used for diagnostic purposes. Accordingly, a fifth aspect of the invention provides a nucleotide probe or primer having a sequence found in or derived by degeneracy of the genetic code from a sequence shown in SEQ ID No: 1, 3 or 5

[0121] The term "probe" as used in the present application refers to DNA (preferably single stranded) or RNA molecules (or modifications or combinations thereof) that hybridize under the stringent conditions, as defined above, to nucleic acid molecules having SEQ ID No: 1, 3 or 5 or to sequences homologous to SEQ ID No:1, 3 or 5, or to its complementary or anti-sense sequence. Generally, probes are significantly shorter than full-length sequences. Such probes contain from about 5 to about 100, preferably from about 10 to about 80, nucleotides. In particular, probes have sequences that are at least 75%, preferably at least 85%, more preferably 95% homologous to a portion of SEQ ID No:1, 3 or 5 or that are complementary to such sequences. Probes may contain modified bases such as inosine, methyl-

5-deoxycytidine, deoxyuridine, dimethylamino-5-deoxyuridine, or diamino-2, 6-purine. Sugar or phosphate residues may also be modified or substituted. For example, a deoxyribose residue may be replaced by a polyamide (Nielsen et al., *Science* (1991) 254:1497) and phosphate residues may be replaced by ester groups such as diphosphate, alkyl, arylphosphonate and phosphorothioate esters. In addition, the 2'-hydroxyl group on ribonucleotides may be modified by including such groups as alkyl groups.

[0122] Probes of the invention are used in diagnostic tests, as capture or detection probes. Such capture probes are conventionally immobilized on a solid support, directly or indirectly, by covalent means or by passive adsorption. A detection probe is labeled by a detection marker selected from: radioactive isotopes, enzymes such as peroxidase, alkaline phosphatase, and enzymes able to hydrolyze a chromogenic, fluorogenic, or luminescent substrate, compounds that are chromogenic, fluorogenic, or luminescent, nucleotide base analogs, and biotin.

[0123] Probes of the invention are used in any conventional hybridization technique, such as dot blot (Maniatis et al., *Molecular Cloning: A Laboratory Manual* (1982) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), Southern blot (Southern, *J. Mol. Biol.* (1975) 98:503), northern blot (identical to Southern blot with the exception that RNA is used as a target), or the sandwich technique (Dunn et al., *Cell* (1977) 12:23). The latter technique involves the use of a specific capture probe and/or a specific detection probe with nucleotide sequences that at least partially differ from each other.

[0124] A primer is a probe of usually about 10 to about 40 nucleotides that is used to initiate enzymatic polymerization of DNA in an amplification process (e.g., PCR), in an elongation process, or in a reverse transcription method. Primers used in diagnostic methods involving PCR are labeled by methods known in the art.

[0125] As described herein, the invention also encompasses (i) a reagent comprising a probe of the invention for detecting and/or identifying the presence of Chlamydia in a biological material; (ii) a method for detecting and/or identifying the presence of Chlamydia in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA or RNA is extracted from the material and denatured, and (c) exposed to a probe of the invention, for example, a capture, detection probe or both, under stringent hybridization conditions, such that hybridization is detected; and (iii) a method for detecting and/or identifying the presence of Chlamydia in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA is extracted therefrom, (c) the extracted DNA is primed with at least one, and preferably two, primers of the invention and amplified by polymerase chain reaction, and (d) the amplified DNA fragment is produced.

[0126] It is apparent that disclosure of polynucleotide sequences of SEQ ID No: 1, 3 or 5, its homologs and partial sequences enable their corresponding amino acid sequences. Accordingly, a sixth aspect of the invention features a substantially purified polypeptide or polypeptide derivative having an amino acid sequence encoded by a polynucleotide of the invention.

[0127] A "substantially purified polypeptide" as used herein is defined as a polypeptide that is separated from the

environment in which it naturally occurs and/or that is free of the majority of the polypeptides that are present in the environment in which it was synthesized. For example, a substantially purified polypeptide is free from cytoplasmic polypeptides. Those skilled in the art would readily understand that the polypeptides of the invention may be purified from a natural source, i.e., a Chlamydia strain, or produced by recombinant means.

[0128] Consistent with the sixth aspect of the invention are polypeptides, homologs or fragments which are modified or treated to enhance their immunogenicity in the target animal, in whom the polypeptide, homolog or fragments are intended to confer protection against Chlamydia. Such modifications or treatments include: amino acid substitutions with an amino acid derivative such as 3-methylhistidine, 4-hydroxyproline, 5-hydroxylysine etc., modifications or deletions which are carried out after preparation of the polypeptide, homolog or fragment, such as the modification of free amino, carboxyl or hydroxyl side groups of the amino acids.

[0129] Identification of homologous polypeptides or polypeptide derivatives encoded by polynucleotides of the invention which have specific antigenicity is achieved by screening for cross-reactivity with an antiserum raised against the polypeptide of reference having an amino acid sequence of SEQ ID No: 1, 3 or 5. The procedure is as follows: a monospecific hyperimmune antiserum is raised against a purified reference polypeptide, a fusion polypeptide (for example, an expression product of MBP, GST, or His-tag systems, the description and instructions for use of which are contained in Invitrogen product manuals for pcDNA3.1/Myc-His(+) A, B, and C and for the Xpress™ System Protein Purification), or a synthetic peptide predicted to be antigenic. Where an antiserum is raised against a fusion polypeptide, two different fusion systems are employed. Specific antigenicity can be determined according to a number of methods, including Western blot (Towbin et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:4350), dot blot, and ELISA, as described below.

[0130] In a Western blot assay, the product to be screened, either as a purified preparation or a total *E. coli* extract, is submitted to SDS-Page electrophoresis as described by Laemmli (*Nature* (1970) 227:680). After transfer to a nitrocellulose membrane, the material is further incubated with the monospecific hyperimmune antiserum diluted in the range of dilutions from about 1:5 to about 1:5000, preferably from about 1:100 to about 1:500. Specific antigenicity is shown once a band corresponding to the product exhibits reactivity at any of the dilutions in the above range.

[0131] In an ELISA assay, the product to be screened is preferably used as the coating antigen. A purified preparation is preferred, although a whole cell extract can also be used. Briefly, about 100 μ l of a preparation at about 10 μ g protein/ml are distributed into wells of a 96-well polycarbonate ELISA plate. The plate is incubated for 2 hours at 37° C. then overnight at 4° C. The plate is washed with phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBS/Tween buffer). The wells are saturated with 250 μ l PBS containing 1% bovine serum albumin (BSA) to prevent non-specific antibody binding. After 1 hour incubation at 37°C., the plate is washed with PBS/Tween buffer. The antiserum is serially diluted in PBS/Tween buffer containing

0.5% BSA. 100 μ l of dilutions are added per well. The plate is incubated for 90 minutes at 37° C., washed and evaluated according to standard procedures. For example, a goat anti-rabbit peroxidase conjugate is added to the wells when specific antibodies were raised in rabbits. Incubation is carried out for 90 minutes at 37° C. and the plate is washed. The reaction is developed with the appropriate substrate and the reaction is measured by colorimetry (absorbance measured spectrophotometrically). Under the above experimental conditions, a positive reaction is shown by O.D. values greater than a non immune control serum.

[0132] In a dot blot assay, a purified product is preferred, although a whole cell extract can also be used. Briefly, a solution of the product at about 100 μ g/ml is serially two-fold diluted in 50 mM Tris-HCl (pH 7.5). 100 μ l of each dilution are applied to a nitrocellulose membrane 0.45 μ m set in a 96-well dot blot apparatus (Biorad). The buffer is removed by applying vacuum to the system. Wells are washed by addition of 50 mM Tris-HCl (pH 7.5) and the membrane is air-dried. The membrane is saturated in blocking buffer (50 mM Tris-HCl (pH 7.5) 0.15 M NaCl, 10 g/L skim milk) and incubated with an antiserum dilution from about 1:50 to about 1:5000, preferably about 1:500. The reaction is revealed according to standard procedures. For example, a goat anti-rabbit peroxidase conjugate is added to the wells when rabbit antibodies are used. Incubation is carried out 90 minutes at 37° C. and the blot is washed. The reaction is developed with the appropriate substrate and stopped. The reaction is measured visually by the appearance of a colored spot, e.g., by colorimetry. Under the above experimental conditions, a positive reaction is shown once a colored spot is associated with a dilution of at least about 1:5, preferably of at least about 1:500.

[0133] Therapeutic or prophylactic efficacy of a polypeptide or derivative of the invention can be evaluated as described below. A seventh aspect of the invention provides (i) a composition of matter comprising a polypeptide of the invention together with a diluent or carrier; specifically (ii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a polypeptide of the invention; (iii) a method for inducing an immune response against Chlamydia in a mammal, by administering to the mammal an immunogenically effective amount of a polypeptide of the invention to elicit a protective immune response to Chlamydia; and particularly, (iv) a method for preventing and/or treating a Chlamydia (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, or *C. pecorum*) infection, by administering a prophylactic or therapeutic amount of a polypeptide of the invention to an infected individual. Additionally, the seventh aspect of the invention encompasses the use of a polypeptide of the invention in the preparation of a medicament for preventing and/or treating Chlamydia infection.

[0134] As used herein, the immunogenic compositions of the invention are administered by conventional routes known the vaccine field, in particular to a mucosal (e.g., ocular, intranasal, pulmonary, oral, gastric, intestinal, rectal, vaginal, or urinary tract) surface or via the parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. The choice of administration route depends upon a number of parameters, such as the adjuvant associated with the polypeptide. If a mucosal adjuvant is used, the intranasal or oral route is preferred. If a lipid

formulation or an aluminum compound is used, the parenteral route is preferred with the sub-cutaneous or intramuscular route being most preferred. The choice also depends upon the nature of the vaccine agent. For example, a polypeptide of the invention fused to CTB or LTB is best administered to a mucosal surface.

[0135] As used herein, the composition of the invention contains one or several polypeptides or derivatives of the invention. The composition optionally contains at least one additional Chlamydia antigen, or a subunit, fragment, homolog, mutant, or derivative thereof.

[0136] For use in a composition of the invention, a polypeptide or derivative thereof is formulated into or with liposomes, preferably neutral or anionic liposomes, microspheres, ISCOMS, or virus-like-particles (VLPs) to facilitate delivery and/or enhance the immune response. These compounds are readily available to one skilled in the art; for example, see Liposomes: A Practical Approach, RCP New Ed, IRL press (1990).

[0137] Adjuvants other than liposomes and the like are also used and are known in the art. Adjuvants may protect the antigen from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. An appropriate selection can conventionally be made by those skilled in the art, for example, from those described below (under the eleventh aspect of the invention).

[0138] Treatment is achieved in a single dose or repeated as necessary at intervals, as can be determined readily by one skilled in the art. For example, a priming dose is followed by three booster doses at weekly or monthly intervals. An appropriate dose depends on various parameters including the recipient (e.g., adult or infant), the particular vaccine antigen, the route and frequency of administration, the presence/absence or type of adjuvant, and the desired effect (e.g., protection and/or treatment), as can be determined by one skilled in the art. In general, a vaccine antigen of the invention is administered by a mucosal route in an amount from about 10 μ g to about 500 mg, preferably from about 1 mg to about 200 mg. For the parenteral route of administration, the dose usually does not exceed about 1 mg, preferably about 100 μ g.

[0139] When used as vaccine agents, polynucleotides and polypeptides of the invention may be used sequentially as part of a multistep immunization process. For example, a mammal is initially primed with a vaccine vector of the invention such as a pox virus, e.g., via the parenteral route, and then boosted twice with the polypeptide encoded by the vaccine vector, e.g., via the mucosal route. In another example, liposomes associated with a polypeptide or derivative of the invention is also used for priming, with boosting being carried out mucosally using a soluble polypeptide or derivative of the invention in combination with a mucosal adjuvant (e.g., LT).

[0140] A polypeptide derivative of the invention is also used in accordance with the seventh aspect as a diagnostic reagent for detecting the presence of anti-Chlamydia antibodies, e.g., in a blood sample. Such polypeptides are about 5 to about 80, preferably about 10 to about 50 amino acids in length. They are either labeled or unlabeled, depending

upon the diagnostic method. Diagnostic methods involving such a reagent are described below.

[0141] Upon expression of a DNA molecule of the invention, a polypeptide or polypeptide derivative is produced and purified using known laboratory techniques. As described above, the polypeptide or polypeptide derivative may be produced as a fusion protein containing a fused tail that facilitates purification. The fusion product is used to immunize a small mammal, e.g., a mouse or a rabbit, in order to raise antibodies against the polypeptide or polypeptide derivative (monospecific antibodies). Accordingly, an eighth aspect of the invention provides a monospecific antibody that binds to a polypeptide or polypeptide derivative of the invention.

[0142] By "monospecific antibody" is meant an antibody that is capable of reacting with a unique naturally-occurring Chlamydia polypeptide. An antibody of the invention is either polyclonal or monoclonal. Monospecific antibodies may be recombinant, e.g., chimeric (e.g., constituted by a variable region of murine origin associated with a human constant region), humanized (a human immunoglobulin constant backbone together with hypervariable region of animal, e.g., murine, origin), and/or single chain. Both polyclonal and monospecific antibodies may also be in the form of immunoglobulin fragments, e.g., F(ab)'2 or Fab fragments. The antibodies of the invention are of any isotype, e.g., IgG or IgA, and polyclonal antibodies are of a single isotype or a mixture of isotypes.

[0143] Antibodies against the polypeptides, homologs or fragments of the present invention are generated by immunization of a mammal with a composition comprising said polypeptide, homolog or fragment. Such antibodies may be polyclonal or monoclonal. Methods to produce polyclonal or monoclonal antibodies are well known in the art. For a review, see "Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Eds. E. Harlow and D. Lane (1988), and D. E. Yelton et al., 1981. Ann. Rev. Biochem. 50:657-680. For monoclonal antibodies, see Kohler & Milstein (1975) Nature 256:495-497.

[0144] The antibodies of the invention, which are raised to a polypeptide or polypeptide derivative of the invention, are produced and identified using standard immunological assays, e.g., Western blot analysis, dot blot assay, or ELISA (see, e.g., Coligan et al., Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, N.Y.). The antibodies are used in diagnostic methods to detect the presence of a Chlamydia antigen in a sample, such as a biological sample. The antibodies are also used in affinity chromatography for purifying a polypeptide or polypeptide derivative of the invention. As is discussed further below, such antibodies may be used in prophylactic and therapeutic passive immunization methods.

[0145] Accordingly, a ninth aspect of the invention provides (i) a reagent for detecting the presence of Chlamydia in a biological sample that contains an antibody, polypeptide, or polypeptide derivative of the invention; and (ii) a diagnostic method for detecting the presence of Chlamydia in a biological sample, by contacting the biological sample with an antibody, a polypeptide, or a polypeptide derivative of the invention, such that an immune complex is formed, and by detecting such complex to indicate the presence of Chlamydia in the sample or the organism from which the sample is derived.

[0146] Those skilled in the art will readily understand that the immune complex is formed between a component of the sample and the antibody, polypeptide, or polypeptide derivative, whichever is used, and that any unbound material is removed prior to detecting the complex. It is understood that a polypeptide reagent is useful for detecting the presence of anti-Chlamydia antibodies in a sample, e.g., a blood sample, while an antibody of the invention is used for screening a sample, such as a gastric extract or biopsy, for the presence of Chlamydia polypeptides.

[0147] For diagnostic applications, the reagent (i.e., the antibody, polypeptide, or polypeptide derivative of the invention) is either in a free state or immobilized on a solid support, such as a tube, a bead, or any other conventional support used in the field. Immobilization is achieved using direct or indirect means. Direct means include passive adsorption (non-covalent binding) or covalent binding between the support and the reagent. By "indirect means" is meant that an anti-reagent compound that interacts with a reagent is first attached to the solid support. For example, if a polypeptide reagent is used, an antibody that binds to it can serve as an anti-reagent, provided that it binds to an epitope that is not involved in the recognition of antibodies in biological samples. Indirect means may also employ a ligand-receptor system, for example, where a molecule such as a vitamin is grafted onto the polypeptide reagent and the corresponding receptor immobilized on the solid phase. This is illustrated by the biotin-streptavidin system. Alternatively, a peptide tail is added chemically or by genetic engineering to the reagent and the grafted or fused product immobilized by passive adsorption or covalent linkage of the peptide tail.

[0148] Such diagnostic agents may be included in a kit which also comprises instructions for use. The reagent is labeled with a detection means which allows for the detection of the reagent when it is bound to its target. The detection means may be a fluorescent agent such as fluorescein isocyanate or fluorescein isothiocyanate, or an enzyme such as horse radish peroxidase or luciferase or alkaline phosphatase, or a radioactive element such as ¹²⁵I or ⁵¹Cr.

[0149] Accordingly, a tenth aspect of the invention provides a process for purifying, from a biological sample, a polypeptide or polypeptide derivative of the invention, which involves carrying out antibody-based affinity chromatography with the biological sample, wherein the antibody is a monospecific antibody of the invention.

[0150] For use in a purification process of the invention, the antibody is either polyclonal or monospecific, and preferably is of the IgG type. Purified IgGs is prepared from an antiserum using standard methods (see, e.g., Coligan et al., Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, N.Y.). Conventional chromatography supports, as well as standard methods for grafting antibodies, are described in, e.g., Antibodies: A Laboratory Manual, D. Lane, E. Harlow, Eds. (1988) and outlined below.

[0151] Briefly, a biological sample, such as an *C. pneumoniae* extract preferably in a buffer solution, is applied to a chromatography material, preferably equilibrated with the buffer used to dilute the biological sample so that the polypeptide or polypeptide derivative of the invention (i.e., the antigen) is allowed to adsorb onto the material. The chromatography material, such as a gel or a resin coupled to

an antibody of the invention, is in either a batch form or a column. The unbound components are washed off and the antigen is then eluted with an appropriate elution buffer, such as a glycine buffer or a buffer containing a chaotropic agent, e.g., guanidine HCl, or high salt concentration (e.g., 3 M MgCl₂). Eluted fractions are recovered and the presence of the antigen is detected, e.g., by measuring the absorbance at 280 nm.

[0152] An eleventh aspect of the invention provides (i) a composition of matter comprising a monospecific antibody of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a monospecific antibody of the invention, and (iii) a method for treating or preventing a Chlamydia (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae* or *C. pecorum*) infection, by administering a therapeutic or prophylactic amount of a monospecific antibody of the invention to an infected individual. Additionally, the eleventh aspect of the invention encompasses the use of a monospecific antibody of the invention in the preparation of a medicament for treating or preventing Chlamydia infection.

[0153] The monospecific antibody is either polyclonal or monoclonal, preferably of the IgA isotype (predominantly). In passive immunization, the antibody is administered to a mucosal surface of a mammal, e.g., the gastric mucosa, e.g., orally or intragastrically, advantageously, in the presence of a bicarbonate buffer. Alternatively, systemic administration, not requiring a bicarbonate buffer, is carried out. A monospecific antibody of the invention is administered as a single active component or as a mixture with at least one monospecific antibody specific for a different Chlamydia polypeptide. The amount of antibody and the particular regimen used are readily determined by one skilled in the art. For example, daily administration of about 100 to 1,000 mg of antibodies over one week, or three doses per day of about 100 to 1,000 mg of antibodies over two or three days, are effective regimens for most purposes.

[0154] Therapeutic or prophylactic efficacy are evaluated using standard methods in the art, e.g., by measuring induction of a mucosal immune response or induction of protective and/or therapeutic immunity, using, e.g., the *C. pneumoniae* mouse model. Those skilled in the art will readily recognize that the *C. pneumoniae* strain of the model may be replaced with another Chlamydia strain. For example, the efficacy of DNA molecules and polypeptides from *C. pneumoniae* is preferably evaluated in a mouse model using *C. pneumoniae* strain. Protection is determined by comparing the degree of Chlamydia infection to that of a control group. Protection is shown when infection is reduced by comparison to the control group. Such an evaluation is made for polynucleotides, vaccine vectors, polypeptides and derivatives thereof, as well as antibodies of the invention.

[0155] Adjuvants useful in any of the vaccine compositions described above are as follows.

[0156] Adjuvants for parenteral administration include aluminum compounds, such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate. The antigen is precipitated with, or adsorbed onto, the aluminum compound according to standard protocols. Other adjuvants, such as RIBI (ImmunoChem, Hamilton, Mont.), are used in parenteral administration.

[0157] Adjuvants for mucosal administration include bacterial toxins, e.g., the cholera toxin (CT), the *E. coli* heat-labile toxin (LT), the *Clostridium difficile* toxin A and the pertussis toxin (PT), or combinations, subunits, toxoids, or mutants thereof such as a purified preparation of native cholera toxin subunit B (CTB). Fragments, homologs, derivatives, and fusions to any of these toxins are also suitable, provided that they retain adjuvant activity. Preferably, a mutant having reduced toxicity is used. Suitable mutants are described, e.g., in WO 95/17211 (Arg-7-Lys CT mutant), WO 96/06627 (Arg-192-Gly LT mutant), and WO 95/34323 (Arg-9-Lys and Glu-129-Gly PT mutant). Additional LT mutants that are used in the methods and compositions of the invention include, e.g., Ser-63-Lys, Ala-69Gly, Glu-110-Asp, and Glu-112-Asp mutants. Other adjuvants, such as a bacterial monophosphoryl lipid A (MPLA) of, e.g., *E. coli*, *Salmonella minnesota*, *Salmonella typhimurium*, or *Shigella flexneri*; saponins, or polylactide glycolide (PLGA) microspheres, is also be used in mucosal administration.

[0158] Adjuvants useful for both mucosal and parenteral administrations include polyphosphazene (WO 95/02415), DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol; U.S. Pat. No. 5,283,185 and wO 96/14831) and QS-21 (WO 88/09336).

[0159] Any pharmaceutical composition of the invention containing a polynucleotide, a polypeptide, a polypeptide derivative, or an antibody of the invention, is manufactured in a conventional manner. In particular, it is formulated with a pharmaceutically acceptable diluent or carrier, e.g., water or a saline solution such as phosphate buffer saline. In general, a diluent or carrier is selected on the basis of the mode and route of administration, and standard pharmaceutical practice. Suitable pharmaceutical carriers or diluents, as well as pharmaceutical necessities for their use in pharmaceutical formulations, are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field and in the USP/NF.

[0160] The invention also includes methods in which Chlamydia infection are treated by oral administration of a Chlamydia polypeptide of the invention and a mucosal adjuvant, in combination with an antibiotic, an antacid, sucralfate, or a combination thereof. Examples of such compounds that can be administered with the vaccine antigen and the adjuvant are antibiotics, including, e.g., macrolides, tetracyclines, and derivatives thereof (specific examples of antibiotics that can be used include azithromycin or doxycyclin or immunomodulators such as cytokines or steroids). In addition, compounds containing more than one of the above-listed components coupled together, are used. The invention also includes compositions for carrying out these methods, i.e., compositions containing a Chlamydia antigen (or antigens) of the invention, an adjuvant, and one or more of the above-listed compounds, in a pharmaceutically acceptable carrier or diluent.

[0161] It has recently been shown that the 9 kDa cysteine rich membrane protein contains a sequence cross-reactive with the murine alpha-myosin heavy chain epitope M7A-alpha, an epitope conserved in humans (Bachmaier et al., *Science* (1999) 283:1335). This cross-reactivity is proposed to contribute to the development of cardiovascular disease, so it may be beneficial to remove this epitope, and any other epitopes cross-reactive with human antigens, from the pro-

tein if it is to be used as a vaccine. Accordingly, a further embodiment of the present invention includes the modification of the coding sequence, for example, by deletion or substitution of the nucleotides encoding the epitope from polynucleotides encoding the protein, as to improve the efficacy and safety of the protein as a vaccine. A similar approach may be appropriate for any protective antigen found to have unwanted homologies or cross-reactivities with human antigens.

[0162] Amounts of the above-listed compounds used in the methods and compositions of the invention are readily determined by one skilled in the art. Treatment/immunization schedules are also known and readily designed by one skilled in the art. For example, the non-vaccine components can be administered on days 1-14, and the vaccine antigen +adjuvant can be administered on days 7, 14, 21, and 28.

EXAMPLES

[0163] The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Example 1

[0164] This example illustrates the preparation of a plasmid vector pCACPNM555a containing the full length 76 kDa protein gene.

[0165] The full-length 76 kDa protein gene was amplified from Chlamydia pneumoniae genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAAT-GCGGCCGC CACCATGGTTAACCTATTGGTCCAGG 3') (SEQ ID No:9) and a 3' primer (5' GCGCCGGATCC CTTGGAGATAACCAGAAATAGAG 3') (SEQ ID No:10). The 5' primer contains a Not I restriction site, a ribosome binding site, an initiation codon and a sequence close to the 5' end of the full-length 76 kDa protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the 76 kDa protein and a Bam HI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

[0166] After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen) and then digested with Not I and Bam HI and cloned into the pCA-Myc-His eukaryotic expression vector describe in Example 2 (FIG. 4) with transcription under control of the human CMV promoter.

Example 2

[0167] This example illustrates the preparation of the eukaryotic expression vector pCA/Myc-His.

[0168] Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012

(Vical) was isolated on a Spe I/Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR fragment containing the full-length 76 kDa protein gene was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCACPNM555a (FIG. 4).

[0169] The resulting plasmid, pCACPNM555a, was transferred by electroporation into *E. coli* XL-1 blue (Stratagene) which was grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

Example 3

[0170] This example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C. pneumoniae*.

[0171] It has been previously demonstrated (Yang et. al., 1993) that mice are susceptible to intranasal infection with different isolates of *C. pneumoniae*. Strain AR-39 (Grayston, 1989) was used in Balb/c mice as a challenge infection model to examine the capacity of Chlamydia gene products delivered as naked DNA to elicit a protective response against a sublethal *C. pneumoniae* lung infection. Protective immunity is defined as an accelerated clearance of pulmonary infection.

[0172] Groups of 7 to 9 week old male Balb/c mice (7 to 10 per group) were immunized intramuscularly (i.m.) plus intranasally (i.n.) with plasmid DNA containing the coding sequence of *C. pneumoniae* full-length 76kDa protein as described in Examples 1 and 2. Saline or the plasmid vector lacking an inserted Chlamydial gene was given to groups of control animals.

[0173] For i.m. immunization alternate left and right quadriceps were injected with 100 µg of DNA in 50 µl of PBS on three occasions at 0, 3 and 6 weeks. For i.n. immunization, anaesthetized mice aspirated 50 µl of PBS containing 50 µg DNA on three occasions at 0, 3 and 6 weeks. At week 8, immunized mice were inoculated i.n. with 5×10⁵ IFU of *C. pneumoniae*, strain AR39 in 100 µl of SPG buffer to test their ability to limit the growth of a sublethal *C. pneumoniae* challenge.

[0174] Lungs were taken from mice at days 5 and 9 post-challenge and immediately homogenised in SPG buffer (7.5% sucrose, 5 mM glutamate, 12.5 mM phosphate pH7.5). The homogenate was stored frozen at -70° C. until assay. Dilutions of the homogenate were assayed for the presence of infectious Chlamydia by inoculation onto monolayers of susceptible cells. The inoculum was centrifuged onto the cells at 3000 rpm for 1 hour, then the cells were incubated for three days at 35° C. in the presence of 1 µg/ml cycloheximide. After incubation the monolayers were fixed with formalin and methanol then immunoperoxidase stained for the presence of Chlamydial inclusions using convalescent sera from rabbits infected with *C. pneumoniae* and metal-enhanced DAB as a peroxidase substrate.

[0175] FIG. 7 and Table 1 show that mice immunized i.n. and i.m. with pCACPNM555a had Chlamydial lung titers less than 30,000 IFU/lung (mean 23,550) in 5 of 6 cases at day 9 whereas the range of values for control mice sham immunized with saline were 20,800 to 323,300 IFU/lung (mean 206,375) for (Table 1). DNA immunisation per se was not responsible for the observed protective effect since two other plasmid DNA constructs, pCACPNM806 and pCACPNM760, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice. The constructs pCACPNM806 and pCACPNM760 are identical to pCACPNM555a except that the nucleotide sequence encoding the full-length 76kDa protein is replaced with *C. pneumoniae* nucleotide sequences encoding an unrelated sequence.

Example 5

[0179] This example illustrates the preparation of the eukaryotic expression vector pCA/Myc-His.

[0180] Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Dam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I/Dam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR fragment containing the 5' truncated 76 kDa protein gene was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCAI555 (FIG. 5).

TABLE 1

BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS				
IMMUNIZING CONSTRUCT				
MOUSE	Saline Day 9	pCACPNM806 Day 9	pCACPNM760 Day 9	pCACPNM555a Day 9
1	225900	36700	140300	27300
2	20800	238700	128400	15200
3	286100	52300	88700	34600
4	106700	109600	25600	20500
5	323300	290000	37200	22000
6	144300	298800	5900	21700
7	261700			
8	282200			
MEAN	206375	171016.667	71016.6667	23550
SD	105183.9	119141.32	56306.57	6648.53
Wilcoxon p		0.8518	0.0293	0.008

Example 4

[0176] This example illustrates the preparation of a plasmid vector pCAI555 containing a 5'-truncated 76 kDa protein gene.

[0177] The 5' truncated 76 kDa protein gene was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' ATAAGAAT-GCGGCCGCCACCATGAGTCTGGCA-GATAAGCTGGG 3') (SEQ ID No:7 and a 3' primer (5' GCGCCGGATCC CTTGGAGATAACCAGAAATA 3') (SEQ ID No:8). The 5' primer contains a Not I restriction site, a ribosome binding site, an initiation codon and a sequence at the second Met codon of the 76 kDa protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the 3' 76 kDa protein and a Bam HI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

[0178] After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen) and then digested with Not I and Bam HI and cloned into the pCA-Myc-His eukaryotic expression vector described in Example 5 (FIG. 5) with transcription under control of the human CMV promoter.

[0181] The resulting plasmid, pCAI555, was transferred by electroporation into *E. coli* XL-1 blue (Stratagene) which was grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

Example 6

[0182] This Example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C. pneumoniae*. The procedures are described in Example 3 above, except that the DNA plasmid used for immunization contains the coding sequence of *C. pneumoniae* 5'-truncated 76 kDa protein, as described in Examples 4 and 5.

[0183] FIG. 8 and Table 2 show that mice immunized i.n. and i.m. with pCAI555 had Chlamydial lung titers less than 13000 IFU/lung (mean 6050) in 6 of 6 cases at day 9 whereas the range of values for control mice sham immunized with saline were 106,100 IFU/lung (mean 39,625) for (Table 2). DNA immunisation per se was not responsible for the observed protective effect since two other plasmid DNA constructs, pCAI116 and pCAI178, failed to protect, with

lung titers in immunised mice similar to those obtained for saline-immunized control mice. The constructs pCAI116 and pCAI178 are identical to pCAI555 except that the nucleotide sequence encoding the 5'-truncated 76 kDa protein is replaced with a *C. pneumoniae* nucleotide sequence encoding an unprotective sequence and the nucleoside 5'-diphosphate phosphotransferase protein.

TABLE 2

BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS

MOUSE	IMMUNIZING CONSTRUCT			
	Saline Day 9	pCAI116 Day 9	pCAI178 Day 9	pCAI555 Day 9
1	1700	47700	80600	6100
2	36200	12600	31900	10700
3	106100	28600	30600	500
4	33500	17700	6500	5100
5	70400	77300	53000	1100
6	48700	17600	79500	12800
7	600			
8	19800			
9	29500			
10	100000			
11	15000			
12	56600			
13	60300			
14	88800			
15	30400			
16	69300			
17	47500			
18	96500			
19	30200			
20	84800			
21	3800			
22	65900			
23	33000			
MEAN	49069.57	33583.33	47016.67	6050
SD	32120.48	24832.67	29524.32	4967.80

Example 7

[0184] This example illustrates the preparation of a plasmid vector pCAD76kDa containing a 3'-truncated 76 kDa protein gene.

[0185] The 3'-truncated 76 kDa protein gene was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5' GCTCTA-GACGCCATGACAAAAAAACATTATGCTTGGG 3') (SEQ ID No:9) and a 3' primer (5' CGGGATCC ATAGAACT-TGCTGCAGCGGG 3') (SEQ ID No:10). The 5' primer contains a Xba I restriction site, a ribosome binding site, an initiation codon and a sequence 765 bp upstream of the 5' end of the 76 kDa protein coding sequence. The 3' primer includes a 21 bp the sequence downstream of codon 452 of the 76 kDa protein and a Bam HI restriction site. An additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag. Note that inclusion of the 765

bp 5' region and the 21 bp 3' regions were inadvertent. These sequences are not part of the 76 kDa protein gene. Nevertheless, immunoprotection was achieved using this sequence (Example 6).

[0186] After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen) and then digested with Xba I and Bam HI and cloned into the pCA-Myc-His eukaryotic expression vector describe in Example 8 (FIG. 6) with transcription under control of the human CMV promoter.

Example 8

[0187] This Example illustrates the preparation of the eukaryotic expression vector pCA/Myc-His.

[0188] Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I/Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Xba I/Bam HI restricted PCR fragment containing a 3'-truncated 76 kDa protein gene was ligated into the Xba I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCAD76kDa (FIG. 6).

[0189] The resulting plasmid, pCAD76kDa, was transferred by electroporation into *E. coli* XL-1 blue (Stratagene) which was grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

Example 9

[0190] This example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C. pneumoniae*. The procedures are as described in Example 3 above, except that the DNA plasmid used for immunization contains the coding sequence of *C. pneumoniae* 3'-truncated 76 kDa protein, as described in Examples 7 and 8.

[0191] FIG. 9 and Table 3 show that mice immunized i.n. and i.m. with pCAD76kDa had Chlamydial lung titers less than 2400 in 5 of 5 cases whereas the range of values for control mice were 1800-23100 IFU/lung (mean 11811) and 16600-26100 IFU/lung (mean 22100) for sham immunized with saline or immunized with the unmodified vector respectively (Table 2). The lack of protection with the unmodified vector confirms that DNA per se was not responsible for the observed protective effect. This is further supported by the results obtained for one additional plasmid DNA construct, pdagA, that failed to protect, and for which the mean lung titers were similar to those obtained for saline-immunized control mice. The construct pdagA is identical to pCAD76kDa except that the nucleotide sequence encoding the 3'-truncated 76 kDa protein is replaced with a *C. pneumoniae* nucleotide sequence encoding the protein dagA.

TABLE 3

BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS

<u>IMMUNIZING CONSTRUCT</u>				
MOUSE	Saline	Vector	pdagA	pCAD76kDa
1	17700	19900	16000	1700
2	3900	16600	500	2000
3	1800	24300	18500	2300
4	16400	26100	12800	2100
5	11700	23600	6400	600
6	23100			
7	12000			
8	5300			
9	14400			
10	18700			
11	7300			

TABLE 3-continued

BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS

IMMUNIZING CONSTRUCT

[0192]

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 14

```
<210> SEQ ID NO 1
<211> LENGTH: 2156
<212> TYPE: DNA
<213> ORGANISM: Chlamydia pneumoniae
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (101)..(2053)
```

<400> SEQUENCE: 1

ataaaatctt taaaaacagg ctgcattaa ttattatgtga gagctttttt ttatttttt 60

ataataaaac taaaagat ttattat tttt ttgatgtttt atg gtt aat cct att 115
Met Val Asn Pro Ile
1 5

```

ggc cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat ctt 163
Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp Leu
          10          15          20

```

```

tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa gct 211
Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu Ala
          25           30           35

```

```

caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc gat 259
Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr Asp
          40           45           50

```

tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc atg 307
Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu Met
55 60 65

```

agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct tct 355
Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser Ser
    70          75          80          85

```

```

act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct acg      403
Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro Thr
90          95          100

```

```

cct cct cca ccc acg ttt gat gat tat aag act caa gcg cca aca gct      451
Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr Ala
          105           110           115

```

-continued

tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct gct	499
Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala Ala	
120 125 130	
ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gca gct	547
Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala Ala	
135 140 145	
act gat gag gaa acc gca atc gct gct gat gaa act aat aat gac	595
Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn Ala	
150 155 160 165	
gat gca gtt aaa gtt ggc gca att aca gaa tta gca aaa tat gct	643
Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr Ala	
170 175 180	
tcg gat aac caa gca att ctt gac tct tta ggt aaa ctg act tcc ttc	691
Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser Phe	
185 190 195	
gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac aaa	739
Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn Lys	
200 205 210	
gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca ggg	787
Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly	
215 220 225	
aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct aca	835
Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala Thr	
230 235 240 245	
gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat ttt	883
Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr Phe	
250 255 260	
gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat aac	931
Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn Asn	
265 270 275	
agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct aag	979
Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala Lys	
280 285 290	
aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att ctt	1027
Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile Leu	
295 300 305	
caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa aat	1075
Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys Asn	
310 315 320 325	
atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca gtt	1123
Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr Val	
330 335 340	
gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt tcc	1171
Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val Ser	
345 350 355	
atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg tct	1219
Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met Ser	
360 365 370	
ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat tct	1267
Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp Ser	
375 380 385	
caa gct gcc caa caa gag ctc gca gca caa gct aga gca gca aaa gcc	1315
Gln Ala Ala Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys Ala	
390 395 400 405	
gct gga gat gac agt gct gct gca gca gat gct cag aaa gct	1363
Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys Ala	
410 415 420	

-continued

tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc aat Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu Asn 425 430 435	1411
gct tta gga cag atc gct tct gct gtt gtg agc gca gga gtt cct Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val Pro 440 445 450	1459
ccc gct gca gca agt tct ata ggg tca tct gta aaa cag ctt tac aag Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val Lys Gln Leu Tyr Lys 455 460 465	1507
acc tca aaa tct aca ggt tct gat tat aaa aca cag ata tca gca ggt Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala Gly 470 475 480 485	1555
tat gat gct tac aaa tcc atc aat gat gcc tat ggt agg gca cga aat Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg Asn 490 495 500	1603
gat gcg act cgt gat gtg ata aac aat gta agt acc ccc gct ctc aca Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser Thr Pro Ala Leu Thr 505 510 515	1651
cga tcc gtt cct aga gca cga aca gaa gct cga gga cca gaa aaa aca Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys Thr 520 525 530	1699
gat caa gcc ctc gct agg gtg att tct ggc aat agc aga act ctt gga Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu Gly 535 540 545	1747
gat gtc tat agt caa gtt tcg gca cta caa tct gta atg cag atc atc Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile Ile 550 555 560 565	1795
cag tcg aat cct caa gcg aat aat gag gag atc aga caa aag ctt aca Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile Arg Gln Lys Leu Thr 570 575 580	1843
tcg gca gtg aca aag cct cca cag ttt ggc tat cct tat gtg caa ctt Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln Leu 585 590 595	1891
tct aat gac tct aca cag aag ttc ata gct aaa tta gaa agt ttg ttt Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys Leu Glu Ser Leu Phe 600 605 610	1939
gct gaa gga tct agg aca gca gct gaa ata aaa gca ctt tcc ttt gaa Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys Ala Leu Ser Phe Glu 615 620 625	1987
acg aac tcc ttg ttt att cag cag gtg ctg gtc aat atc ggc tct cta Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser Leu 630 635 640 645	2035
tat tct ggt tat ctc caa taacaacacc taagtgttcg tttggagaga Tyr Ser Gly Tyr Leu Gln 650	2083
ttattatgtg ctttgtaag gccttggttt aggccttacc aacacactag aacgatcttc	2143
aataaataaa aga	2156

<210> SEQ ID NO 2
<211> LENGTH: 651
<212> TYPE: PRT
<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 2

Met Val Asn Pro Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr
1 5 10 15

Pro Pro Ala Asp Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn

-continued

20	25	30
Lys Ser Ala Glu Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys		
35	40	45
Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala		
50	55	60
Val Asn Ala Leu Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser		
65	70	75
Asn Ser Ser Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr		
85	90	95
Ala Thr Ala Pro Thr Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr		
100	105	110
Gln Ala Gln Thr Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala		
115	120	125
Asp Ile Gln Ala Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile		
130	135	140
Lys Asp Thr Ala Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp		
145	150	155
Glu Thr Lys Asn Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu		
165	170	175
Leu Ala Lys Tyr Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly		
180	185	190
Lys Leu Thr Ser Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val		
195	200	205
Ala Asn Asn Asn Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn		
210	215	220
Pro Val Val Pro Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp		
225	230	235
Gln Thr Asp Ala Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile		
245	250	255
Arg Asp Ala Tyr Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn		
260	265	270
Ala Lys Ser Asn Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala		
275	280	285
Ile Ala Thr Ala Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro		
290	295	300
Asp Ser Pro Ile Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu		
305	310	315
Lys Asp Leu Lys Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn		
325	330	335
Pro Gly Thr Thr Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly		
340	345	350
Ser Ile Arg Val Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala		
355	360	365
Ser Ile Leu Met Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr		
370	375	380
Glu Asn Pro Asp Ser Gln Ala Ala Gln Glu Leu Ala Ala Gln Ala		
385	390	395
Arg Ala Ala Lys Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala		
405	410	415
Asp Ala Gln Lys Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln		
420	425	430

-continued

Gln Gly Ile Leu Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val
 435 440 445
 Ser Ala Gly Val Pro Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val
 450 455 460
 Lys Gln Leu Tyr Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr
 465 470 475 480
 Gln Ile Ser Ala Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr
 485 490 495
 Gly Arg Ala Arg Asn Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser
 500 505 510
 Thr Pro Ala Leu Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg
 515 520 525
 Gly Pro Glu Lys Thr Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn
 530 535 540
 Ser Arg Thr Leu Gly Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser
 545 550 555 560
 Val Met Gln Ile Ile Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile
 565 570 575
 Arg Gln Lys Leu Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr
 580 585 590
 Pro Tyr Val Gln Leu Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys
 595 600 605
 Leu Glu Ser Leu Phe Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys
 610 615 620
 Ala Leu Ser Phe Glu Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val
 625 630 635 640
 Asn Ile Gly Ser Leu Tyr Ser Gly Tyr Leu Gln
 645 650

<210> SEQ ID NO 3
 <211> LENGTH: 1852
 <212> TYPE: DNA
 <213> ORGANISM: Chlamydia pneumoniae
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1749)

<400> SEQUENCE: 3

atg	agt	ctg	gca	gat	aag	ctg	ggt	att	gct	tct	agt	aac	agc	tcg	tct	48
Met	Ser	Leu	Ala	Asp	Lys	Leu	Gly	Ile	Ala	Ser	Ser	Asn	Ser	Ser	Ser	
1					5			10							15	
tct	act	agc	aga	tct	gca	gac	gtg	gac	tca	acg	aca	gca	acc	gca	cct	96
Ser	Thr	Ser	Arg	Ser	Ala	Asp	Val	Asp	Ser	Thr	Thr	Ala	Thr	Ala	Pro	
20					25				30							
acg	cct	cca	ccc	acg	ttt	gat	gat	tat	aag	act	caa	gca	caa	aca		144
Thr	Pro	Pro	Pro	Thr	Phe	Asp	Asp	Tyr	Lys	Thr	Gln	Ala	Gln	Thr		
35					40				45							
gct	tac	gat	act	atc	ttt	acc	tca	aca	tca	cta	gct	gac	ata	cag	gct	192
Ala	Tyr	Asp	Thr	Ile	Phe	Thr	Ser	Thr	Ser	Leu	Ala	Asp	Ile	Gln	Ala	
50					55				60							
gct	ttg	gtg	agc	ctc	cag	gat	gct	gtc	act	aat	ata	aag	gat	aca	gct	240
Ala	Leu	Val	Ser	Leu	Gln	Asp	Ala	Val	Thr	Asn	Ile	Lys	Asp	Thr	Ala	
65					70				75			80				
gct	act	gat	gag	gaa	acc	gca	atc	gct	gcg	gag	tgg	gaa	act	aag	aat	288
Ala	Thr	Asp	Glu	Glu	Thr	Ala	Ile	Ala	Ala	Glu	Trp	Glu	Thr	Lys	Asn	

-continued

85	90	95	
gcc gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr 100	105	110	336
gct tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser 115	120	125	384
ttc gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn 130	135	140	432
aaa gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro 145	150	155	480
ggg aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala 165	170	175	528
aca gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr 180	185	190	576
ttt gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn 195	200	205	624
aac agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala 210	215	220	672
aag aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att Lys Thr Gln Ile Ala Glu Ala Gln Lys Phe Pro Asp Ser Pro Ile 225	230	235	720
ctt caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys 245	250	255	768
aat atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr 260	265	270	816
gtt gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val 275	280	285	864
tcc atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met 290	295	300	912
tct ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp 305	310	315	960
tct caa gct gcc caa cag gag ctc gca gca caa gct aga gca gcg aaa Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys 325	330	335	1008
gcc gct gga gat gac agt gct gca gcg ctg gca gat gct cag aaa Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys 340	345	350	1056
gct tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gly Ile Leu 355	360	365	1104
aat gct tta gga cag atc gct tct gct gct gtt gtg agc gca gga gtt Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val 370	375	380	1152
cct ccc gct gca gca agt tct ata ggg tca tct gta aaa cag ctt tac Pro Pro Ala Ala Ala Ser Ser Ile Gly Ser Ser Val Val Gln Leu Tyr 390			1200

-continued

385	390	395	400	
aag acc tca aaa tct aca ggt tct gat tat aaa aca cag ata tca gca Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala 405 410 415				1248
ggt tat gat gct tac aaa tcc atc aat gat gcc tat ggt agg gca cga Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg 420 425 430				1296
aat gat gcg act cgt gat gtg ata aac aat gta agt acc ccc gct ctc Asn Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser Thr Pro Ala Leu 435 440 445				1344
aca cga tcc gtt cct aga gca cga aca gaa gct cga gga cca gaa aaa Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys 450 455 460				1392
aca gat caa gcc ctc gct agg gtg att tct ggc aat agc aga act ctt Thr Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu 465 470 475 480				1440
gga gat gtc tat agt caa gtt tcg gca cta caa tct gta atg cag atc Gly Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile 485 490 495				1488
act cag tcg aat cct caa gcg aat aat gag gag atc aga caa aag ctt Thr Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile Arg Gln Lys Leu 500 505 510				1536
aca tcg gca gtg aca aag cct cca cag ttt ggc tat cct tat gtg caa Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln 515 520 525				1584
ctt tct aat gac tct aca cag aag ttc ata gct aaa tta gaa agt ttg Leu Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys Leu Glu Ser Leu 530 535 540				1632
ttt gct gaa gga tct agg aca gca gct gaa ata aaa gca ctt tcc ttt Phe Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys Ala Leu Ser Phe 545 550 555 560				1680
gaa acg aac tcc ttg ttt att cag cag gtg ctg gtc aat atc ggc tct Glu Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser 565 570 575				1728
cta tat tct ggt tat ctc caa taacaacacc taagtgtcgt tttggagaga Leu Tyr Ser Gly Tyr Leu Gln 580				1779
tttattatgtc ctttgtaag gccttgcgtt aggccttacc aacacactag aacgatctc				1839
aataaataaa aga				1852

<210> SEQ ID NO 4
<211> LENGTH: 583
<212> TYPE: PRT
<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 4

Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser 1 5 10 15
Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro 20 25 30
Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr 35 40 45
Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala 50 55 60
Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala 65 70 75 80

-continued

Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn
 85 90 95

Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr
 100 105 110

Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser
 115 120 125

Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn
 130 135 140

Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro
 145 150 155 160

Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala
 165 170 175

Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr
 180 185 190

Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn
 195 200 205

Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala
 210 215 220

Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile
 225 230 235 240

Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys
 245 250 255

Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr
 260 265 270

Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val
 275 280 285

Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met
 290 295 300

Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp
 305 310 315 320

Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys
 325 330 335

Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys
 340 345 350

Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu
 355 360 365

Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val
 370 375 380

Pro Pro Ala Ala Ser Ser Ile Gly Ser Ser Val Lys Gln Leu Tyr
 385 390 395 400

Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala
 405 410 415

Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg
 420 425 430

Asn Asp Ala Thr Arg Asp Val Ile Asn Asn Val Ser Thr Pro Ala Leu
 435 440 445

Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys
 450 455 460

Thr Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu
 465 470 475 480

-continued

Gly Asp Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile
 485 490 495
 Thr Gln Ser Asn Pro Gln Ala Asn Asn Glu Glu Ile Arg Gln Lys Leu
 500 505 510
 Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln
 515 520 525
 Leu Ser Asn Asp Ser Thr Gln Lys Phe Ile Ala Lys Leu Glu Ser Leu
 530 535 540
 Phe Ala Glu Gly Ser Arg Thr Ala Ala Glu Ile Lys Ala Leu Ser Phe
 545 550 555 560
 Glu Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser
 565 570 575
 Leu Tyr Ser Gly Tyr Leu Gln
 580

<210> SEQ ID NO 5
 <211> LENGTH: 1456
 <212> TYPE: DNA
 <213> ORGANISM: Chlamydia pneumoniae
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (101)..(1456)

 <400> SEQUENCE: 5

ataaaatctt	taaaaaacagg	ctcgcattaa	ttattagtga	gagctttttt	tttatttttt	60															
ataataaaac	taaaagattt	ttattttttt	ttgagttttt	atg gtt aat cct att	Met Val Asn Pro Ile	115															
Gly	Pro	Ile	Asp	Glu	Thr	Arg	Thr	Pro	Pro	Ala	Asp	Leu	10	15	20	163					
tct	gtc	caa	gga	tgg	gag	gcg	agt	gca	aat	aag	agt	gcg	gaa	gct	25	30	35	211			
Ser	Ala	Gln	Gly	Leu	Glu	Ala	Ser	Ala	Ala	Asn	Lys	Ser	Ala	Glu	Ala	40	45	50	259		
tct	gtc	gaa	ggt	gca	gaa	gct	aag	cct	aaa	gaa	tct	aag	acc	gat	55	60	65	307			
Ser	Val	Glu	Arg	Trp	Ser	Ile	Leu	Arg	Ser	Ala	Val	Asn	Ala	Leu	Met	70	75	80	355		
agt	ctg	gca	gat	aag	ctg	ggt	att	gct	tct	agt	aac	agc	tcg	tct	tct	90	95	100	403		
Ser	Leu	Ala	Asp	Lys	Leu	Gly	Ile	Ala	Ser	Ser	Asn	Ser	Ser	Ser	Ser	105	110	115	451		
cct	cct	cca	ccc	acg	ttt	gat	gat	tat	aag	act	caa	gca	acc	gca	cct	act	95	100	105	499	
Tyr	Asp	Thr	Ile	Phe	Asp	Asp	Tyr	Lys	Thr	Gln	Ala	Gln	Thr	Ala	Pro	Thr	120	125	130	547	
ttg	gtg	agc	ctc	cag	gat	gct	gtc	act	aat	ata	aag	gat	aca	gcg	gct	act	135	140	145	595	
Leu	Val	Ser	Leu	Gln	Asp	Ala	Val	Thr	Asn	Ile	Lys	Asp	Thr	Ala	Ala	Thr	Asp	Glu	Thr	Ala	Ala

-continued

150	155	160	165	
gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat gct				643
Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr Ala				
170	175	180		
tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc ttc				691
Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser Phe				
185	190	195		
gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac aaa				739
Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn Lys				
200	205	210		
gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca ggg				787
Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly				
215	220	225		
aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct aca				835
Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala Thr				
230	235	240	245	
gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat ttt				883
Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr Phe				
250	255	260		
gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat aac				931
Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn Asn				
265	270	275		
agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct aag				979
Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala Lys				
280	285	290		
aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att ctt				1027
Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile Leu				
295	300	305		
caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa aat				1075
Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys Asn				
310	315	320	325	
atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca gtt				1123
Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr Val				
330	335	340		
gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt tcc				1171
Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val Ser				
345	350	355		
atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg tct				1219
Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met Ser				
360	365	370		
ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat tct				1267
Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp Ser				
375	380	385		
caa gct gcc caa cag gag ctc gca gca caa gct aga gca gcg aaa gcc				1315
Gln Ala Ala Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys Ala				
390	395	400	405	
gct gga gat gac agt gct gca gcg ctg gca gat gct cag aaa gct				1363
Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys Ala				
410	415	420		
tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc aat				1411
Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gly Ile Leu Asn				
425	430	435		
gct tta gga cag atc gct tct gct gct gtt gtg agc gca gga gta				1456
Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val				
440	445	450		

-continued

<211> LENGTH: 452

<212> TYPE: PRT

<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 6

Met Val Asn Pro Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr
1 5 10 15

Pro Pro Ala Asp Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn
20 25 30

Lys Ser Ala Glu Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys
35 40 45

Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala
50 55 60

Val Asn Ala Leu Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser
65 70 75 80

Asn Ser Ser Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr
85 90 95

Ala Thr Ala Pro Thr Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr
100 105 110

Gln Ala Gln Thr Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala
115 120 125

Asp Ile Gln Ala Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile
130 135 140

Lys Asp Thr Ala Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp
145 150 155 160

Glu Thr Lys Asn Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu
165 170 175

Leu Ala Lys Tyr Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly
180 185 190

Lys Leu Thr Ser Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val
195 200 205

Ala Asn Asn Asn Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn
210 215 220

Pro Val Val Pro Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp
225 230 235 240

Gln Thr Asp Ala Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile
245 250 255

Arg Asp Ala Tyr Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn
260 265 270

Ala Lys Ser Asn Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala
275 280 285

Ile Ala Thr Ala Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro
290 295 300

Asp Ser Pro Ile Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu
305 310 315 320

Lys Asp Leu Lys Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn
325 330 335

Pro Gly Thr Thr Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly
340 345 350

Ser Ile Arg Val Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala
355 360 365

Ser Ile Leu Met Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr

-continued

370	375	380	
Glu Asn Pro Asp Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala			
385	390	395	400
Arg Ala Ala Lys Ala Ala Gly Asp Asp Ser Ala Ala Ala Ala Leu Ala			
405	410	415	
Asp Ala Gln Lys Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln			
420	425	430	
Gln Gly Ile Leu Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val			
435	440	445	
Ser Ala Gly Val			
450			

```
<210> SEQ ID NO 7
<211> LENGTH: 2238
<212> TYPE: DNA
<213> ORGANISM: Chlamydia pneumoniae
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (766)..(2235)
```

<400> SEQUENCE: 7

atgacaaaaaa aacattatgc ttgggttgc gaaggattc tcaatcgaaa gcctaaacag	120
ttttttgtga aatgttagtgt tgtcgactgg aacacattcg ttccttcaga aacccact	180
acagaaaaag ctgctacaaa cgctatgaaa tacaataact gtgttggca gtggctgc	240
ggaaagcata gtcaggttcc ttggatcaat ggacagaaaa agcctctata tctttatgga	300
gcttcttaa tgaaccctt agcaaaggct acgaagacta cgtaaatgg aaaagaaaac	360
ctagcttggt ttattggagg aacttttaggg ggactcagaa aagctggaga ctggctgc	420
acagttacgtt atgagttatgt cgaaggcttgc tcggttccag aatagatgt ttcaggatt	480
ggccgtggta atttattaaa gtttggtcc gcccaagcaa ttgctgttca ctatgtatc	540
aaagaggctt atggttttac aaattataaa ggattttccg ctctatataat gtatggcatc	600
acagattctc tatcattcag agctttaggg gcttactcata aaccagcaaa cgataaactc	660
ggcagtgatt ttactttccg aaagtttgcgat ctaggtataa ttcaagcggtt ttaagtcaaa	720
tttttataaa atctttaaa acaggctcgc attaattttt agtgagagct ttttttttat	777
tttttataat aaaactaaaa gatttttattt attttttgat tttttt atg gtt aat cct	825
Met Val Asn Pro	
1	
att ggt cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gca gat	873
Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp	
5 10 15 20	
ctt tct gct caa gga ttg gag gcg agt gca gca aat aag agt gcg gaa	921
Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu	
25 30 35	
gct caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc	969
Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr	
40 45 50	
gat tct gta gag cga tgg agc atc ttg cgt tct gca gtg aat gct ctc	1017
Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu	
55 60 65	
atg agt ctg gca gat aag ctg ggt att gct tct agt aac agc tcg tct	
Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser	
70 75 80	

-continued

tct act agc aga tct gca gac gtg gac tca acg aca gcg acc gca cct	1065
Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr Ala Thr Ala Pro	
85 90 95 100	
acg cct cct cca ccc acg ttt gat gat tat aag act caa gcg caa aca	1113
Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr	
105 110 115	
gct tac gat act atc ttt acc tca aca tca cta gct gac ata cag gct	1161
Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala	
120 125 130	
gct ttg gtg agc ctc cag gat gct gtc act aat ata aag gat aca gcg	1209
Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala	
135 140 145	
gct act gat gag gaa acc gca atc gct gcg gag tgg gaa act aag aat	1257
Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn	
150 155 160	
gcc gat gca gtt aaa gtt ggc gcg caa att aca gaa tta gcg aaa tat	1305
Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr	
165 170 175 180	
gct tcg gat aac caa gcg att ctt gac tct tta ggt aaa ctg act tcc	1353
Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser	
185 190 195	
ttc gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac	1401
Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn	
200 205 210	
aaa gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca	1449
Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro	
215 220 225	
ggg aaa acg cct gca att gct caa tct tta gtt gat cag aca gat gct	1497
Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala	
230 235 240	
aca gcg aca cag ata gag aaa gat gga aat gcg att agg gat gca tat	1545
Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr	
245 250 255 260	
ttt gca gga cag aac gct agt gga gct gta gaa aat gct aaa tct aat	1593
Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn	
265 270 275	
aac agt ata agc aac ata gat tca gct aaa gca gca atc gct act gct	1641
Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala	
280 285 290	
aag aca caa ata gct gaa gct cag aaa aag ttc ccc gac tct cca att	1689
Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile	
295 300 305	
ctt caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa	1737
Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu Lys Asp Leu Lys	
310 315 320	
aat atc aaa cct gca gat ggt tct gat gtt cca aat cca gga act aca	1785
Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr	
325 330 335 340	
gtt gga ggc tcc aag caa caa gga agt agt att ggt agt att cgt gtt	1833
Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly Ser Ile Arg Val	
345 350 355	
tcc atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg	1881
Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met	
360 365 370	
tct ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat	1929
Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp	
375 380 385	

-continued

tct caa gct gcc caa cag gag ctc gca gca caa gct aga gca gca gaa Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys 390 395 400	1977
gcc gct gga gat gac agt gct gca gca gct gtc gca gat gct cag aaa Ala Ala Gly Asp Asp Ser Ala Ala Ala Ala Leu Ala Asp Ala Gln Lys 405 410 415 420	2025
gct tta gaa gcg gct cta ggt aaa gct ggg caa caa cag ggc ata ctc Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln Gln Gly Ile Leu 425 430 435	2073
aat gct tta gga cag atc gct tct gct gct gtt gtc agc gca gga gta Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val 440 445 450	2121
ctc ccg ctg cag caa gtt cta tgg atc cga gct cgg tac caa gct tac Leu Pro Leu Gln Gln Val Leu Trp Ile Arg Ala Arg Tyr Gln Ala Tyr 455 460 465	2169
gta gaa caa aaa ctc atc tca gaa gag gat ctg aat agc gcc gtc gac Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp 470 475 480	2217
cat cat cat cat cat cat tga His His His His His His 485 490	2238

<210> SEQ ID NO: 8

<211> LENGTH: 490

<212> TYPE: PRT

<213> ORGANISM: Chlamydia pneumoniae

<400> SEQUENCE: 8

Met Val Asn Pro Ile Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr 1 5 10 15
Pro Pro Ala Asp Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn 20 25 30
Lys Ser Ala Glu Ala Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys 35 40 45
Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala 50 55 60
Val Asn Ala Leu Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser 65 70 75 80
Asn Ser Ser Ser Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Thr 85 90 95
Ala Thr Ala Pro Thr Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr 100 105 110
Gln Ala Gln Thr Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala 115 120 125
Asp Ile Gln Ala Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile 130 135 140
Lys Asp Thr Ala Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp 145 150 155 160
Glu Thr Lys Asn Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu 165 170 175
Leu Ala Lys Tyr Ala Ser Asp Asn Gln Ala Ile Leu Asp Ser Leu Gly 180 185 190
Lys Leu Thr Ser Phe Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val 195 200 205
Ala Asn Asn Asn Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn

-continued

210	215	220
Pro Val Val Pro Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp		
225	230	235
Gln Thr Asp Ala Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile		
245	250	255
Arg Asp Ala Tyr Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn		
260	265	270
Ala Lys Ser Asn Asn Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala		
275	280	285
Ile Ala Thr Ala Lys Thr Gln Ile Ala Glu Ala Gln Lys Lys Phe Pro		
290	295	300
Asp Ser Pro Ile Leu Gln Glu Ala Glu Gln Met Val Ile Gln Ala Glu		
305	310	315
Lys Asp Leu Lys Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn		
325	330	335
Pro Gly Thr Thr Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Ile Gly		
340	345	350
Ser Ile Arg Val Ser Met Leu Leu Asp Asp Ala Glu Asn Glu Thr Ala		
355	360	365
Ser Ile Leu Met Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr		
370	375	380
Glu Asn Pro Asp Ser Gln Ala Ala Gln Gln Glu Leu Ala Ala Gln Ala		
385	390	395
Arg Ala Ala Lys Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala		
405	410	415
Asp Ala Gln Lys Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln		
420	425	430
Gln Gly Ile Leu Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val		
435	440	445
Ser Ala Gly Val Leu Pro Leu Gln Gln Val Leu Trp Ile Arg Ala Arg		
450	455	460
Tyr Gln Ala Tyr Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn		
465	470	475
Ser Ala Val Asp His His His His His		
485	490	

<210> SEQ ID NO 9
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 9

ataagaatgc ggccgccacc atggtaatc ctattggtcc agg 43

<210> SEQ ID NO 10
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 10

gcgccccatc ccttggatc aaccagaata tagag 35

-continued

```

<210> SEQ ID NO 11
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 11

```

ataagaatgc ggccggcacc atgagtcgg cagataagct ggg 43

```

<210> SEQ ID NO 12
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 12

```

gcgcggatc ccttggat aaccagaata ta 32

```

<210> SEQ ID NO 13
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 13

```

gctctagacc gccatgacaa aaaaacatta tgcttggg 38

```

<210> SEQ ID NO 14
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 14

```

cgggatccat agaacttgcg gcagcggg 28

1. A nucleic acid molecule comprising a nucleic acid sequence which encodes a polypeptide selected from any of:

- (a) SEQ ID No: 2;
- (b) SEQ ID No. 4;
- (c) SEQ ID No. 6;
- (d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and
- (e) a polypeptide of any one of (a) to (d) which has been modified to improve its immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d).

2. A nucleic acid molecule comprising a nucleic acid sequence selected from any of:

- (a) SEQ ID No: 1;
- (b) SEQ ID No: 3;
- (c) SEQ ID No: 5;

(d) a sequence which encodes a polypeptide encoded by any one of SEQ ID Nos: 1, 3 and 5;

(e) a sequence comprising at least 38 consecutive nucleotides from any one of the nucleic acid sequences of (a) to (d); and

(f) a sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptides encoded by any one of SEQ ID Nos: 1, 3 and 5.

3. A nucleic acid molecule comprising a nucleic acid sequence which is anti-sense to the nucleic acid molecule of claim 1.

4. A nucleic acid molecule comprising a nucleic acid sequence which encodes a fusion protein, said fusion protein comprising a polypeptide encoded by a nucleic acid molecule according to claim 1 and an additional polypeptide.

5. The nucleic acid molecule of claim 4 wherein the additional polypeptide is a heterologous signal peptide.

6. The nucleic acid molecule of claim 4 wherein the additional polypeptide has adjuvant activity.

7. The nucleic acid molecule according to claim 1, operatively linked to one or more expression control sequences.

8. A vaccine comprising at least one first nucleic acid according to claim 1, and a vaccine vector wherein each first nucleic acid is expressed as a polypeptide, the vaccine optionally comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by said first nucleic acid.

9. The vaccine of claim 8 wherein the second nucleic acid encodes an additional Chlamydia polypeptide.

10. A pharmaceutical composition comprising a nucleic acid according to claim 1 and a pharmaceutically acceptable carrier.

11. A pharmaceutical composition comprising a vaccine according to claim 8 and a pharmaceutically acceptable carrier.

12. A unicellular host transformed with the nucleic acid molecule of claim 7.

13. A nucleic acid probe of 5 to 100 nucleotides which hybridizes under stringent conditions to the nucleic acid molecule of SEQ ID No: 1, or to a homolog or complementary or anti-sense sequence of said nucleic acid molecule.

14. A primer of 10 to 40 nucleotides which hybridizes under stringent conditions to the nucleic acid molecules of SEQ ID No: 1, or to a homolog or complementary or anti-sense sequence of said nucleic acid molecule.

15. A polypeptide comprising an amino acid sequence selected from any of:

- (a) SEQ ID No: 2;
- (b) SEQ ID No: 4;
- (c) SEQ ID No: 6;
- (d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and
- (e) a polypeptide of any one of (a) to (d) which has been modified to improve its immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d).

16. A fusion polypeptide comprising the polypeptide of claim 15 and an additional polypeptide.

17. The fusion polypeptide of claim 16 wherein the additional polypeptide is a heterologous signal peptide.

18. The fusion protein of claim 16 wherein the additional polypeptide has adjuvant activity.

19. A method for producing a polypeptide of claim 15, comprising the step of culturing a unicellular host according to claim 12.

20. An antibody against the polypeptide of claim 15.

21. A vaccine comprising at least one first polypeptide according to claim 15 and a pharmaceutically acceptable carrier, optionally comprising a second polypeptide which enhances the immune response to the first polypeptide.

22. The vaccine of claim 21 wherein the second polypeptide comprises an additional Chlamydia polypeptide.

23. A pharmaceutical composition comprising a polypeptide according to claim 15 and a pharmaceutically acceptable carrier.

24. A pharmaceutical composition comprising a vaccine according to claim 21 and a pharmaceutically acceptable carrier.

25. A pharmaceutical composition comprising an antibody according to claim 20 and a pharmaceutically acceptable carrier.

26. A method for preventing or treating Chlamydia infection using the nucleic acid of claim 1.

27. A method for preventing or treating Chlamydia infection using the vaccine of claim 8.

28. A method for preventing or treating Chlamydia infection using the pharmaceutical composition of claim 10.

29. A method for preventing or treating Chlamydia infection using the polypeptide of claim 15.

30. A method for preventing or treating Chlamydia infection using the antibody of claim 20.

31. A method of detecting Chlamydia infection comprising the step of assaying a body fluid of a mammal to be tested with the nucleic acid of claim 1.

32. A method of detecting Chlamydia infection comprising the step of assaying a body fluid of a mammal to be tested with the polypeptide of claim 15.

33. A method of detecting Chlamydia infection comprising the step of assaying a body fluid of a mammal to be tested with the antibody of claim 20.

34. A method for identifying the polypeptide of claim 15 which induces an immune response effective to prevent or lessen the severity of Chlamydia infection in a mammal previously immunized with polypeptide, comprising the steps of:

- (a) immunizing a mouse with the polypeptide; and
- (b) inoculating the immunized mouse with Chlamydia; wherein the polypeptide which prevents or lessens the severity of Chlamydia infection in the immunized mouse compared to a non-immunized control mouse is identified.

35. An expression plasmid selected from the group consisting of pCACP NM555a, pCAI555 and pCAD76kDa.

36. A nucleic acid molecule selected from the group consisting of SEQ ID Nos: 1, 3, 5 and 7.

37. A polypeptide selected from the group consisting of SEQ ID Nos: 2, 4, 6 and 8.

38. An isolated 76 kDa protein from Chlamydia.

* * * * *