Identification and Characterization of T Helper Cell Epitopes of the Major Outer Membrane Protein of *Chlamydia trachomatis*

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Summary

Chlamydia trachomatis serovars A, B, and C are the causative agents of trachoma, the world's leading cause of preventable blindness. Immunoprophylaxis is a possible approach to control trachoma. The chlamydial major outer membrane protein (MOMP) is thought to play an important role in the development of protective immunity against chlamydial infection, and is therefore considered to be a promising candidate antigen in the development of a trachoma vaccine. Much effort has been focused on the molecular characterization of B cell sites of the MOMP that elicit neutralizing antibodies. Neutralizing sites have been identified as linear epitopes that reside within variable domains (VDs) of the protein whose primary sequences vary among different serovars. No information exists on MOMP T helper (Th) cell antigenic determinants, which are likely critical components for the development of a successful chlamydial vaccine. We used overlapping synthetic peptides (25 mers) representing the entire primary sequence of serovar A MOMP in T cell proliferation assays to identify T cell antigenic determinants of this molecule. Eight synthetic peptides (A-2, A-3, A-7, A-8, A-11, A-22, A-23, and A-24) stimulated proliferative responses of splenic T cells isolated from MOMP-immunized A/J mice. To ascertain if these peptides functioned as Th cell antigens, we determined their ability to prime A/J mice in vivo to produce an anamnestic IgG response specific to the MOMP. Mice primed with synthetic peptides A-8 (106–130) or A-23 (331–355) produced IgG antibodies reactive with the native MOMP and with the synthetic peptides corresponding to surface-accessible serovar-specific epitopes located in VD I and serogroup-specific epitopes located in VD IV of the protein. We synthesized the A-8 and A-23 peptides with the VD I sequence as colinear chimeric peptides. Immunization of mice with the T/B cell peptides produced high titered antibodies against the VD I sequence, and these antibodies reacted with the native MOMP and intact chlamydiae. The MOMP sequences containing these Th cell epitopes are conserved among the MOMP genes of different *C. trachomatis* serovars, indicating that they are common Th cell antigenic sites. Thus, the Th cell epitopes contained within these peptides, in combination with different trachoma serovar-specific B cell neutralizing determinants, may be useful in the development of a synthetic or recombinant trivalent trachoma vaccine.

Chlamydia trachomatis serovars A, B, and C are the etiologic agents of trachoma; the world's leading cause of preventable blindness (1). Measures for the prevention or control of blindness due to trachoma are needed, and immunoprophylaxis is considered to be a possible means for accomplishing this goal. Early vaccination attempts in humans using parenterally administered, noninfectious bacteria were ineffective and, in fact, proved to be deleterious in that they prompted a more severe disease that was attributed to vaccine-induced hypersensitivity (2–4). These difficulties, combined with a limited knowledge about chlamydial antigenic structure and host immune responses to chlamydial antigens, impaired further progress toward the development of a trachoma vaccine.

Recently, significant progress in understanding immunity to chlamydial infection has restored both the interest in and the feasibility of the development of a trachoma vaccine. Ocular chlamydial infection in guinea pigs and nonhuman primates induces both protective and deleterious immune responses, and the antigens that elicit these responses are distinct. The deleterious immune response is cell mediated and characterized as a delayed hypersensitivity response (5, 6). The antigen that elicits deleterious delayed hypersensitivity is common

to the genus and has been identified as a 57-kD chlamydial stress-response protein (7, 8). Conversely, protective immunity to ocular infections is largely serovar specific (9–11). Although the immune mechanism(s) that functions in protection is unknown, resistance to infection correlates with the presence of neutralizing serovar-specific IgA antibodies in tears (12, 13) suggesting that secretory antibody plays a critical role in protective immunity. The chlamydial major outer membrane protein (MOMP)[1] is considered to be important in protective immunity since it contains antigenic determinants that elicit serovar-specific neutralizing antibodies (14). Thus, current trachoma vaccine strategies are focused on the development of recombinant or synthetic peptide MOMP vaccines that induce serovar-specific mucosal immunity and circumvent sensitization with chlamydial antigens that evoke deleterious immune responses.

The MOMP genes of trachoma serovars A, B, and C have been sequenced (15, 16). The proteins are highly conserved, being interspersed by four short variable domains (VDs I–IV) whose sequences vary among the different MOMPs. Serovar-specific neutralizing mAbs map to linear epitopes within VDs I of the MOMP of serovars A and C and VD II of the MOMP of serovar B (15, 17). We envision that these serovar-specific MOMP epitopes will ultimately be combined in a synthetic or recombinant product to form a trivalent trachoma vaccine. However, an effective trachoma vaccine will likely rely on natural boosting of immunized individuals by exposure to or reinfection with chlamydiae. This requirement for T cell immunity would not be met by vaccination with peptide antigens consisting only of serovar-specific B cell epitopes or through the use of irrelevant carriers, but will require the incorporation of MOMP-derived Th cell epitopes.

In this report, we identify potentially important Th cell determinants of the MOMP and show that synthetic colinear chimeric peptides composed of MOMP Th and B cell antigenic determinants are immunogenic and stimulate high titered serovar-specific antibodies reactive with both the native MOMP and intact chlamydiae. These findings will be useful for developing a synthetic or recombinant trachoma vaccine.

Materials and Methods

Chlamydiae. *C. trachomatis* serovar A (strain Har-13) was grown in HeLa 229 cells, and elementary bodies (EBs) were purified by centrifugation in discontinuous Renografin gradients (18).

Purification of A MOMP. Serovar A MOMP was purified by preparative SDS-PAGE and electroelution as previously described (19).

Peptide Synthesis. Peptides were synthesized on RapidAmide resin using a RaMPS (DuPont Co., Wilmington, DE) manual synthesizer or by using an automated synthesizer (430; Applied Biosystems, Foster City, CA) according to the directions of the manufacturer. The synthesis used pentafluorophenyl esters of fluorenylmethyloxycarbonyl-L amino acids, except for serine and threonine, where oxobenzotriazine esters were used, and arginine, which was coupled as the symmetric anhydride. Peptides were cleaved from the resin with trifluoroacetic acid, extracted four times with diethyl ether and twice with ethyl acetate/diethyl ether (1.5:1). The peptides were precipitated from ether with water and the water precipitated peptides were lyophilized. Soluble peptides were purified by reverse phase HPLC using a semipreparative C18 ultrasphere column (Beckman Instruments, Inc., Fullerton, CA) and a water/acetonitrile/0.1% trifluoroacetic acid elution gradient. Insoluble peptides were purified by desalting through a Sephadex G-10 column using 50% acetic acid. The composition of peptides was verified by amino acid analysis. The cysteine residue was blocked with a *t*-butyl group in all peptides containing this residue.

Mice. Female A/J (H-2^a), C57BL/10SnJ (H-2^b), BALB/cJ (H-2^d), CBA/J (H-2^k), DBA/1J (H-2^q), and SJL/J (H-2^s) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and used at 8–12 wk of age.

Immunogenicity of MOMP in Strains of Mice Differing at H-2. Groups of five mice were immunized intraperitoneally with 0.2 ml CFA containing 5 μg of purified serovar A MOMP. 3 wk after immunization, mice were bled and sera were assayed by ELISA for IgG antibodies specific to the MOMP and synthetic peptides corresponding to each of the MOMP VDs. The synthetic peptide antigens used were VD I (residues 61–85), VD II (residues 136–160), VD III (residues 226–250), the NH_2-terminal end of VD IV (residues 286–310), and the COOH-terminal end of VD IV (residues 301–325).

Preparation of APC. Peritoneal exudate cells were elicited by intraperitoneal injection with 1.0 ml 2.5% oyster glycogen (Type II; Sigma Chemical Co., St. Louis, MO) in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.2 (PBS). Peritoneal exudate cells were harvested 3–4 d after injection, washed twice in PBS, and irradiated (2,000 rad) in a ^{137}Cs γ irradiator. Irradiated peritoneal exudate cells were washed once and resuspended to 10^7 cells/ml in prewarmed phosphate buffered balanced salt solution, pH 7.2 (PBBS), containing 10% FCS (HyClone Laboratories, Logan, UT). An equal volume of peritoneal exudate cells (10^7 cells/ml) and synthetic peptides (1 mg/ml) were mixed and incubated for 1 h at 37°C. These antigen-pulsed peritoneal exudate cells were washed three times with PBBS and resuspended to a concentration of 10^6 cells/ml in Click's medium (20) supplemented with 5% FCS and 50 μg/ml gentamicin sulfate. Control peritoneal exudate cells were prepared similarly, except cells were mixed with an equal volume of medium instead of antigen.

T Cell Proliferation Assays. A/J mice were immunized intraperitoneally with 0.2 ml of CFA containing 5 μg of MOMP on day 0 and boosted intraperitoneally 3 wk later with 0.2 ml of IFA containing 5 μg of MOMP. 2–3 wk after the booster immunization, mice were killed, splenic T cells isolated, and proliferation assays performed. Nylon wool–passed T cells were prepared (21, 22) from spleens of mice immunized with either serovar A MOMP in CFA or CFA alone. Approximately 5% of the nylon wool–passed cells expressed surface Ig^+, as determined by staining with FITC-conjugated goat anti-mouse Ig serum (CooperBiomedical, Inc., Malvern, PA). The cells were washed twice with PBBS and resuspended to 5×10^6 cells/ml in Click's medium. 100 μl of T cell suspension and 100 μl of APC were placed into wells of 96-well flat-bottomed microtiter plates (Linbro; Flow Laboratories, McLean, VA) and incubated for 5 d at 37°C and 5% CO_2. Cells were pulsed with 1 μCi/well [^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear, Boston, MA) for the final 16 h, then harvested with an automatic cell harvester (Cambridge Technology, Inc., Water-

[1] *Abbreviations used in this paper*: AS, amphipathic scores; EB, elementary body; g.m.t., geometric mean titers; MOMP, chlamydial major outer membrane protein; PBBS, phosphate-buffered balanced salt solution; VD, variable domain.

town, MA), and the incorporation of [^{3}H]thymidine into DNA was measured using a liquid scintillation counter (LS9000; Beckman Instruments, Inc.). All cultures were done in triplicate. Results are presented as Δcpm (cpm of experimental cultures minus cpm of cultures of T cells alone).

In Vivo Priming of Th Cells with Synthetic Peptides. Groups of five A/J mice were immunized intraperitoneally with 0.2 ml of CFA containing either 100 μg of synthetic peptide or PBS alone. 3 wk later, mice were immunized intraperitoneally with 0.2 ml of IFA containing 5 μg of purified MOMP. Mice were bled before immunization with purified MOMP (day 0) and 12 d after challenge immunization. Sera were collected and pooled by groups. The sera were assayed by ELISA for IgG antibodies specific to the MOMP and to synthetic peptides corresponding to sequences contained within the different MOMP VDs.

Immunogenicity of Colinear Th Cell/B Cell Peptides. Groups of five A/J mice were immunized intraperitoneally with 0.2 ml CFA containing 100 μg of colinear peptides (40 mers) corresponding to Th cell and B cell sites or the free peptides alone. Colinear peptides were synthesized to combine Th cell epitope residues 106–130 or 331–355 with B cell epitope 66–80 (corresponding to VD I) and designated as A-8 VD I (^{106}ALNIWDRFDVFCTLGATTGY-LKGNS$^{130}{}_{66}$PTTSDVAGLEKDPVA$_{80}$) and A-23 VD I (^{331}KMK-SRKSCGIAVGTTVVDADKYAVT$^{355}{}_{66}$PTTSDVAGLEKDPVA$_{80}$), respectively. Mice were bled 3 wk later, and their sera were assayed by ELISA for IgG antibody reactive with intact EBs, purified MOMP, and synthetic peptides corresponding to the Th cell and B cell portions of the colinear peptides.

ELISA. Microtiter plates (Immulon 2, 96-well, U-bottomed; Dynatech Laboratories, Inc., Alexandria, VA) were coated with 100 μl of purified A MOMP (0.5 μg/ml), serovar A EBs (10 μg/ml), or synthetic peptides (5 μg/ml) in 0.05 M Tris buffer (pH 7.5) containing 0.15 M NaCl. The microtiter plates were sealed and incubated overnight at 4°C. Wells were emptied and washed three times with PBS containing 0.05% Tween 20 (PBS-Tween) and then incubated with 200 μl blocking buffer (PBS-Tween containing 2% BSA) for 2 h at 37°C. The plates were washed once in PBS-Tween, and then 100 μl of immune or control mouse serum diluted in blocking buffer was added to appropriate wells and incubated for 1.5 h at 37°C. The plates were washed five times with PBS-Tween, and a 1:500 dilution of alkaline phosphatase–conjugated rabbit anti-mouse IgG (γ chain specific; Zymed Laboratories, San Francisco, CA) was added to the wells and the plates incubated for 1 h at 37°C. After washing, 100 μl of substrate (5 mg *p*-nitrophenyl phosphate in 10 ml of 0.1 M 2, 2 amino-2-methyl-1, 3-propandiol, pH 10.3) was added, and the plates incubated for 30 min at 37°C. The enzymatic reaction was terminated by the addition of 50 μl of 5 N NaOH to each well. OD was read at 405 nm. Endpoint titrations were the highest dilutions of sera giving 0.3 OD units at 405 nm.

Sequence Analysis. The primary sequence of A MOMP was analyzed by the AMPHI algorithm developed by Margalit et al. (23) for regions with periodic variation in the hydrophobicity consistent with the formation of an amphipathic α helix. Overlapping blocks of 11 amino acids were used in the analysis, and Fauchere-Pliska (24) hydrophobicity values of amino acids were used in the calculations. Only those segments of the sequence with amphipathic scores (AS) of eight or greater are shown in the MOMP sequence. The computer analysis was kindly performed by Dr. Hanah Margalit, Laboratory of Mathematical Biology and Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD. Structural motifs (charged residues or glycine, followed by two or three hydrophobic residues, and then polar residues or glycine) described by Rothbard and Taylor (25) were identified by visual inspection of the MOMP primary sequence.

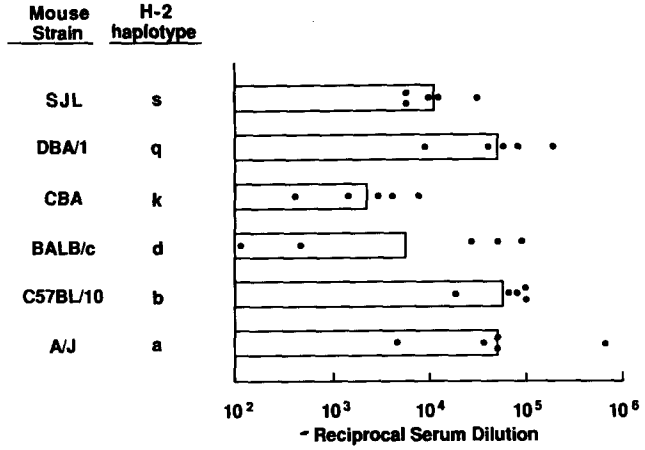

Figure 1. Antibody responses of mice of different H-2 haplotypes immunized with purified serovar A MOMP. Bars represent geometric mean anti-MOMP IgG titers of pooled sera of five mice after a single immunization with purified MOMP. The antibody titers for individual mice are also shown (●). Endpoint titrations were the highest dilutions of sera giving an absorbance reading of 0.3.

Results

Antibody Response to the MOMP in Mice Differing in H-2 Haplotype. Initially, inbred strains of mice were tested for their ability to produce an anti-MOMP antibody response. Six inbred strains of mice of different H-2 haplotype were immunized with purified A MOMP, and the IgG response specific to the MOMP was determined by ELISA (Fig. 1). All strains tested produced IgG geometric mean titers (g.m.t.) of $\log_{10}$ 3.3 or greater after a single immunization, however, certain strains of mice produced higher titered anti-MOMP responses (A/J [g.m.t. = $\log_{10}$ 4.7], C57BL/10 [g.m.t. = $\log_{10}$ 4.8], and DBA/1 [g.m.t. = $\log_{10}$ 4.7]).

The molecular specificity of the anti-MOMP response of different strains of mice was determined by assaying the IgG antibody responses to synthetic peptides corresponding to sequences contained within the four VDs of the MOMP. The molecular specificity of the anti-MOMP response for each mouse strain is shown in Table 1. VD I (residues 61–85) and the NH_2-terminal end of VD IV (residues 286–310) were found to be the immunodominant regions of the MOMP recognized by mouse strains differing in H-2 haplotype. CBA mice responded less intensely to VD I compared with other strains, whereas C57BL/10 and SJL mice had lower IgG antibody titers specific to the NH_2-terminal end of VD IV than other mouse strains.

To determine whether these same regions of MOMP were immunodominant when the protein was presented in its native state, A/J mice were immunized with formalin-fixed intact serovar A EBs, and their sera were analyzed for antibodies reactive with peptides corresponding to the different MOMP VDs. As shown in Table 2, immunization with intact chlamydial EBs produced IgG antibody responses primarily reac-

Table 1. *Molecular Specificity of Anti-MOMP Antibody Responses of Different Inbred Strains of Mice Immunized with Serovar A MOMP*

Strain of mice	H-2 haplotype	ELISA antibody titer				
		VD I p61-85	VD II p136-160	VD III p226-250	N'-VD IV p286-310	C'-VD IV p301-325
A/J	a	2,048	64	64	2,048	64
C57BL/10	b	2,048	16	256	256	<16
BALB/c	d	2,048	<16	64	1,024	128
CBA	k	256	<16	<16	4,096	<16
DBA/1	q	2,048	16	64	1,024	512
SJL	s	512	<16	128	128	<16

ELISA titers are expressed as the reciprocal of pooled serum dilutions giving an absorbance reading of 0.3. Absorbance values of normal mouse sera were 0.1 or less. Synthetic peptides corresponding to sequences of each VD of serovar A MOMP were used as ELISA antigens. Because VD IV contains 30 residues, overlapping peptides p286–310 and p301–325, representing the NH_2- and COOH-terminal portions of the domain, respectively, were assayed separately.

tive with synthetic peptides corresponding to VD I and the NH_2-terminal end of VD IV. Thus, the antibody titer and molecular specificity of the IgG response were similar after immunization with intact EBs or purified MOMP, and the immunogenicity of the contiguous B cell sites located in VD I and IV of the MOMP did not significantly differ when MOMP was presented either in its denatured purified form or native state.

These findings demonstrated that serovar A MOMP VD I and the NH_2-terminal end of VD IV were immunodominant in mouse strains disparate at H-2 suggesting that Th cell determinants of the protein that induce B cell responses to these sites are recognized in association with several MHC haplotypes. Because mouse strains disparate at H-2 were capable of producing anti-MOMP antibodies with similar specificities, we used a single strain (A/J) to further identify MOMP Th cell epitopes.

Screening of Synthetic Peptides in T Cell Proliferation Assays. Our approach was to identify regions of the MOMP containing T cell determinants and then to define these T cell sites functionally. We first examined the T cell–stimulating ability of synthetic peptides corresponding to MOMP sequences. A series of 25 overlapping peptides (25 mers) that represented the entire MOMP sequence was synthesized and tested in T cell proliferation assays. The synthetic peptides were designated A-1 through A-25. The position of the peptides in relation to their location in the primary sequence of serovar A MOMP is shown in Fig. 2. Screening for T cell–proliferative responses was first done using mixtures of synthetic peptides with each group containing equal amounts of three different peptides. T cells from mice primed with MOMP responded to five groups of mixed synthetic peptides: A-(1–3), A-(7–9), A-(9–11), A-(20–22), and A-(23–25) (Fig. 3 *A*). Peptides contained within each of these groups were then tested individually in T cell proliferation assays. MOMP-primed T cells proliferated when cultured with peptides A-2, A-3, A-7, A-8, A-11, A-22, A-23, and A-24 (Fig. 3 *B*). Because peptides A-2 and A-3, A-7 and A-8, and A-22, A-23, and A-24 contain overlapping sequences, it is possible that the T cell–stimulating ability of these peptides resulted from shared T cell epitope(s) among the peptides in each of the three groups.

Table 2. *Molecular Specificity of Anti-MOMP Antibody Responses of A/J Mice Immunized with Serovar A EBs or Serovar A MOMP*

Immunogen	ELISA antibody titer					
	A MOMP	VD I p61-85	VD II p136-160	VD III p226-250	N'-VD IV p286-310	C'-VD IV p301-325
A EBS	2,048	1,024	16	64	1,024	64
A MOMP	8,192	2,048	128	128	1,024	64

ELISA titers are expressed as the reciprocal of pooled serum dilutions giving absorbance of reading of 0.3. Absorbance values of normal mouse sera were 0.1 or less. Groups of five A/J mice were immunized with 1.3×10^7 IFUs of formalin-killed serovar A EBs or 5 μg of serovar A MOMP purified by preparatory SDS-PAGE.

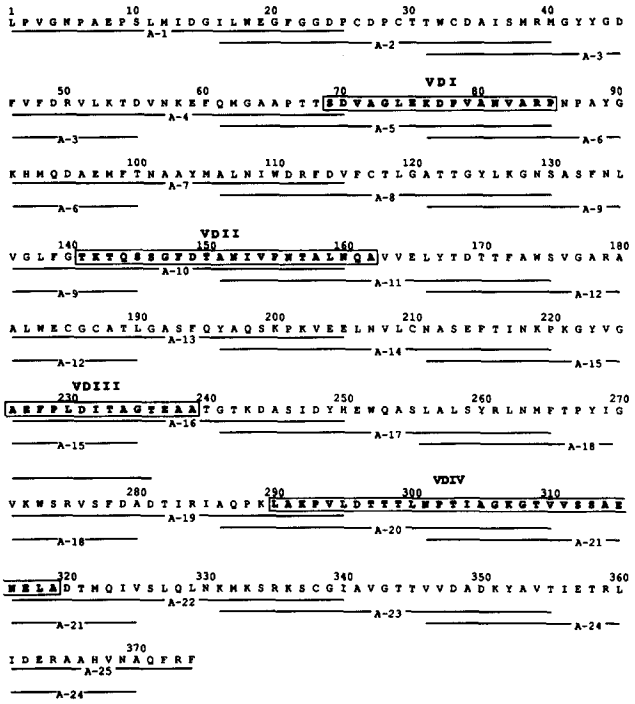

Figure 2. Amino acid sequence of the mature serovar A MOMP and the locations of overlapping synthetic peptides. Synthetic peptides consisted of 25 residues each and overlapped by 10 residues. Peptides were designated A-1 through A-25. The VDs of serovar A MOMP are also identified: VD I (residues 69–85), VD II (residues 141–162), VD III (residues 226–239), and VD IV (residues 290–319). VD I is the location of the serovar A-specific neutralizing site (residues 70–80). Neutralizing serogroup-specific and non-neutralizing species-specific epitopes have been mapped to VD IV.

Ability of Synthetic Peptides to Prime Th Cells In Vivo. We next determined whether the synthetic peptides that stimulated in vitro T cell–proliferative responses were capable of functioning as Th cell epitopes. For those studies, we used an in vivo assay described by Milich et al. (26) for the identification of Hepatitis B virus nucleocapsid Th cell epitopes. A/J mice were immunized with synthetic peptides that stimulated in vitro T cell–proliferative responses (A-2, A-3, A-7, A-8, A-11, A-22, A-23, and A-24), and 3 wk later were boosted with purified MOMP. Mice were bled before boosting with MOMP (day 0) and 12 d after the booster immunization. Sera were pooled by groups and assayed by ELISA for IgG antibody specific to MOMP, synthetic peptides corresponding to sequences contained within VD I–IV, and the homologous immunizing peptide. The rationale of this experimental approach is that memory Th cells are primed by immunizing with the synthetic peptide and are recalled to induce B cells to produce MOMP-specific IgG antibody after booster immunization with the native molecule. Immunological recall by native MOMP indicates that the peptide recognition site is relevant to the native antigen. By assaying IgG responses to defined synthetic peptide sequences of the protein, it is possible to define the specificity of the Th cell response at the molecular level.

The IgG antibody responses of A/J mice primed with synthetic peptides and challenged with purified MOMP are shown in Fig. 4. Mice primed with CFA alone and boosted with the MOMP in IFA did not produce antibody specific to MOMP or to synthetic peptides corresponding to VD sequences. These results demonstrated that immunization with 5 μg of MOMP in IFA was a suboptimal immunizing dose that was incapable of inducing an IgG anti-MOMP response. With the exception of peptide A-3, each of the synthetic peptides tested effectively primed A/J mice to produce IgG antibodies specific to the MOMP. However, only peptides A-7, A-8, and A-23 primed mice to produce significant amounts of IgG antibodies specific to B cell determinants located within VD I and the NH_2-terminal end of VD IV. These same sera did not possess significant levels of antibodies reactive with sequences corresponding to VD II, VD III, or the homologous priming peptide. These results indicated that peptides A-7, A-8, and A-23 contain Th cell epitopes that direct B cell clones to produce antibodies specific to VD I and VD IV. Peptides A-11 and A-22 also primed mice to produce antibodies reactive with VD IV, suggesting that MOMP con-

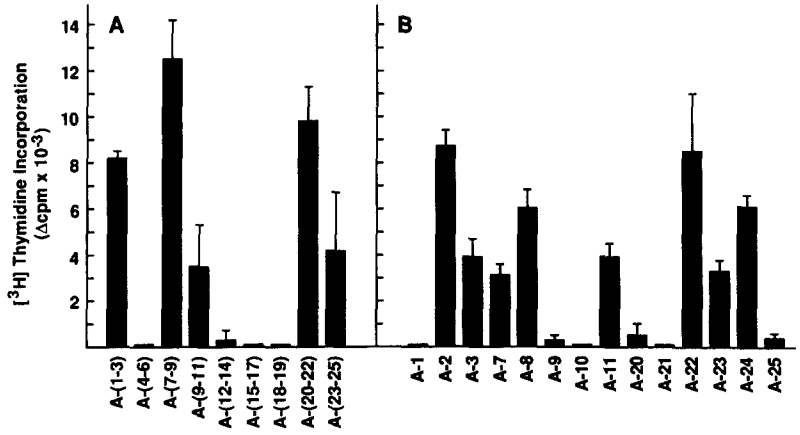

Figure 3. Proliferative responses of MOMP-primed T cells to overlapping synthetic peptides (25 mers) corresponding to the entire serovar A MOMP sequence. (*A*) Proliferative responses to pooled synthetic peptides. (*B*) Proliferative responses to individual peptides. A/J mice were immunized intraperitoneally with either 5 μg of serovar A MOMP in CFA or CFA alone. 3 wk after immunization, splenic T cells were isolated and cultured with either pools of synthetic peptides or individual synthetic peptides. Proliferative responses were measured after 5 d of in vitro culture, as described in Materials and Methods. No antigen backgrounds (media alone) were: *A*, 2,190 cpm; *B*, 4,037 cpm. Proliferative responses to the various peptide antigens of T cells isolated from mice immunized with CFA alone were: *A*, Δcpm <1,000, except A-(7–9), with Δcpm = 1,850; *B*, Δcpm <500, except A-2, with Δcpm = 4,056.

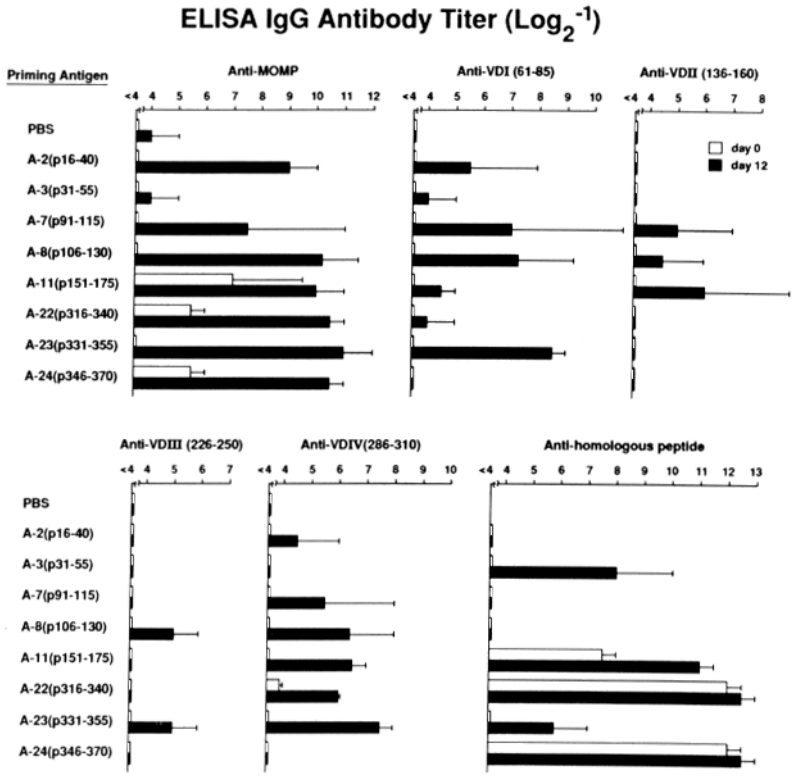

Figure 4. Synthetic peptides containing T cell determinants of MOMP primed mice to produce an augmented anti-MOMP IgG response in vivo. Groups of five A/J mice were primed with synthetic peptides and then challenged with purified MOMP. Mice were bled before booster immunization (day 0) and 12 d after challenge immunization. Sera were collected, pooled, and analyzed by ELISA for IgG antibody specific to MOMP; synthetic peptides corresponding to VD I, II, III, and the NH_2-terminal end of VD IV; and the homologous immunizing peptide. Endpoint titers are expressed as the reciprocal of the $\log_2$ of the highest serum dilution giving an absorbance reading of 0.3. Data are the mean titers of two experiments for all peptides except A-8 and A-23, which were tested three times.

tains multiple Th cell sites capable of directing antibody responses to this domain. Considerable experimental variation was observed in the antibody responses specific to MOMP in mice primed with peptide A-7. The reason for this experimental variation is unknown, however, peptide A-7 may contain a partial Th cell determinant (see Discussion) that could, in part, account for these findings.

Peptides A-11, A-22, and A-24 appeared to contain both Th and B cell determinants since mice immunized with these peptides produced high titered antibodies to the homologous immunizing peptide before immunization with the native MOMP. The B cell determinants associated with these peptides are not likely relevant to chlamydial vaccine development since they are not known to be surface accessible on intact serovar A EBs. Likewise, it is unlikely that the Th cell determinants present in these peptides would be useful components of a chlamydial vaccine since they do not provide Th cell function for antigenic sites located in VD I or IV. Sera from mice primed with peptide A-2 contained antibodies specific to MOMP, however, these antibodies reacted only poorly with VD I and IV sequences and did not recognize the homologous immunizing peptide suggesting that the antibody was specific to B cell sites located outside the MOMP VDs or reacts with structurally dependent VD determinants that are not present in the short synthetic peptide antigens.

Collectively, the above results indicate that peptides A-8 and A-23 contain Th cell epitopes and that these epitopes function in a cooperative and directional manner in inducing B cell clones to produce antibodies specific to surface-exposed neutralizing determinants located in MOMP VD I and IV. Since peptides A-8 and A-23 primed mice to produce higher titered antibodies specific to these important B cell sites, we chose them for further investigation.

Immunogenicity of Colinear Chimeric Peptides Containing Th and B Cell Determinants. To test conclusively that peptides A-8 and A-23 contained Th cell epitopes, we synthesized them as colinear peptides with the VD I sequence and used them to immunize mice. Peptide A-8 VD I was colinearly synthesized to contain residues 106–130 and 66–80, and peptide A-23 VD I was colinearly synthesized to contain residues 331– 355 and 66–80. Groups of five A/J mice were immunized with the colinear peptides A-8 VD I, A-23 VD I, or the free peptides A-8, A-23, or VD I. Their sera were tested by ELISA for IgG antibody reactive against intact EBs, purified MOMP, a synthetic peptide corresponding to the VD I sequences, and synthetic peptides containing T cell sites only (Fig. 5). Mice immunized with peptides A-8 (106–130), A-23 (331–355), or VD I (63–83) did not produce antibodies reactive with the purified MOMP, intact EBs, or VD I sequences. In contrast, when mice were immunized with the colinear peptides A-8 VD I or A-23 VD I, significant antibody responses reactive with intact EBs, MOMP, and the synthetic peptide corresponding to VD I were detected. The colinear peptide A-8 VD I was a superior immunogen since mice immunized with this peptide consistently produced higher titered antibodies reactive with EBs, MOMP, and VD I. Sera from mice immunized with A-8 VD I and A-23 VD I peptides did not react with the synthetic peptides A-8 or A-23, demonstrating that the antibody response elicited by colinear peptides was directed at B cell sites located in the VD I se-

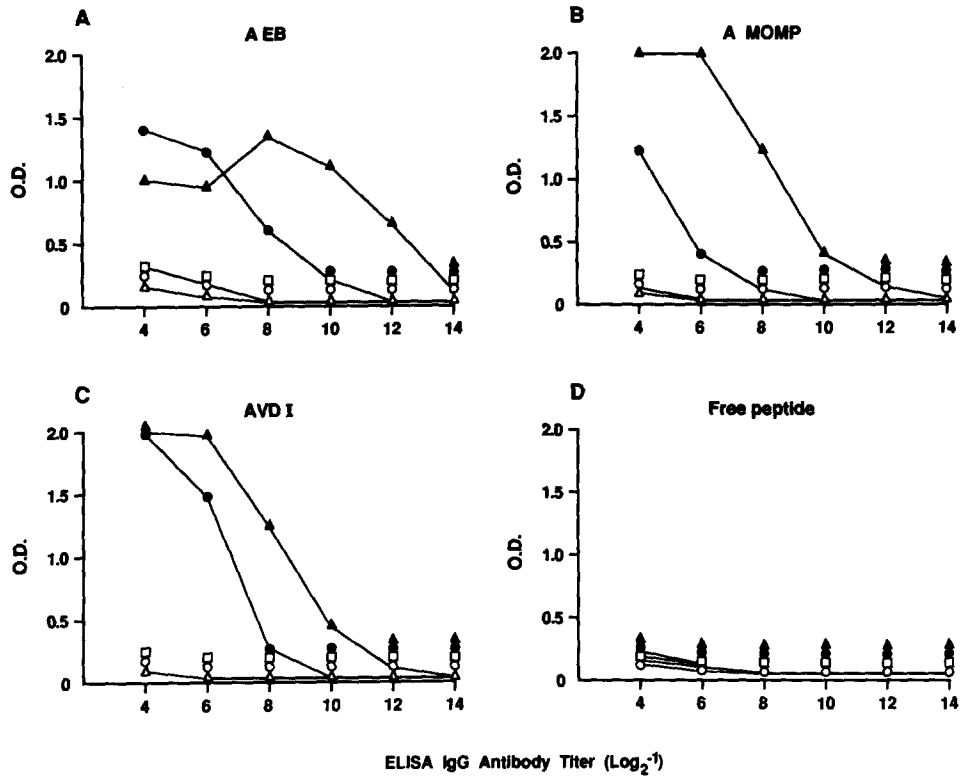

Figure 5. Antibody responses of A/J mice after immunization with chimeric T/B cell peptides or free peptides. Five mice per group were immunized intraperitoneally with 100 μg of peptide in CFA. 3 wk after immunization, the mice were bled, their sera pooled, and then analyzed by ELISA. Mouse antisera were (▲) anti-A-8 VD I, (●) anti-A-23 VD I, (△) anti-VD-I, (□) anti-A-8, and (○) anti-A-23. These sera were analyzed by ELISA against (*A*) intact serovar A EBs, (*B*) purified serovar A MOMP, (*C*) peptide VD I (residues 61–85), and (*D*) free peptides A-8 and A-23. Mouse anti-A-8 and anti-A-8 VD I were tested against peptide A-8, and mouse anti-A-23 and anti-A-23 VD I were tested against peptide A-23. Mice immunized with A-8 VD I or A-23 VD I produce high titered antibodies reactive with EBs, MOMP, and VD I. Anti-A-8 VD I or A-23 VD I sera did not react with the A-8 or A-23 peptides, demonstrating that the antibody response was directed at the VD I sequence of the chimeric peptides. Mice immunized with the VD I peptide did not produce antibodies reactive with the VD I peptide, demonstrating that this sequence alone was not immunogenic.

quence. Importantly, the VD I–specific antibodies produced after immunization with the colinear peptides reacted with both intact EBs and purified MOMP demonstrating that the antibody specificity produced by these synthetic peptides is relevant to the native MOMP structure.

Discussion

The *C. trachomatis* MOMP is a major target antigen for the development of a trachoma vaccine. A MOMP-based vaccine will require either a recombinant or synthetic antigen since it is not realistic to obtain the quantities of native MOMP that would be necessary for vaccination. A synthetic trivalent antigen composed of trachoma serovar-specific MOMP epitopes is a credible vaccine strategy. However, it is unlikely that these B cell determinants alone will be sufficient since they lack MOMP-derived Th cell determinants capable of recalling antigen-specific immune responses; this will most likely be a requirement of a successful trachoma vaccine. The goal of this study was to identify Th cell epitopes of the MOMP that direct B cell clones to produce antibodies specific to serovar-specific epitopes. These Th epitopes could then be incorporated with neutralizing B cell epitopes in a peptide-based trachoma vaccine.

Initially, we investigated the immunogenicity of the purified MOMP in six inbred mouse strains differing at H-2 to determine if variation in antibody response to the MOMP was influenced by MHC haplotype. These studies were done to identify high responder strains that would then be selected for further investigations to identify MOMP Th cell determinants. Our findings showed that inbred strains differing at H-2 produced significant antibody responses to the MOMP and that VD I and the NH_2-terminal end of VD IV were the immunodominant B cell sites recognized by strains of mice disparate at H-2. VDs I and IV were also found to be immunodominant when intact EBs were used as immunogens. At the surface of serovar A EBs, portions of VD I and VD IV are exposed, whereas VD II and III are not exposed (15; and our unpublished observations). The surface exposure of these domains likely contributes to their immunogenicity on EBs, but it is unclear why these same VDs are also immunodominant in the purified protein. Nevertheless, our findings clearly demonstrate that linear determinants contained within serovar A MOMP VD I and VD IV are immunodominant B cell epitopes that are recognized in association with multiple H-2 haplotypes.

To identify T cell sites of the MOMP, 25 overlapping peptides representing the entire primary sequence of serovar A MOMP were synthesized and tested in T cell proliferation assays. Our findings demonstrated that eight synthetic peptides contained T cell epitopes. We compared our experimental data with the AMPHI (23) and the motif (25) methods for

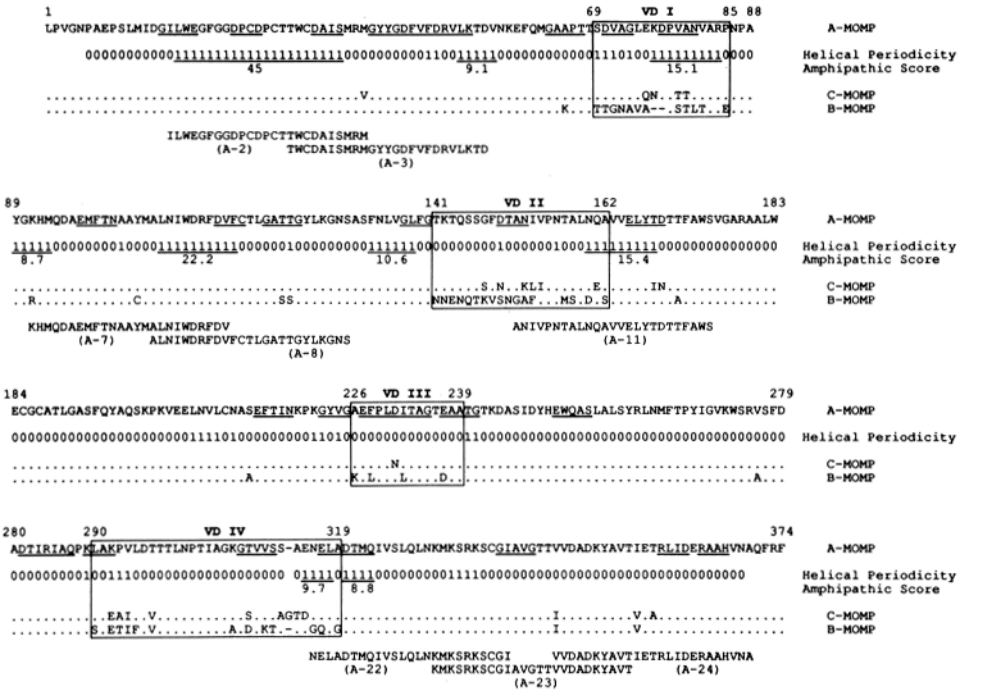

Figure 6. Schematic diagram comparing experimental data defining MOMP T cell epitopes with those identified using AMPHI and motif T cell epitope prediction algorithms. The primary sequences of serovars A, C, and B MOMP are shown. The A MOMP sequence is presented as the prototype sequence for comparative purposes. Dots in the B and C MOMP sequences represent identical amino acid residues with serovar A MOMP. T cell sites predicted by the AMPHI algorithm and motif methods are identified in the serovar A MOMP sequence. Helical periodicity in the sequence is denoted by *0* or *1*, and the amphipathic scores are given below the individual amphipathic segments. T cell motifs (charged residue or glycine, followed by two or three hydrophobic residues and then polar residues or glycine) are underlined in the A MOMP sequence. Those synthetic peptides that produced positive proliferative responses in MOMP-primed T cell assays are shown below their corresponding sequences in the primary MOMP sequences. Each of the peptides contained T cell sites predicted by one or both of the prediction methods. The majority of MOMP peptides containing T cell determinants map to regions of the molecule that are conserved among different *C. trachomatis* MOMP genes. The MOMP VDs are identified in the sequences by the boxes. VD I of serovar A MOMP is the location of neutralizing serovar-specific antigenic determinants. VD II and VD III are not surface accessible on intact serovar A EBs. VD IV is surface accessible and is the location of serogroup-specific neutralizing antigenic determinants.

the prediction of likely T cell sites of the MOMP molecule (Fig. 6). Both methods predicted a rather large proportion of the molecule as potential T cell sites; 35% of the primary sequence contained T cell motifs and 21% amphipathic segments. Six of the eight MOMP synthetic peptides that produced positive T cell proliferative responses (A-2, A-3, A-7, A-8, A-11, and A-22) contained amphipathic segments with AS of eight or greater. All eight of the peptides possessed T cell motifs (charged residues or glycine followed by two or three hydrophobic residues and then polar residues or glycine) in their sequences. Peptides A-2, A-7, and A-8, which correspond to regions of MOMP that produced the highest amphipathic scores (residues 17–37, AS = 45; and 107–116, AS = 22.2), also were ones of those that produced the strongest T cell proliferative responses. In contrast, peptides corresponding to the portion of the MOMP sequence containing the most prominent arrangement of T cell motifs (residues 222–241) were negative in MOMP-primed T cell proliferation assays. This cluster of predicted T cell motifs is located in VD III, the least variable domain of the MOMPs and the only domain to which B cell sites have not mapped. T cell sites that map to this domain would be of interest since they would provide antigenic diversity of the MOMP at the T cell level. It is possible that this site might be immunogenic in mouse strains different at H-2 or in other species.

In our attempts to define peptides that provide functional Th cell responses, we used an in vivo assay that depends on the priming peptide to augment IgG antibody responses specific to MOMP after booster immunization. We found that peptides A-7, A-8, and A-23 primed mice to produce high titered antibodies specific to the immunodominant B cell sites located in VD I and IV of the protein. It is likely that peptides A-7 and A-8 carry a common immunodominant Th cell epitope since they are overlapping peptides that share the sequence ^{106}ALNIWDRFDV^{115}, which has the second highest amphipathic score (AS = 22.2) in the MOMP primary sequence. It is interesting to note that in vivo priming with peptide A-7 produced more variable results in its ability to augment antibody responses to VD I and VD IV than those observed with peptide A-8. This difference may result because peptide A-8 contains the entire predicted T cell amphipathic segment (residues 107–116), whereas peptide A-7 is missing the COOH-terminal phenylalanine residue of the predicted site.

To directly demonstrate that peptides A-8 and A-23 contained functional Th cell epitopes, we synthesized these peptides as colinear peptides with the VD I sequence and tested their immunogenicity. Our findings clearly demonstrate that the T/B cell colinear peptides were highly immunogenic. A single immunization with the peptides produced high titered antibodies specific to the serovar-specific B cell determinants located in VD I. More importantly, these antibodies were reactive with both the MOMP and intact serovar A EBs, demonstrating that they recognize VD I determinants in the native protein. We have shown that peptides A-8 and A-23 are capable of directing B cell clones to produce antibodies

specific to different sites (VD I and VD IV) on serovar A MOMP. Further studies are needed to determine if these Th cell determinants are capable of directing B cell clones to produce antibodies specific to sites located in the MOMP VDs of other *C. trachomatis* serovars. The Th cell epitopes located in peptides A-8 and A-23 likely have common T cell antigenic properties that are shared with other MOMPs since sequences contained within these peptides are conserved among different MOMP genes (Fig. 6) (15, 16, 27, 28). A more exact description of the molecular structure of MOMP Th cell determinants contained within peptides A-8 and A-23 will require fine mapping studies using nested sets of overlapping peptides. Additionally, it will be important to determine their immunogenicity in congeneic strains of mice differing at H-2 to describe precisely the genetic restriction of the T cell repertoire capable of responding to these determinants.

One of the concerns facing peptide-based vaccines is their ability to induce effective immunity against the pathogen. Peptide vaccines corresponding to B cell sites alone are often ineffective immunogens, whereas peptides corresponding to B cell sites conjugated to irrelevant carriers may be ineffective in priming or boosting immunity upon re-exposure to the pathogen since they are incapable of inducing functional Th cell activity to the native antigen. In attempts to circumvent these potential problems in the development of a synthetic peptide vaccine for trachoma, we have identified Th cell determinants of the MOMP and incorporated these determinants as chimeric peptides with an immunodominant MOMP B cell determinant. In theory, these chimeric peptides should be capable of either priming or boosting neutralizing serovar-specific antibody responses upon natural exposure or reinfection with chlamydiae. Studies are planned to evaluate the immunogenicity of these chimeric peptides in primates and to determine their vaccine potential in a primate model of *C. trachomatis* infection.

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