

Immunogenicity of a Chimeric Peptide Corresponding to T Helper and B Cell Epitopes of the *Chlamydia trachomatis* Major Outer Membrane Protein

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Summary

The immunogenicity of a chimeric T/B cell peptide corresponding to antigenically characterized epitopes of the *Chlamydia trachomatis* major outer membrane protein (MOMP) was studied in mice to further define its potential use in the development of a subunit vaccine in preventing blinding trachoma in humans. The chimeric peptide, designated A8-VDI, corresponds to a conserved MOMP T helper (Th) cell epitope(s) (A8, residues 106–130) and serovar A VDI (residues 66–80), which contains the serovar-specific neutralizing epitope ⁷¹VAGLEK⁷⁶. Mice immunized with peptide A8-VDI produced high-titered polyclonal IgG antibodies which recognized the VAGLEK-neutralizing epitope. Peptide A8-VDI primed A/J mice to produce high-titered serum-neutralizing antibodies in response to a secondary immunization with intact chlamydial elementary bodies (EBs). Peptide A8-VDI, but not peptide VDI alone, was immunogenic in six different inbred strains of mice disparate at H-2, indicating that the Th cell epitope(s) contained in the A8 portion of the chimera was recognized in the context of multiple major histocompatibility complex (MHC) haplotypes. An unexpected finding of this work was that different inbred strains of mice immunized with the chimeric peptide produced antibodies of differing fine specificities to the VDI portion of the chimera. Some mouse strains produced anti-VDI antibodies that did not recognize the VAGLEK-neutralizing epitope. The ability of mice to respond to the VAGLEK-neutralizing site was not dependent on MHC haplotype since mouse strains of the same H-2 haplotype produced anti-VDI antibodies of differing fine specificity.

Trachoma, the leading cause of preventable blindness in developing nations of the world, is caused by *Chlamydia trachomatis* serovars A, B, and C. It is estimated that 500 million people suffer from trachoma and as many as 10 million of these individuals are permanently blinded by the disease (1). Because of its tremendous impact on human health, much attention has been focused on the development of a vaccine to prevent or control blindness due to trachoma.

Vaccines employing whole chlamydial organisms have been used in attempts to vaccinate humans against trachoma; however, they have failed in part due to host hypersensitivity reactions produced by the vaccine and the inability of systemic immunization to produce sufficient levels of local antibody to afford protection (2–4). Protective immunity in experimentally infected humans and nonhuman primates to ocular reinfection with *C. trachomatis* is serovar specific (5–7). Furthermore, resistance to ocular reinfection correlates with the presence of serovar-specific tear antibodies (8, 9), suggesting that those chlamydial surface antigens that form the basis for serovar-specific immunity are target antigens for the devel-

opment of a trachoma vaccine. Thus, a strategy for the development of a trachoma vaccine is to avoid chlamydial-immunopathological responses and focus on vaccination with protective antigen(s).

The major outer membrane protein (MOMP)¹ of *C. trachomatis* accounts for 60% of the outer membrane by weight, is surface exposed, and is the primary chlamydial-serotyping antigen (10–12). The genes encoding the MOMPs of trachoma serovars A, B, and C have been sequenced (13, 14). The MOMP is composed of four variable domains (VDs) that are equally interspersed between highly conserved regions. The sequences contained within exposed VDs comprise linear B cell epitopes which are *C. trachomatis*-serotyping determiners and primary targets for neutralizing mAbs. Neutralizing mAbs specific to the MOMP of trachoma serovars A, C, and B map to linear epitopes located in VDs I and II, respectively (13,

¹ Abbreviations used in this paper: EB, elementary body; MOMP, major outer membrane protein; VD, variable domain.

15). These epitopes are considered to be promising target antigens for the development of a synthetic or recombinant subunit trachoma vaccine. In addition to neutralizing B-cell epitopes, an effective subunit trachoma vaccine will likely require native MOMP T helper (Th) cell epitopes to provide specific T cell memory and recall high-titered neutralizing antibodies in vaccinated individuals upon natural reexposure to or infection by chlamydiae.

T cell antigenic determinants of *C. trachomatis* serovar A MOMP have been mapped (16). A 25-amino acid peptide, designated A8, that corresponds to conserved residues 106–130, provides cognate Th cell function for the production of antibodies to serovar A-specific epitopes located in MOMP VDI. Mice immunized with a colinearly synthesized peptide corresponding to A8 and VDI sequences, designated A8-VDI, produced high-titered antibodies specific to VDI that reacted with intact chlamydiae. Because the A8 peptide sequence is conserved among different *C. trachomatis* serovars (13, 14, 17–21), the Th cell epitope(s) contained within its sequence is likely antigenically common. Thus, peptide A8, in combination with trachoma serovar A-, B-, and C-neutralizing epitopes, may be used as a common carrier for the production of protective antibodies against all three trachoma serovars.

To more thoroughly define the utility of peptide A8 in chlamydial-vaccine development, we have performed additional studies to determine: if polyclonal antibodies produced against the chimeric peptide are functional-neutralizing antibodies; the ability of peptide A8 to prime Th and B cell immunity in response to a secondary immunization with intact chlamydial elementary bodies (EBs); and the immunogenicity of the peptide in mouse strains differing at H-2 to delineate the genetic restriction of the Th cell repertoire capable of responding to the peptide.

Our results showed that immunization with peptide A8-VDI induced the production of neutralizing antibodies, and that the peptide was able to prime mice to produce exceptionally high titers of serum-neutralizing antibodies after secondary immunization with intact chlamydiae. Moreover, the A8-VDI peptide was immunogenic in six H-2-disparate inbred strains of mice, suggesting that the Th cell epitope(s) contained within the peptide is recognized by multiple MHC alleles.

Materials and Methods

Chlamydiae

C. trachomatis serovar A (strain Har-13) was grown in HeLa 229 cells and EBs were purified from infected cells by density gradient centrifugation as previously described (10).

Synthetic Peptides

Peptides A8, A VDI, and A8-VDI were synthesized on Rapid-Amide resin using a RaMPS (DuPont Co., Wilmington, DE) manual synthesizer or using an automated synthesizer (430; Applied Biosystems Inc., Foster City, CA) as described previously (16). Peptide A VDI corresponds to serovar A MOMP residues 63–83 (GAAPTSDVAGLEKDPVANVA) and contains the linear serovar

A-specific neutralizing epitope ⁷¹VAGLEK⁷⁶. Peptide A8 corresponds to serovar A MOMP residues 106–130 (ALNIWDRE-DVFCTLGATTGYLKGNS) and contains a functional Th cell epitope(s) that provides cognate help for the production of antibodies to B cell epitopes contained in VDI (16). Peptide A8-VDI is a 40-residue chimeric peptide colinearly synthesized with residues 106–130 at its NH₂ terminus and residues 66–80 at its COOH terminus.

mAb

mAb A-20 is specific to *C. trachomatis* serovar A MOMP and maps to a linear epitope in its VDI (13, 22). mAb A-20 binds to intact serovar A EBs by dot-immunoblot (22) and neutralizes chlamydial infectivity in vitro (23).

ELISA

The ELISA used has been previously described (16). Briefly, microtiter plates (Immulon 2; Dynatech Laboratories, Inc., Alexandria, VA) were coated overnight at 4°C with 100 µl of synthetic peptide (5 µg/ml), purified A MOMP (0.5 µg/ml), or formalin-killed *C. trachomatis* serovar A EBs (10 µg/ml) in 0.05 M Tris buffer (pH 7.5) containing 0.15 M NaCl. Serial twofold-dilutions of mouse sera were tested in duplicate. Mouse IgG was detected using an anti-mouse IgG-alkaline phosphatase conjugate (γ chain specific; Zymed Laboratories Inc., San Francisco, CA) followed by substrate (5 mg *p*-nitrophenyl phosphate in 10 ml of 0.1 M 2:2 amino-2-methyl-1:3-propandiol, pH 10.3). Absorbance at 405 nm was measured with an ELISA reader (Bio-Rad Laboratories, Richmond, CA). Pooled preimmune or normal mouse sera were used for negative controls. ELISA titers were expressed as the highest serum dilution giving an absorbance value of 0.3 OD units.

Fine Specificity of Antibody Response

The peptide-pin-based assay described by Geysen et al. (24, 25) was used to determine the fine specificity of mouse antipeptide sera. Sequential and overlapping octapeptides representing the serovar A MOMP VDI sequence (61–85) were synthesized on prederivatized polyethylene pins, (Epitope Scanning Kit; Cambridge Research Biochemicals Inc., Wilmington, DE) as described by the manufacturer. Reactivity of mouse IgG antibody to the solid phase octapeptides was determined by ELISA using anti-mouse IgG-alkaline phosphatase conjugate as described above for peptide ELISAs.

Neutralization of Chlamydial Infectivity

In vitro neutralization of chlamydial infectivity by mouse antisera was assayed on Syrian hamster kidney (HaK) cells grown in 96-well microtiter plates (Linbro, 96 flat-bottomed wells; Flow Laboratories, Inc., McLean, VA). Briefly, 10⁵ HaK cells were grown in 96-well plates 24 h before inoculation. Purified serovar A EBs were diluted in 0.25 M sucrose, 10 mM sodium phosphate, 5 mM L-glutamic acid (SPG, pH 7.2) to give a final concentration of 4 × 10⁵ inclusion-forming units (IFUs)/ml. Twofold dilutions of pooled mouse sera were mixed with an equal volume of serovar A EBs suspended in SPG buffer. The mixtures were incubated at 37°C for 1 h and 50 µl of the mixture was inoculated in triplicate onto confluent HaK cell monolayers. After a 2-h incubation at 37°C, the inocula were removed, and the monolayers were washed with 100 µl of HBSS. Monolayers were refed with 200 µl of MEM supplemented with 10% FCS (MEM-10) containing 0.5 µg cycloheximide/ml and incubated at 37°C for 70 h. The monolayers were fixed with methanol and chlamydial inclusions identified by

indirect fluorescent antibody staining using a genus-specific mAb to chlamydial LPS. The calculations for determining percent reduction of IFUs by mouse antisera were done as previously described (26).

Immunization of Mice

Immunoreactivity and Neutralizing Activity of Anti-A8-VDI Sera. A/J mice were immunized (five mice per group) intraperitoneally with 50 µg of peptides A8-VDI, VDI, or A8 emulsified in CFA. The mice were given two booster immunizations intraperitoneally with the same concentration of the three peptide immunogens in IFA at 3-wk intervals. Mice were pre-bled and bled again 2 wk after the third immunization by tail bleeding. Sera were tested for IgG antibody reactive to peptide VDI, MOMP, and intact EBs by ELISA, and for functional antibody activity by performing neutralization assays. The fine specificity of anti-A8-VDI antisera was determined using the peptide-pin assay against overlapping sequential-octamer peptides corresponding to the VDI sequence.

The Ability of Peptide A8-VDI to Function as a Priming Immunogen for the Generation of an Anamnestic Neutralizing Antibody Response. A/J mice (five mice per group) were primed with 50 µg i.p. peptide A8-VDI, 3×10^7 IFUs of serovar A EBs, or PBS emulsified in CFA. 5 wk after the primary immunization, mice were injected intraperitoneally with the same concentrations of peptide A8-VDI, serovar A EBs, or PBS emulsified in IFA. Mice were bled 5 wk after the primary immunization and 3 wk after the secondary immunization. The sera from individual mice in experimental groups were pooled and analyzed by ELISA for IgG antibody reactive to peptide VDI and EBs. The neutralizing titers of the pooled antipeptide sera were determined as described above.

Immunogenicity of Peptide A8-VDI in Inbred and H-2 Congenic Mice. The following inbred strains of mice were used: A/J (H-2^a), C57BL/10SnJ (H-2^b), BALB/cByJ (H-2^d), CBA/J (H-2^k), DBA/1J (H-2^q), SJL/J (H-2^v). H-2 congenic mouse strains used were B10.A/SgSnJ (H-2^a), B10.BR/SgSnJ (H-2^b), A.BY/SNJ (H-2^b), and A.SW/SnJ (H-2^v). Mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Both sexes of mice at ~8–12 wk of age were used for experimentation. Groups of five mice were immunized by intraperitoneal injection of 50 µg of A8-VDI or VDI alone emulsified in CFA and boosted twice at 3 wk intervals with the same dose of peptide in IFA. Mice were bled 2 wk after the final immunization. Sera were collected, pooled according to groups, and tested by ELISA against serovar A VDI, MOMP, and EBs.

The fine specificity of the antibody response to linear determinants in the VDI sequence was determined using the peptide-pin assay.

Results

Neutralizing Activity and Molecular Specificity of Antibodies Produced by Mice Immunized with Peptide A8-VDI. A/J mice immunized with the chimeric peptide A8-VDI produced IgG antibodies reactive with the VDI peptide, serovar A MOMP, and EBs (Table 1), whereas sera from mice immunized with the free peptides A8 or VDI were nonreactive. Furthermore, the antibodies produced in response to immunization with peptide A8-VDI recognized MOMP in its native configuration and were neutralizing.

To define the molecular specificity of the anti-VDI antibodies, we assayed the reactivity of the antipeptide sera against sequential and overlapping octameric peptides corresponding to the serovar A MOMP VDI sequence (Fig. 1). Anti-A8-VDI sera reacted strongly with six octamer peptides of the VDI sequence spanning residues 69–81 (Fig. 1, top). These residues contain the ⁷¹VAGLEK⁷⁶ sequence recognized by the serovar A-specific mAb A-20 (Fig. 1, bottom). Thus, the polyclonal IgG antibodies produced by immunization of mice with peptide A8-VDI recognized the same key serovar A-specific epitope as the neutralizing mAb A-20.

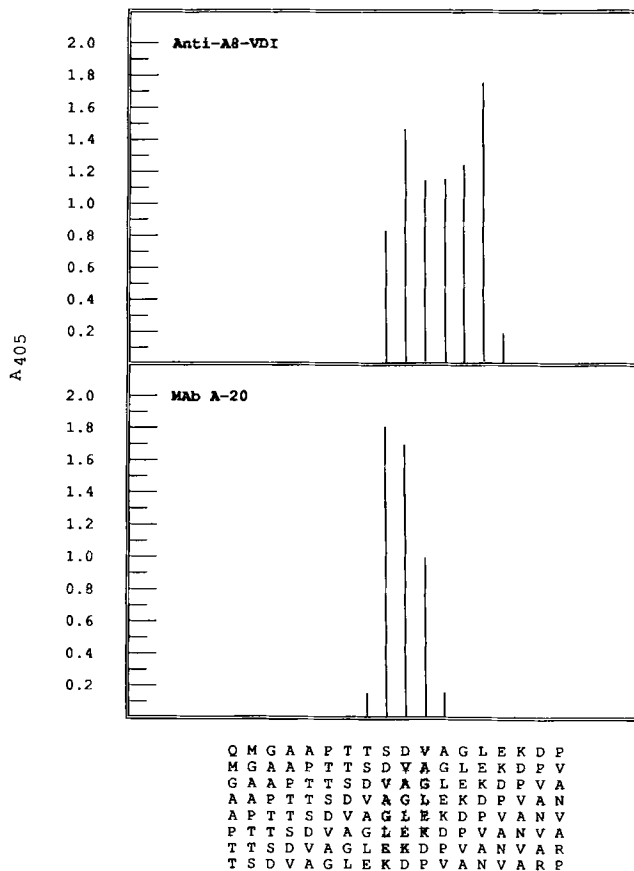
The Ability of Peptide A8-VDI to Function as a Priming Immunogen. An effective trachoma vaccine will likely depend on its ability to prime protective T and B cell immunity so that natural reexposure to or infection by chlamydiae induces an anamnestic-immune response to targeted MOMP-neutralizing epitopes. To determine if the peptide A8-VDI could function in this capacity, A/J mice were: immunized with 50 µg intraperitoneally A8-VDI peptide in CFA and then boosted 5 wk later with 3×10^7 formalin-killed EBs emulsified in IFA; or primed intraperitoneally 3×10^7 EBs in CFA and boosted with peptide A8-VDI in IFA. Mice were bled 5 wk after the primary immunization, and 3 wk after the secondary immunization, and sera were tested by ELISA for IgG antibodies to peptide VDI and intact EBs. The results showed that peptide A8-VDI was an excellent priming im-

Table 1. Immunoreactivity and Neutralizing Properties of Anti-A8-VDI Sera

mAb or mouse antiserum	ELISA IgG antibody titer against serovar A			Neutralizing titer (ND ₅₀)
	VDI	MOMP	EBs	
mAb A-20*	32,768	32,768	32,768	512
Anti-A8-VDI	16,384	16,384	16,384	1,024
Anti-AVDI	<16	<16	<16	<16
Anti-A8	<16	<16	<16	<16

ELISA titers are expressed as the reciprocal of pooled serum dilutions giving an absorbance reading (405 nm) of 0.3. Absorbance values of preimmune or normal mouse sera were 0.1 or less. Neutralization titers are expressed as the reciprocal of pooled serum dilutions that reduced chlamydial IFUs for HaK cells by 50% or more. Preimmune or normal mouse sera were used as negative controls in the neutralization assays. Percent reduction in IFUs was determined as follows: $100 \times [(IFUs \text{ control sera} - IFUs \text{ experimental sera}) / IFUs \text{ Control Sera}]$.

* mAb A-20 contained 1 mg protein/ml before dilution.



A VDI (residues 61-85)

Figure 1. Fine specificity of the antibody response in A/J mice immunized with peptide A8-VDI. Pooled sera from A/J mice immunized with peptide A8-VDI were tested against sequential and overlapping octameric peptides corresponding to the primary sequence of serovar A MOMP VDI (residues 61-85). Antibodies reacted strongly with six octameric peptides spanning VDI residues 69-81 (*top*), which included the serovar A-specific MOMP epitope ⁷¹VAGLEK⁷⁶ recognized by the neutralizing mAb A-20 (*bottom*). The VAGLEK epitope recognized by mAb A-20 is denoted by the stippled pattern in the VDI sequence.

munogen but was not effective as secondary or booster immunogen (Table 2). Mice primed with peptide A8-VDI and then given a secondary immunization with 3×10^7 IFUs of serovar A EBs in IFA produced exceptionally high-titered antibodies to the VDI peptide and serovar A EBs. These sera also had higher titers of chlamydial-specific neutralizing antibodies. In contrast to these findings, peptide A8-VDI was not an effective secondary immunogen for the production of anamnestic antibody response in mice that were primed with intact serovar A EBs. These findings suggest that the A8 Th cell epitope(s) may not be the immunodominant determinant in the context of intact chlamydial EBs. However, once T cell pools specific to the A8 peptide have been induced, they are apparently preferentially expanded in response to secondary immunization with intact chlamydial organisms.

Immunogenicity of Peptide A8-VDI in Inbred Mouse Strains Disparate at H-2. Th cell immunity is MHC restricted and it is generally accepted that in a given haplotype-specific Th cell stimulation is a necessary prerequisite to B cell proliferation (27). To determine whether the Th cell epitopes contained in the A8 portion of peptide A8-VDI could be recognized by multiple MHC haplotypes, we studied its ability to induce an IgG antibody response specific to VDI in inbred strains of mice differing at H-2. As a control for T cell recognition of the A8 portion of the chimeric peptide, mice were also immunized with the VDI peptide alone. If an IgG antibody response specific to VDI was dependent on immunization with the chimeric peptide it was interpreted that the given H-2 haplotype was capable of recognizing the Th cell epitope contained in the A8 sequence. Six inbred strains of mice having different H-2 haplotypes were immunized with peptide A8-VDI or the VDI peptide alone and their sera tested for antibody reactivity to peptide VDI, MOMP, and EBs (Fig. 2). All inbred strains of mice immunized with the A8-VDI peptide produced IgG antibodies reactive with the VDI peptide. In contrast, only C57BL/10 (H-2^b) mice produced anti-VDI antibodies after immunization with the free VDI peptide. These results indicate that multiple H-2 haplotypes recognize the Th cell epitope(s) contained in the A8 sequence. The ability of VDI alone to induce an IgG antibody response in C57BL/10 mice shows that in addition to B cell epitopes the VDI sequence also contains a Th cell epitope(s) recognized by C57BL/10 mice. Although all the inbred strains of mice tested produced anti-VDI antibodies only some strains of mice produced antibodies reactive with the MOMP and intact chlamydial EBs (Fig. 2). A/J, CBA, and DBA mouse strains made antibodies that recognized the MOMP and intact EBs, but C57BL/10, BALB/c, and SJL antisera either reacted weakly or not at all with these antigens.

Neutralizing Activity of Anti-A8-VDI Antibodies Produced in Different Inbred Mouse Strains. Since some mouse strains produced antibodies reactive with the VDI peptide but not intact EBs, it was important to determine if these anti-VDI antibodies were capable of neutralizing chlamydial infectivity. The *in vitro* neutralizing activity of the anti-A8-VDI sera from different inbred mice is shown in Table 3. A/J, DBA, and CBA mouse sera were neutralizing whereas C57BL/10, BALB/c, and SJL sera were nonneutralizing. Therefore, although the A8-VDI peptide evoked antibody responses against peptide VDI in each of the inbred mice tested, only certain strains produced functional neutralizing antibodies. The ability of mouse sera to neutralize infectivity correlated directly with its reactivity to intact EBs by ELISA. A possible explanation for these findings was that the fine specificity of the antibodies produced after immunization of peptide A8-VDI was different among the mouse strains.

Fine Specificity of Anti-A8-VDI Antibodies Produced in Different Inbred Mouse Strains. Anti-A8-VDI sera from each mouse strain were assayed against overlapping octapeptides corresponding to the VDI sequence using the peptide-pin assay (Fig. 3). Anti-A8-VDI mouse sera that were reactive with intact EBs that were neutralizing (A/J, DBA, and CBA) and recognized octapeptides spanning the serovar A-specific neu-

Table 2. *The Ability of Peptide A8-VDI to Function as a Priming Immunogen*

Immunogen		5 wk after primary immunization			3 wk after secondary immunization		
		ELISA antibody titer		Neutralizing titer (ND ₅₀)	ELISA antibody titer		Neutralizing titer (ND ₅₀)
Primary	Secondary	VDI	EBs		VDI	EBs	
PBS	A EBs	<16	<16	ND	64	32	ND
A8-VDI	PBS	4,096	4,096	512	4,096	4,096	1,024
A8-VDI	A EBs	4,096	4,096	1,024	32,768	65,536	8,192
PBS	A8-VDI	<16	<16	ND	128	128	ND
A EBs	PBS	2,048	4,096	1,024	2,048	2,048	1,024
A EBs	A8-VDI	1,024	4,096	2,048	4,096	4,096	2,048

ELISA and neutralization titers are expressed as described in Table 1. Groups of five A/J mice were used for each experiment. Mice primed with peptide A8-VDI were injected intraperitoneally with 50 µg peptide in CFA. Mice boosted with peptide A8-VDI were immunized intraperitoneally with the same concentration of peptide emulsified in IFA. Mice primed with EBs were immunized intraperitoneally with 3 × 10⁷ IFUs in CFA. Mice boosted with EBs were immunized intraperitoneally with the same quantity of EBs in IFA (note that this quantity of EBs was subimmunogenic since it did not elicit a significant antibody response in mice when administered intraperitoneally in IFA).

tralizing epitope VAGLEK. In contrast, those mouse sera that did not react with intact EBs by ELISA and that were non-neutralizing (C57BL/10, BALB/c, and SJL) failed to react with VDI octapeptides containing the VAGLEK sequence, or had very low levels of reactive antibody against the epitope. The fact that peptide A8-VDI did not induce antibodies to the VAGLEK epitope in C57BL/10, BALB/c, and SJL mice provides an explanation for the lack of neutralizing activity observed with these mouse sera (Table 3).

It is unclear why those mouse sera which reacted with the VDI peptide by ELISA did not react with octameric peptides in the peptide-pin assay. It is possible that these sera contain antibodies made against structural determinants of the VDI peptide that are not present in octameric peptides or that they recognized linear epitopes in VDI that exceeded eight amino acid residues.

Antibody Response of H-2 Congenic Mouse Strains Immunized with Peptide A8-VDI. To further characterize the genetic control of the immune response to peptide A8, and to more precisely ascertain if the differences in the molecular specificity of the antibody response observed in inbred strains of mice (Fig. 3) were associated with H-2 haplotype, we immunized groups of A and B10 H-2-congenic mouse strains with peptide A8-VDI. We chose these strains since A/J and C57BL/10 mice represented responder and nonresponder strains, respectively, to the VAGLEK-neutralizing epitope. A corresponding pattern of antibody responsiveness to the VDI peptide and intact EBs was also observed in these same strains of congenic mice (Fig. 4). All three A strain-congenic mice, A/J (H-2^a), A.BY (H-2^b) and A.SW (H-2^s), immunized with the A8-VDI produced antibodies reactive with the VDI peptide and intact serovar A EBs. In contrast, B10-congenic strains of mice, C57BL/10 (H-2^b), B10.A (H-2^a), and B10.BR (H-2^k), produced antibodies reactive with the VDI peptide, but

these antibodies did not react with intact EBs. A.BY (H-2^b) mice immunized with VDI peptide alone produced antibody reactive with the VDI peptide and EBs, further demonstrating that the VDI sequence contains a Th cell epitope(s) whose immunogenicity is H-2^b restricted.

Discussion

In previous studies we showed that peptide A8, which corresponds to serovar A MOMP residues 106–130, contained a Th cell epitope(s) that was capable of providing cognate T cell help for the production of antibody to a serovar A-specific B cell epitope located in VDI (16). We colinearly synthesized A8 and VDI sequences as a chimeric T/B cell peptide, designated A8-VDI, and showed it to be highly immunogenic in mice. Polyclonal antibodies produced in A/J mice against A8-VDI reacted only with the VDI portion of the chimera and recognized the VDI sequence in its native conformation on the surface of intact chlamydial EBs. These preliminary findings were encouraging with respect to the potential use of the chimeric peptide in the design of a synthetic chlamydial vaccine. However, they did not address other functional and immunogenic properties of the chimeric peptide that are important towards this end. Here, we have performed further studies to delineate the immunogenic properties of this chimeric peptide to more thoroughly address its potential use in chlamydial vaccine development. We showed that antibodies produced to the chimeric T/B peptide were functional neutralizing antibodies and that they recognized the same linear serovar A-specific MOMP epitope (VAGLEK) in the VDI sequence as a neutralizing serovar A-specific mAb. We demonstrated that the chimeric peptide could function as an effective priming immunogen for the production of high-titered serum-neutralizing antibodies after secondary immu-

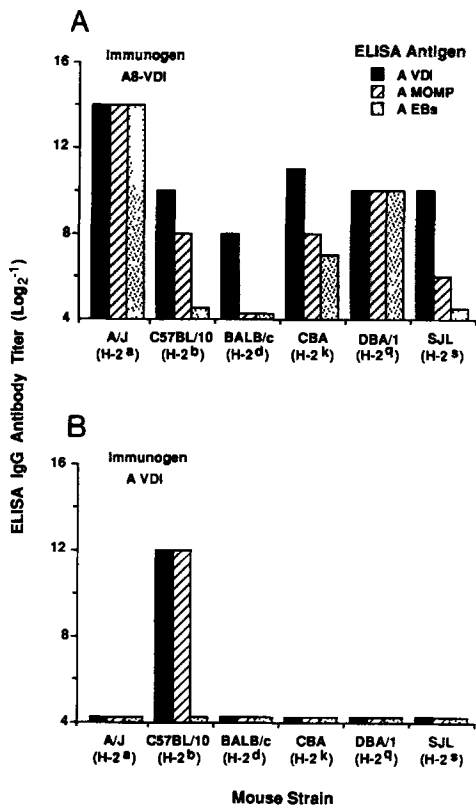


Figure 2. Immunogenicity of peptide A8-VDI in inbred strains of mice having different H-2 haplotypes. Six different inbred strains of mice (five mice per group) were immunized with peptide A8-VDI (A) or VDI (B) alone. The mouse sera were tested by ELISA for IgG antibodies to peptide VDI, serovar A MOMP, and serovar A EBs. All six inbred strains of mice immunized with peptide A8-VDI produced significant IgG antibodies reactive with the VDI peptide. In contrast, immunization with peptide VDI alone did not generate an IgG antibody response in five of the six different inbred strains of mice. The exception was C57BL/10 (H-2^b) mice in which immunization with peptide VDI alone produced antibodies reactive with peptide VDI. Although the six different inbred strains of mice produced anti-VDI antibodies, only strains A/J, DBA, and CBA produced antibodies reactive with the MOMP and intact EBs.

nization with intact EBs. Lastly, we demonstrated that the chimeric T/B cell peptide was immunogenic in mouse strains having different H-2 haplotypes suggesting that the Th cell epitope(s) contained in the A8 portion of the chimera was recognized by multiple MHC haplotypes.

An effective trachoma vaccine will likely depend on boosting protective antibody responses to MOMP in immunized individuals by either exposure to or reinfection by chlamydiae under natural environmental conditions. This will require that the vaccine contain MOMP-derived Th cell epitope(s), and not alien or heterologous carrier molecules. Our data showed that peptide A8-VDI could function as an effective priming immunogen for the production of high-titered serum neutralizing antibodies after secondary challenge immunization with intact chlamydial EBs (Table 2). In contrast, the peptide did not function as an effective secondary immunogen

for boosting serum-neutralizing antibody responses in mice that had been primed with intact EBs. A possible explanation for these findings is that primary immunization with peptide A8-VDI selectively induced Th cell and B cell responses to epitopes contained within this peptide. Upon secondary immunization with intact EBs, these T and B cell pools were preferentially expanded resulting in the production of extremely high-titered neutralizing antibodies. The inability of peptide A8-VDI to function as a boosting immunogen in mice primed with intact EBs indicates that the A8 Th cell epitope(s) is a cryptic determinant that is not immunodominant in the context of the native MOMP molecule. Despite its apparent cryptic nature in the context of native MOMP the A8 T cell determinant(s), when presented in a peptide form, is capable of binding multiple MHC molecules and provides helper T cell function for the production of neutralizing antibody which can be boosted by immunization with intact chlamydiae. These data suggest that the A8 peptide may contain suitable T cell determinant(s) for the development of synthetic trachoma vaccine.

Antigen-specific Th cell responses are MHC class II restricted (28, 29). In the context of vaccine design, immunogens possessing defined Th cell epitopes should be recognized by multiple H-2 alleles to be effective in a genetically diverse population. In attempts to delineate the H-2 restriction in the recognition of the Th cell epitope(s) contained within the A-8 peptide, we studied the immunogenicity of peptide A8-VDI in mouse strains of different H-2 haplotypes. We found that six inbred mouse strains immunized with peptide A8-VDI produced antibodies reactive with the VDI peptide indicating that Th cell epitope(s) contained in the A8 peptide are recognized by multiple H-2 haplotypes. A disturbing aspect of these studies was that the fine specificity of the antibody response to VDI varied among mouse strains. Although all mouse strains tested produced antibodies reactive with the VDI peptide, only certain strains produced antibodies that reacted with intact EBs and recognized the neutralizing epitope VAGLEK in the VDI sequence (Figs. 2 and 3). It is not understood why different mouse strains produced antibodies with different molecular specificities to the VDI peptide. Three H-2-congenic A strain mice made antibodies reactive with intact EBs whereas three H-2-congenic B10 mice did not produce antibodies reactive with EBs. Thus, the differences in antibody-fine specificity to the VDI peptide produced by different mouse strains are likely not H-2 related but are perhaps due to differences in other, possibly Ig genes, that control the production of antibody to VDI. The inability of some mouse strains to respond to the VAGLEK epitope might be explained by a hole in their B cell repertoire that renders them incapable of responding to this epitope. Alternatively, the epitope might not be properly presented by some strains of mice during antigen processing.

The VDI peptide alone was immunogenic in C57BL/10 and A.BY mice, indicating that the VDI sequence contains both Th cell and B cell epitopes. This result is perhaps not unexpected since the VDI peptide contains two predicted Rothbard (30) T cell motif sequences (residues ⁷⁰DVAG⁷³

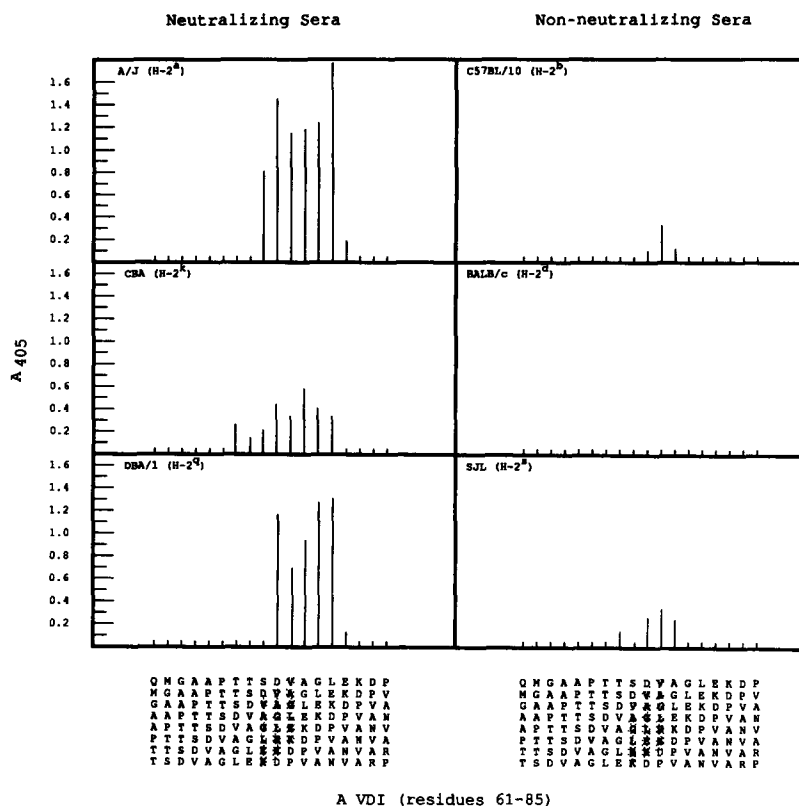


Figure 3. Fine specificity of the antibody response in inbred strains of mice immunized with peptide A8-VDI. Mouse sera were tested at a 1:100 dilution against sequential overlapping octameric peptides corresponding to the VDI sequence. A/J, CBA, and DBA mice produced antibodies reactive with VDI-octameric peptides whereas C57BL/10, BALB/c, and SJL mouse sera were negative or reacted very weakly with VDI octapeptides. A/J and DBA mice produce antibodies reactive with the VAGLEK-neutralizing epitope whereas CBA mice recognized octameric peptides COOH terminal to this epitope. The fine specificity of the antibody response among inbred strains of mice correlates with antibody-neutralizing activity. For example, A/J and DBA mouse sera contained antibodies reactive with the VAGLEK epitope and were neutralizing. In contrast, mouse sera that were nonreactive with VDI octameric peptides or failed to produce antibodies to the VAGLEK sequence were non-neutralizing. The VAGLEK sequence recognized by mAb A-20 is denoted by the stippled pattern.

and ⁷⁷DPVAN⁸¹) and an amphipathic α -helix segment (31) (residues ⁷⁶KDPVANVAR⁸⁴) with an amphipathic score of 15.1. Our findings that MOMP VDs contain Th cell epitopes confirm the findings of Allen et al. (32) who have localized T cell determinants to MOMP VDs. The Th cell epitopes contained in MOMP VDs may not however be particularly attractive for the development of a chlamydial vaccine. For example, as we have shown here, the Th cell epitope(s) in the serovar A MOMP VDI sequence is H-2^b restricted. In addition, the MOMP VDs represent hypervari-

Table 3. Neutralizing Properties of Anti-A8-VDI Sera Produced in Inbred Mice of Different H-2 Haplotypes

Mouse strain	H-2 haplotype	ELISA anti-EB titer	Neutralizing titer (ND ₅₀)
A/J	a	16,384	4,096
C57BL/10	b	<16	<16
BALB/c	d	<16	<16
CBA	k	128	32
DBA/1	q	1,024	256
SJL	s	<16	<16

ELISA and neutralization titers are expressed as the reciprocal of serum dilutions resulting in OD (405 nm) of 0.3 or a 50% reduction in chlamydial IFUs, respectively.

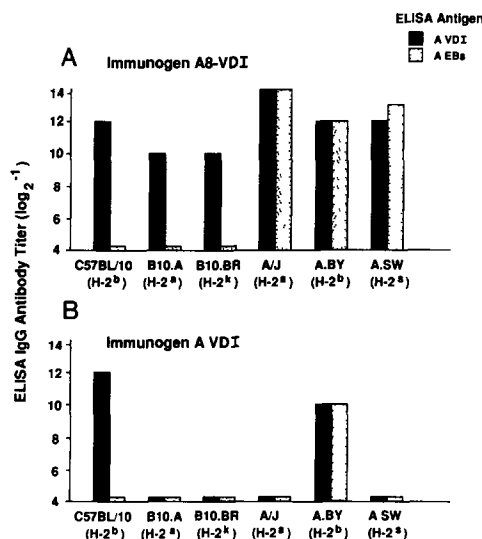


Figure 4. Immunogenicity of peptide A8-VDI in congenic mouse strains differing at H-2. Congenic A and B10 mouse strains differing at H-2 were immunized with peptide A8-VDI (A) and VDI (B), their sera tested by ELISA against VDI and serovar A EBs. Peptide A8-VDI was immunogenic in H-2 congenic A and B10 mouse strains. Congenic A mouse strains produced antibodies reactive with both the VDI peptide and intact serovar A EBs whereas B10-congenic mice made antibodies that reacted only with the peptide VDI. The VDI peptide alone was immunogenic in C57BL/10 (H-2^b) and A.BY (H-2^b) mice demonstrating that the VDI peptide contains a Th cell epitope(s) in addition to B cell epitopes and that this epitope is H-2^b restricted. Thus, the differences observed in antibody fine specificity to VDI epitopes among different mouse strains are not H-2 linked but are more likely due to differences in immunoglobulin genes among the different mouse strains.

able sequences among different *C. trachomatis* MOMP genes (13, 14) indicating that the T cell-antigenic determinants that map to these regions are likely to be antigenically unique among different *C. trachomatis* MOMPs.

The A8 sequence is highly conserved among different *C. trachomatis* MOMP genes indicating that its Th cell epitope(s) is antigenically common among different MOMPs. Our findings indicate that the Th cell determinant(s) in the A8 sequence is capable of priming Th cell immunity that can be recalled by native EBs. Moreover, multiple H-2 haplotypes recognize the A8 Th cell epitope(s). Collectively, these

properties are encouraging in respect to the potential use of the A8 peptide in the design of a synthetic trachoma vaccine. We are currently investigating the immunogenicity of colinear peptides corresponding to the A8 sequence in association with trachoma B and C serovar-specific neutralizing epitopes. Theoretically, a trivalent vaccine consisting of synthetic peptides or recombinant proteins comprised of the A8 sequence in combination with these linear B cell-neutralizing epitopes may be effective in producing protective neutralizing antibodies against all three trachoma serovars.

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