

Isolation of recombinant fragments of the major outer-membrane protein of *Chlamydia trachomatis*: their potential as subunit vaccines

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Recombinant fragments of the major outer-membrane protein (MOMP) of *Chlamydia trachomatis*, expressed at high levels in *Escherichia coli*, were isolated and purified. Antisera to the recombinant proteins reacted preferentially with overlapping synthetic peptides covering the immunologically accessible variable segments of MOMP. These sera also reacted in a species-specific manner with the surface of intact infectious elementary bodies, and in a *Chlamydia* genus-specific manner in assays using denatured or bound chlamydial antigens. The ability of recombinant MOMP preparations to elicit antibody to the surface of chlamydial elementary bodies raises the possibility that these proteins may be useful for chlamydial vaccine development.

Introduction

Chlamydiae are obligate intracellular bacterial pathogens. In developing countries *Chlamydia trachomatis* serovars A, B and C are a major infectious cause of preventable blindness (trachoma). Worldwide, serovars D to K are an important cause of sexually transmitted disease and associated infertility (Robertson & Ward, 1988). Early trachoma vaccine trials using whole chlamydiae were partially successful in that short-term serovar-specific protection was achieved, but disease of enhanced severity was reported in some individuals reinfected with heterologous serovars (reviewed in Schachter & Dawson, 1978). In the ensuing search for a subunit vaccine which excludes these complications much attention has been focused on the major outer-membrane protein (MOMP) of *C. trachomatis*.

MOMP represents up to 60% of the surface protein of the infectious elementary body (EB) and the intracellularly replicative reticulate body (RB; Caldwell *et al.*, 1981). MOMP carries serovar-, subspecies-, species and genus-specific epitopes (Terho *et al.*, 1982; Batteiger *et al.*, 1986; Conlan *et al.*, 1988). Antibodies reactive with these epitopes form the basis for the serological classification of *C. trachomatis* (Wang & Grayston, 1982). The location of these epitopes has been deter-

mined using monoclonal antibodies (mAbs) in conjunction with expressed recombinant DNA (Baehr *et al.*, 1988) or synthetic peptide (Conlan *et al.*, 1988; Stephens *et al.*, 1988) probes. The gene and inferred amino acid sequences of MOMP for all *C. trachomatis* serovars have been either wholly or partially determined (Pickett *et al.*, 1987; Stephens *et al.*, 1987; Baehr *et al.*, 1988; Yuan *et al.*, 1989). The MOMP sequence for *C. psittaci* ewe abortion agent has also been determined (Pickett *et al.*, 1988a). Comparisons of these sequences has provided a molecular rationale for the serological diversity of chlamydiae.

mAbs (Lucero & Kuo, 1985; Zhang *et al.*, 1987) to the surface-exposed serovar- and subspecies-specific epitopes of MOMP neutralize chlamydial infection. Species-specific antibody to MOMP generally fails to neutralize chlamydiae (Zhang *et al.*, 1987) because the corresponding epitope is less well exposed (Kuo & Chi, 1987). However, Collett *et al.* (1989) recently described a species-specific mAb which reacted with a surface-exposed epitope of MOMP. This raises the possibility that a single component vaccine based on MOMP might be effective against all serovars of *C. trachomatis*.

Using recombinant DNA techniques, Pickett *et al.* (1988b) achieved high-level expression of recombinant serovar L1 MOMP (rMOMP) fragments in *Escherichia coli*. The present paper describes: (i) the facile purification of these recombinant protein fragments; (ii) the ability of these antigens to generate antibodies which

Abbreviations: EB, elementary body; mAb, monoclonal antibody; (r)MOMP, (recombinant) major outer-membrane protein; RB, reticulate body; TEM, transmission electron microscopy.

react at the surface of viable chlamydiae in a broad species-specific manner.

Methods

Organisms. The production of stable recombinants of *E. coli* strain K12-JM109 expressing approximately $\frac{3}{4}$, $\frac{1}{2}$ or $\frac{1}{4}$ -length fragments of *C. trachomatis* serovar L1 MOMP as insoluble cytoplasmic inclusions has been described previously (Pickett *et al.*, 1988b). *C. trachomatis* serovar A (strain SA1), serovar B (strain B/Jali20/OT), serovar C (strain UW1), serovar D (strain 1883), serovar F (a recent clinical isolate), serovar G (strain IOL 238), serovar L1 (strain L1/440/LN) and *C. psittaci* (strain EAE A22/M) were grown and purified as previously described (Salari & Ward, 1981).

Isolation of rMOMP. Stocks of recombinant bacteria were stored in liquid nitrogen. Thawed stock cultures were revived on Luria agar (16 h at 37 °C) containing ampicillin (50 mg l⁻¹) and IPTG (0.1 mM) as inducer. These cultures were used to inoculate 100 ml volumes of Luria broth (as above but with the agar omitted) which were incubated aerobically at 37 °C for 16 h in 500 ml conical flasks on an orbital shaker. Cells were harvested by centrifugation and treated with lysozyme-EDTA (Osborn & Munson, 1974). The resulting spheroplasts were lysed by sonication (MSE Soniprep, 3 × 15 s at maximum amplitude) and the insoluble rMOMP inclusions were harvested by centrifugation (12000 r.p.m. for 5 min, microcentrifuge) and washed twice in 20 mM-Tris/HCl, pH 7.0. Few intact cells remained as determined by interference microscopy. The washed pellet was treated with RNAase and DNAase for 1 h at 37 °C in the presence of MgCl₂ (50 mM), washed again, then solubilized at 50 °C for 30 min in 20 mM-Tris/HCl, pH 7.0 containing 5% (w/v) SDS. β -Mercaptoethanol was added to 5% (v/v), the mixture heated to 100 °C for 5 min, cooled and applied to a Sephadex G50 column (1.8 × 28 cm). The column was eluted with 50 mM-Tris/HCl, pH 7.0, containing 10 mM-EDTA, 1 mM-phenylmethylsulphonyl fluoride (PMSF), 0.1% (w/v) SDS and 0.05% (w/v) sodium azide at a flow rate of 22 ml h⁻¹ and 5 ml fractions were collected. Fractions were analysed individually by dot immunoblot using a mAb specific for *C. trachomatis* MOMP. Protein content of the fractions was determined with the Bradford reagent (Bio-Rad). The yield of rMOMP was 10–30 mg (protein) per litre of culture.

Antisera. For antibody production pooled fractions from the Sephadex column were dialysed against distilled water, the resulting pellet resuspended in PBS and emulsified with incomplete Freund's adjuvant (IFA). Alternatively, the pooled fractions were adsorbed directly to alhydrogel without prior dialysis and washed twice in phosphate-buffered saline (Dulbecco A). Half-lop rabbits were prebled then immunized at two intramuscular and two subcutaneous sites with 200 μ g protein in either Freund's adjuvant or alhydrogel on days 1, 28, 63, 114 and serum obtained on day 140. Rabbit antiserum against *E. coli* strain JM109 was kindly provided by Dr John Heckels of this department. This was produced by immunization with 100 μ g (as protein) antigen at multiple subcutaneous sites on days 1, 14, 28, 45 and 80; primary immunization was in complete Freund's adjuvant and all booster injections were in IFA. Antiserum was obtained on day 92. mAbs 6E and 2.3 were produced in Balb/c mice by standard techniques (Terho *et al.*, 1982) using purified *C. trachomatis* serovar L1 organisms as antigen.

ELISA for antibody. Antisera were titrated by an indirect ELISA in 96-well PVC microtitre trays (high activated, Flow Laboratories) using a published method (Robertson *et al.*, 1987). For the detection of antibodies against chlamydiae and *E. coli*, the wells were coated overnight at room temperature with whole organisms (5 μ g protein ml⁻¹, 100 μ l per well) in 0.05 M-carbonate buffer, pH 9.6, containing

0.01% (w/v) sodium azide. For the detection of antibodies to rMOMP, washed inclusions were solubilized in 8 M-urea and diluted to 1 μ g ml⁻¹ in coating buffer immediately prior to use. The test sera were titrated through a threefold dilution series in assays with rMOMP fragments or *C. trachomatis* and through a twofold series in assays with *E. coli*. End-point titres were defined as the last well to give a positive reaction compared to controls.

Immunoblotting. Immunoblotting of chlamydia was done as previously described (Conlan *et al.*, 1989b). Primary rabbit antisera were used at 1:200 dilution; mAbs were used at 1:1000 dilution and were detected with goat anti-mouse IgG (H + L), alkaline phosphatase conjugate (Bio-Rad, 1:3000).

Immunogold-labelling. Surface-exposed epitopes of MOMP on intact chlamydiae were detected by transmission electron microscopy (TEM) using immunogold as previously described (Conlan *et al.*, 1989b). Test antisera were used at dilutions of 1:10 and 1:50; the protein G-gold conjugate was used at 1:10.

Epitope mapping. Solid-phase peptides for epitope mapping were synthesized according to a published method (Geysen *et al.*, 1984) using a commercial kit (Cambridge Research Biochemicals). Synthesis was done according to manufacturer's instructions and as previously described (Conlan *et al.*, 1988). A series of duplicate decapeptides spanning the entire serovar L1 MOMP sequence (Pickett *et al.*, 1987) was synthesized. Each peptide overlapped the preceding decapeptide by five amino acids.

Results

Analysis of rMOMP inclusions

Cytoplasmically located, insoluble rMOMP inclusions were isolated, solubilized and analysed by SDS-PAGE (Fig. 1). Major proteins with apparent molecular masses of 13.5 and 32.7, 28.2 and 13.2 kDa were obtained from the *E. coli* recombinants expressing respectively $\frac{3}{4}$, $\frac{1}{2}$ or $\frac{1}{4}$ -length fragments of serovar L1 MOMP (molecular mass approx. 40 kDa). All samples contained numerous faint minor bands, several of which reacted on Western blots with a mAb specific for *C. trachomatis* MOMP (Fig. 2). These were not removed by gel filtration on Sephadex G50. This mAb did not react with the 13.5 kDa band of the $\frac{3}{4}$ -rMOMP preparation nor with normal *E. coli* (not shown). The identity of this 13.5 kDa band, which was not present in the other recombinant samples, is unknown.

Production of antisera against rMOMP fragments

Immunization with recombinant proteins in both alhydrogel and Freund's adjuvants produced high-titre antibodies reactive with all MOMP fragments on ELISA (Table 1). All of the antisera reacted in a genus-specific manner when whole chlamydiae were used as ELISA antigen. Pre-immune sera were negative for both assays. Antiserum raised against *E. coli* strain JM109 reacted with all rMOMP preparations, presumably due to the

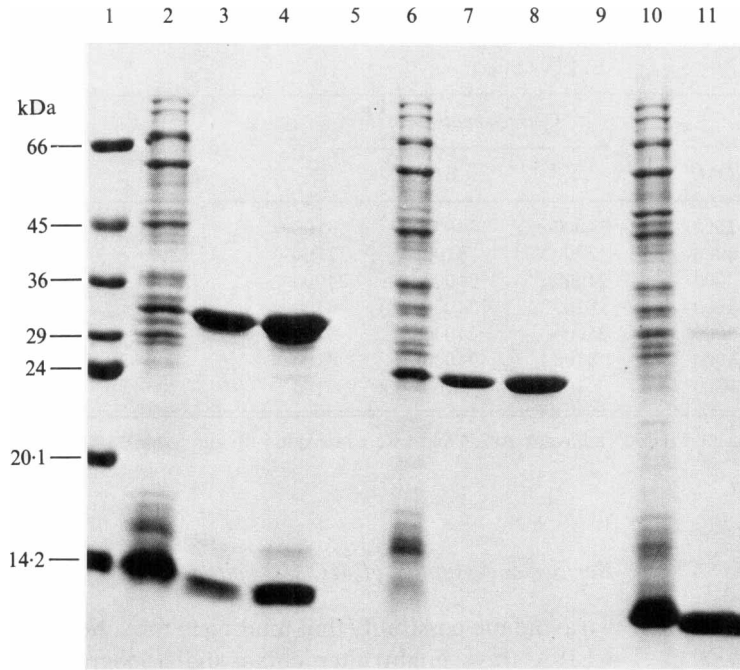


Fig. 1. SDS-PAGE analysis of rMOMP fragments. Lanes: 1, molecular mass markers; 2, *E. coli* expressing $\frac{1}{4}$ rMOMP; 3, isolated $\frac{1}{4}$ rMOMP inclusions; 4, $\frac{1}{4}$ rMOMP from Sephadex G50; 5, blank; 6, *E. coli* expressing $\frac{1}{2}$ rMOMP; 7, $\frac{1}{2}$ rMOMP inclusions; 8, $\frac{1}{2}$ rMOMP from Sephadex G50; 9, blank; 10, *E. coli* expressing $\frac{3}{4}$ rMOMP; 11, $\frac{3}{4}$ rMOMP inclusions.

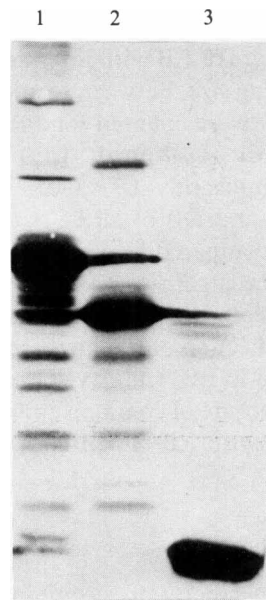


Fig. 2. Western blot of rMOMP fragments. Lanes: 1, $\frac{1}{4}$ rMOMP purified inclusions; 2, $\frac{1}{2}$ rMOMP purified inclusions; 3, $\frac{3}{4}$ rMOMP purified inclusions. Lanes 1–3 probed with mAb reactive with a species-specific epitope on *C. trachomatis* MOMP.

presence of *E. coli* contaminants, since the same serum did not react with whole chlamydial antigens. Likewise rMOMP antisera reacted with whole *E. coli* antigens. This reactivity was abrogated by adsorption of the rMOMP antisera with whole *E. coli* cells. This procedure did not affect the titre of the rMOMP antisera with whole *C. trachomatis* antigens. Apparent variations in

reactivity of these sera with different antigen preparations were noted. In particular, the lower reactivity of antisera 2 and 4 with rMOMP fragments was not matched by an equal decrease in reactivity to whole *C. trachomatis* antigens. Whether this is a reproducible phenomenon reflecting differences in antigen presentation or is a result of variability in the immune response of individual animals is unknown.

Reactivity of antibodies to rMOMP with chlamydiae

The reactivity of antisera to rMOMP with reduced and denatured antigens of chlamydiae on Western blots was exemplified by As1 (Fig. 3, lanes 1–4). This antiserum reacted with multiple bands from *C. trachomatis* serovars L1, B (B-complex), C (C-complex) but with far fewer bands for *C. psittaci*; the positions of the 'native' MOMP bands are indicated. One explanation for this might be the presence of cross-reactive epitopes on multiple chlamydial antigens. All antisera reacted in an identical manner. The most significant difference between the antisera was a marked decrease in the staining intensity of *C. psittaci* bands with antisera raised using alhydrogel as adjuvant. mAbs which were specific for MOMP reacted only with a single band on Western blot (Fig. 3, lane 5). The position of chlamydial LPS was determined using a *Chlamydia* genus-specific mAb (lane 6). All of the sera also reacted with *E. coli* strain JM109 on Western blots (Fig. 3, lanes 8 and 9). As1, 2 and 5 gave similar banding patterns with *E. coli* whilst the different blotting pattern of As6 was similar to that obtained with As3 and

Table 1. Reactivity of antisera (ELISA titre) with rMOMP fragments and whole chlamydiae

Antiserum*	ELISA antigen							
	Recombinant fragments			<i>C. trachomatis</i> serovars:			<i>C. psittaci</i> (EAE)	<i>E. coli</i> (JM109)
	$\frac{3}{4}$ -MOMP	$\frac{1}{2}$ -MOMP	$\frac{1}{4}$ -MOMP	L1	B	C		
As1	437000	437000	437000	24000	24000	8100	8100	4000
As2	16000	16000	16000	2700	8100	2700	300	12000
As3	437000	437000	437000	24000	24000	24000	72000	4000
As4	48000	48000	48000	8100	24000	8100	900	4000
As5	437000	437000	146000	24000	24000	8100	2700	4000
As6	146000	146000	146000	8100	24000	8100	900	4000
<i>E. coli</i> antiserum	1800	5400	1800	<100	<100	<100	<100	36000

* As1, 3 and 5 were raised to $\frac{3}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$ -rMOMP respectively using Freund's adjuvant. As2, 4 and 6 were raised to $\frac{3}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$ -rMOMP respectively using alhydrogel as adjuvant.

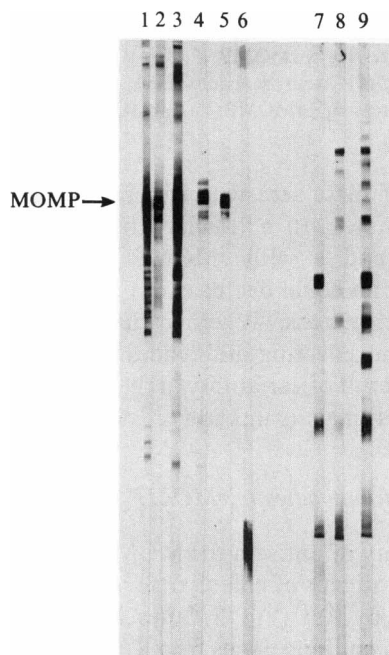


Fig. 3. Western blots of whole *C. trachomatis* with antisera against rMOMP. Antigens were: lanes 1–3, *C. trachomatis* serovars L1, B and C, respectively; lane 4, *C. psittaci*; lanes 5 and 6, *C. trachomatis* serovar C; lanes 7–9, *E. coli* strain JM109. Lanes 1–4 were probed with antisera to rMOMP; lane 5 with *C. trachomatis* MOMP-specific mAb; lane 6 with chlamydia LPS-specific mAb; lane 7 with anti-JM109 antiserum; lanes 8 and 9 with antiserum to rMOMP.

4. When As1 and 4 were adsorbed with whole *E. coli* cells their reactivity to *E. coli* was abolished but the pattern of reactivity observed against chlamydiae was identical to blots using unadsorbed sera (not shown). Antiserum raised against *E. coli* did not react with chlamydiae on Western blots.

Surface accessibility of MOMP epitopes

To avoid the possibility that binding to the solid phase of ELISA trays might alter chlamydial topography, the surface accessibility of MOMP epitopes was determined by immunogold-labelling of intact chlamydiae in suspension prior to TEM. The purified chlamydial preparations used were a mixture of EB with intermediate bodies and some RB of varying size. Between 15 and 30 organisms of each morphology were counted for each antiserum and chlamydial serovar examined. In general, the test sera reacted significantly ($P < 0.05$, *t*-test of sample means) with EB and RB of all *C. trachomatis* serovars examined when compared to pre-immune sera or to the antiserum produced against *E. coli* (Table 2). In all cases RB showed a greater degree of labelling than did EB. This may be a reflection of the increased surface area of the RB compared to EB, and developmental differences in the surface exposure of common epitopes. None of the test sera reacted with EB or RB of *C. psittaci*.

Epitope mapping

The molecular specificity of recombinant antisera was examined against a series of solid-phase peptides spanning the entire serovar L1 MOMP sequence (Fig. 4). None of the test sera reacted with peptides corresponding to the N-terminal- $\frac{1}{4}$ of L1 MOMP which is not present in the recombinant antigens (not shown). Antisera raised using Freund's adjuvant showed a wider spectrum of reactivity against peptides than did sera which employed alhydrogel as adjuvant. Pre-immune sera and antiserum to *E. coli* were negative in these assays. All antisera, with the exception of As5, reacted only with peptides which occur naturally in the respective immunizing MOMP fragments. We speculate that

Table 2. Labelling of intact chlamydiae with rMOMP antisera by immunogold TEM

Figures represent the mean number of gold particles per organism.

Organism		Antiserum							anti-JM109
		Pre-bleed*	As1	As2	As3	As4	As5	As6	
Serovar A	EB	0.19	ND	ND	ND	ND	11.9	8.2	ND
	RB	0.69	ND	ND	ND	ND	43.2	24.7	ND
Serovar B	EB	0.69	14.9	1.4	14.1	11.7	6.7	11.7	0.04
	RB	0.60	40.6	35.2	38.3	58.7	32.0	41.5	4.7
Serovar C	EB	0.27	4.6	5.9	ND	5.4	3.7	10.4	0.73
	RB	0.31	24.6	23.0	ND	26.9	20.1	34.7	4.1
Serovar D	EB	0.5	ND	ND	ND	ND	10.1	16.6	ND
	RB	1.0	ND	ND	ND	ND	30.2	22.9	ND
Serovar F	EB	0.1	13.6	5.3	10.4	14.3	14.2	25.7	ND
	RB	0.59	25.1	13.5	37.2	34.1	35.1	45.1	ND
Serovar G	EB	ND	ND	ND	ND	ND	11.1	12.2	ND
	RB	ND	ND	ND	ND	ND	33.8	ND	ND
Serovar L1	EB	0.14	15.9	5.5	12.0	17.9	12.1	19.3	0.18
	RB	0	51.3	41.0	89.9	48.2	60.2	49.1	3.4
<i>C. psittaci</i>	EB	0.36	0.6	0.4	1.3	0.5	0.2	0.3	ND
	RB	2.2	3.1	1.5	3.4	6.2	1.8	0.9	ND

ND, Not determined.

* Pre-immune serum from rabbit As6. Essentially similar results were obtained for each serovar on at least two separate occasions.

the VS III peptides recognized by As5 may be molecular mimics (mimotopes) of natural epitopes present elsewhere in the protein and recognized by low stringency antibodies. Antibodies reactive with peptides spanning the variable loop domains of MOMP were predominant.

Discussion

The immunopathological side effects of immunization with whole-cell chlamydial preparations has emphasized the need for a subunit vaccine. One difficulty with this approach, is the large-scale production of distinct, purified antigens from this fastidious intracellular bacterium. For MOMP, one approach has been the identification of continuous B cell epitopes using either λ gt11-expressing fragments or synthetic peptides in conjunction with mAbs of known specificity (Baehr *et al.*, 1988; Conlan *et al.*, 1988; Stephens *et al.*, 1988). This rationale may lead to a synthetic peptide vaccine. Indeed, antibodies raised to synthetic peptide homologues of MOMP, recognize the native structure on EB (Conlan *et al.*, 1989b). However, the presentation of suitably adjuvanted synthetic peptides to the immune system and the probable requirements for additional T cell epitopes as well as carrier molecules remains problematic, especially as regards human usage (Shinnick *et al.*, 1983). In these respects, larger recombinant proteins may be particularly useful in providing their

own T-helper epitopes, thus making them inherently more immunogenic.

The ideal chlamydial vaccine should protect against infection with a wide spectrum of serovars. To date, neutralization studies have shown that antibodies reactive with serovar- and subspecies-specific epitopes exposed at the surface of the EB prevented chlamydial infectivity *in vivo* (Zhang *et al.*, 1987) and *in vitro* (Caldwell & Perry, 1982; Lucero & Kuo, 1985). By contrast, antibodies directed against a hidden species-specific epitope did not abrogate infectivity (Zhang *et al.*, 1987). Whether protection is a result simply of antibody binding to the MOMP surface is not known. The finding that antibody to the surface-accessible, genus-specific LPS failed to neutralize chlamydial infectivity (Zhang *et al.*, 1987) might suggest that binding alone is insufficient for effective neutralization. In *in vitro* studies, MOMP-specific neutralizing antibodies did not prevent the attachment and uptake of EB, but did halt their differentiation to replicating RB (Caldwell & Perry, 1982). This differentiation has been shown to be dependent upon relaxation of the sulphhydryl cross-linkages in EB MOMP (Bavoil *et al.*, 1984; Hatch *et al.*, 1984). Neutralizing antibodies might also function by cross-linking the MOMP porin structure, thus preventing differentiation events. If this is so then antibodies solely directed to linear epitopes may not be protective. Although type-, subspecies- and species-specific MOMP domains consisting of short segments of

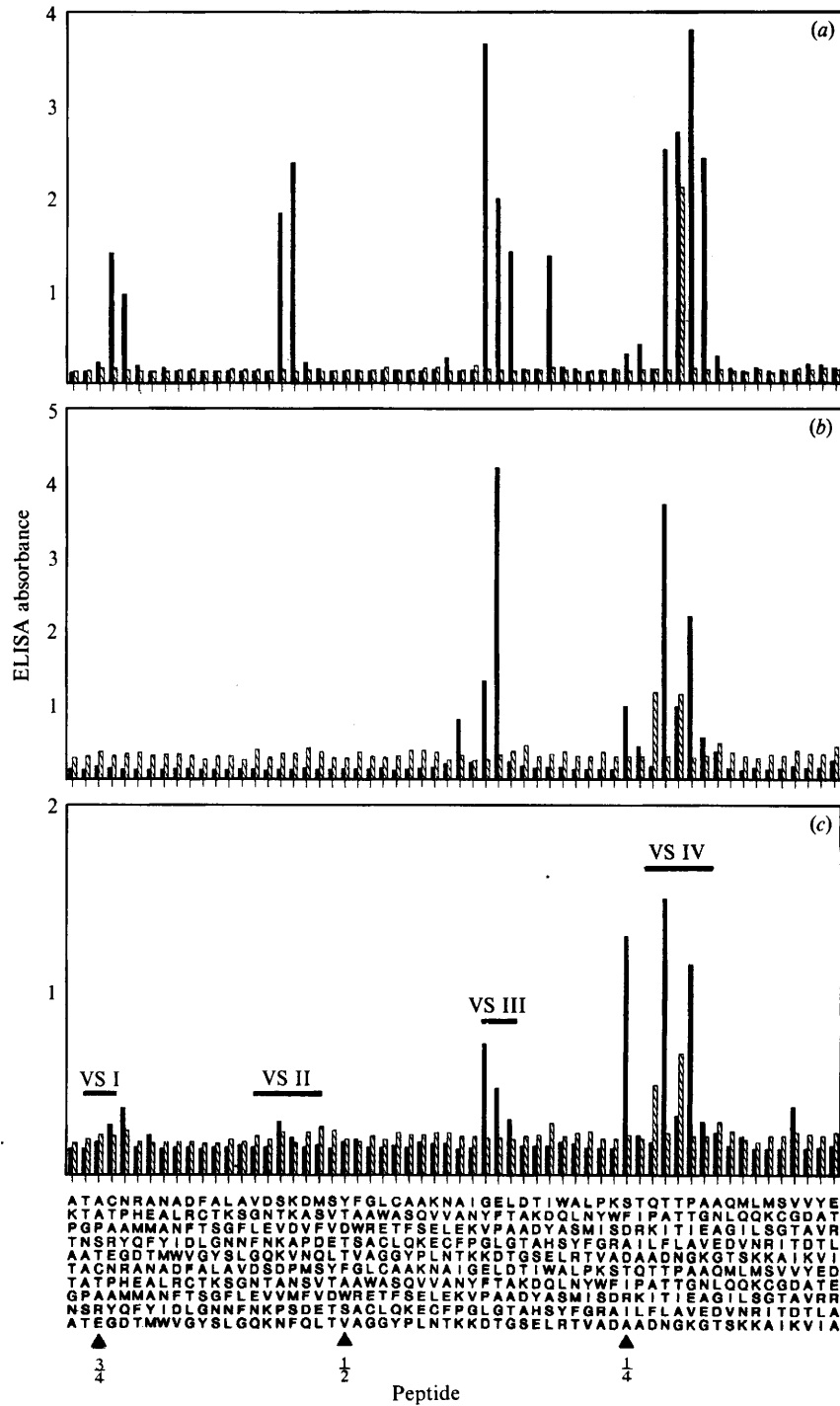


Fig. 4. Reactivity of rMOMP antisera with solid-phase synthetic peptides. As1, 3 and 5 were prepared using Freund's adjuvant. As2, 4 and 6 were prepared using alhydrogel. (a) Reactivity of As1 (■) and As2 (▨); (b) reactivity of As3 (■) and As4 (▨); (c) reactivity of As5 (■) and As6 (▨). The origins of $\frac{3}{4}$ -, $\frac{1}{2}$ - and $\frac{1}{4}$ -MOMP translation are indicated. VS, variable segment.

continuous amino acid sequence have been identified, we have recently obtained evidence that more than one of these loop regions may be spanned by a single mAb paratope (Conlan *et al.*, 1990). Thus, it is likely that these

linear antigenic segments interact to form the paratope contact regions of a discontinuous epitope.

The rMOMP fragments used in the present study were expressed as insoluble inclusions located in the cyto-

plasm (Pickett *et al.*, 1988b). This enabled substantial purification by the simple expedient of spheroplasting followed by lysis and centrifugation. Although immunochemical assays demonstrated the presence of contaminating *E. coli* antigens in the recombinant preparations, these appeared to be minor components, possibly associated with host-cell ribosomes occluded within the rMOMP inclusions. Control experiments showed that antibodies raised against contaminating antigenic material were not responsible for the specific reactivity with chlamydiae of antisera to rMOMP. The multiple bands observed on Western-blotting the rMOMP preparations against a MOMP-specific mAb indicated the presence of MOMP aggregates and processing fragments. These may also occur in chlamydiae themselves, which might explain the multiple bands of *C. trachomatis* antigens reactive with high titre antisera to the rMOMP.

Immunization with all three rMOMP fragments produced *Chlamydia* genus-reactive antibodies by ELISA and Western blotting. However, in immunogold assays, where intact chlamydiae were labelled in suspension, only *C. trachomatis* species-reactive antibodies were detected. This emphasizes the importance of using native bacteria whose topography is unaltered by binding to a solid phase when searching for immunologically accessible surface epitopes. Whether those antibodies which reacted at the EB surface also possessed useful biological functions remains to be determined. It was encouraging, however, that even immunization with the smallest MOMP fragment produced antibodies to known neutralizing epitopes in VS IV, as this recombinant appeared to be the easiest to purify as assessed by SDS-PAGE and Western blots. Moreover, adsorption of recombinant fragments to alhydrogel, a human-approved adjuvant, evoked antibodies to exposed epitopes of MOMP.

Epitope mapping studies with solid-phase peptides were employed in an attempt to explain the serological specificity of recombinant MOMP antisera in molecular terms. The antisera reacted almost exclusively with peptides spanning the variable segments of MOMP, as is the case for neutralizing MOMP mAbs produced by immunization with whole chlamydiae (Zhang *et al.*, 1987). This was surprising given the harsh denaturing conditions to which the MOMP fragments were subjected prior to immunization, but it offers the possibility that rMOMP might be useful for vaccine development. It is possible that MOMP fragments refolded into native conformation following isolation and solubilization. Alternatively, the variable segments may contain epitopes which are immunodominant because they occur in mobile loop regions or because of the functional juxtaposition of strong T-helper epitopes. An examination of the peptides reacting with MOMP antisera did

not provide a ready explanation for the serological reactivity observed in immunochemical assays with whole or denatured organisms. Thus, none of the antisera bound to peptides containing genus-specific sequences, despite their reactivity with denatured *C. psittaci* antigens on Western blot and in ELISA. All of the antisera reacted with peptides from VS IV, even though the species-specific peptide LNPTIAG located in this region is only poorly exposed, if at all, on the EB surface of most serovars (Conlan *et al.*, 1989a; Kuo & Chi, 1987; Zhang *et al.*, 1987). Hence, this reactivity is unlikely to account for the observed species-specific labelling of intact chlamydiae. However, VS IV appears to be immunologically complex, as two surface-exposed, sub-species-specific epitopes flank each side of the species-specific region (Conlan *et al.*, 1988, 1989b).

MOMP is currently the main candidate for the development of a chlamydial vaccine. Monospecific polyclonal antibodies to MOMP, with their high binding affinity, may also be useful for the development of diagnostic tests for chlamydial antigen. The observation reported here that rMOMP preparations in conjunction with human-acceptable alhydrogel adjuvant generate high-titre antibodies, of similar epitope-specificity to mAbs produced by immunization with whole chlamydiae (Conlan *et al.*, 1988), is therefore of major interest. Collaborative studies in progress also indicate that these same rMOMP preparations can be presented, via dendritic cells, to stimulate primary and secondary T cell responses (Dr A. Stagg, personal communication). The ability of rMOMP fragments to stimulate protective immunity *in vivo* is currently being investigated using a mouse model of human *C. trachomatis* infection.

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