Antigen Conformation Dependence of *Chlamydia trachomatis* Infectivity Neutralization

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*Chlamydia trachomatis* infections cause the most common notifiable diseases in the United States, reflecting the successful adaptation of these organisms to persist in their obligate human host population. Antigenic variation of the quantitatively predominant major outer membrane protein (MOMP) is considered to play an important role in this adaptation as a means of immune evasion. The relative capacity of murine polyvalent sera produced following infection, recovery, and challenge to neutralize infectivity was highly serovar-specific and dependent upon thermolabile antigens. The structural complexity of these immunodominant antigens was mimicked by chlamydial MOMP expressed in *Escherichia coli*, as antibodies that neutralize infectivity by recognition of conformation-dependent antigens were specifically removed from sera following absorption using MOMP expressed in *E. coli*.

*Chlamydia trachomatis* is the cause of several important human diseases including trachoma (the leading cause of preventable blindness), pelvic inflammatory disease, ectopic pregnancy, chronic pelvic pain, epididymitis, and infant pneumonia [1]. *C. trachomatis* genital tract infections also significantly increase the risk for human immunodeficiency virus infection [2, 3]. Chlamydial serovariant-specific antigens may be the most effective immune-protective targets because challenge with the homologous serovar results in immune protection, whereas challenge with heterologous serovars results in infection [4, 5]. Studies using *Chlamydia*-specific [1] monoclonal antibodies have suggested that the chlamydial major outer membrane protein (MOMP) contains the serovar-defining antigens and that MOMP serovar-specific antigens are the primary targets that mediate in vitro neutralization of infectivity [6–8]. The antigenic differences among chlamydial strains are defined by variant sequence regions of MOMP, and two discontinuous variant sequence regions (VS1 and VS2) have been associated with serovar specificity because they bind serovar-specific monoclonal antibodies [9, 10]. Epidemiologic data support the view that naturally acquired immunity to *C. trachomatis* is related to serovar-specific immunity, as there is a change in the distribution of MOMP allelic variants over time when this has been evaluated for trachoma [11] and for sexually transmitted infections [12].

Antigenic variation of surface proteins that are the focus of antibody-mediated protection is a common theme for pathogenic microorganisms [13, 14]. The combination of a wide variety of MOMP-specific monoclonal antibodies with different specificities and knowledge of the primary sequence structure of different MOMP variants initially promised to facilitate identification of MOMP epitopes that would be useful for vaccine development. Immunization using VS1-based peptides has been shown to elicit antibody responses that can neutralize infectivity in vitro [15, 16]; however, vaccination with such MOMP peptides has not been successful in eliciting protective immunity in vivo. This may be because the identification of single peptide epitopes and their use as immunogens have typically resulted in eliciting high antipeptide responses but relatively low reactivity to native elementary bodies (EBs), the infectious form of the organism [10, 17, 18]. Although either monospecific polyvalent sera or monoclonal antibodies specific for individual MOMP VS regions can neutralize chlamydial infectivity in vitro, there is evidence that other MOMP antigens more appropriately reflect the complexity indicated by the primary sequence of MOMP and may be defined by conformational determinants [6, 19, 20].

While the use of monoclonal antibodies or synthetic peptides is ideal for some purposes, their singular specificity, even analyzed in the aggregate, has not provided a holistic understanding of native conformation-dependent antigenic structure. *C. trachomatis* provides an attractive model to investigate conformation-dependent immune neutralization because of the availability of a wide variety of serovars and an in vitro neutralization assay. We tested the ability of polyvalent murine antisera derived using native chlamydial organisms representing a variety of serovars to neutralize infectivity in vitro in an effort to evaluate the specificity of neutralization among serovars.
Assessments of neutralization following absorption of sera with synthetic peptides or native MOMP expressed in *Escherichia coli* were employed to dissect the specificity characteristics of antibodies that dominate the ability of polyvalent sera to neutralize chlamydial infectivity.

**Materials and Methods**

**Bacterial strains.** *C. trachomatis* strains used were B/TW-5/OT and C/TW-3/OT, which were originally obtained from C.-C. Kuo (University of Washington, Seattle), and A/HAR-13, D/UW-3/Cx, G/UW-57/Cx, and I/UW-12/Ur, which were provided by H. D. Caldwell (Rocky Mountain Laboratories, Hamilton, MT). All strains were grown in L929 cells, and EB developmental forms were purified on diatrizoate (Renografin; E. R. Squibb & Sons, Princeton, NJ) gradients, aliquoted, and stored at −70°C. EBs were isolated from infected host cells by sonication on ice using several 10-s pulses from a probe sonicator (Braun-Sonic 2000; Braun Biotech, Allentown, PA) to the point that little host cell debris was pelleted following centrifugation at 850 g. EBs were pelleted by centrifugation at 10,000 g for 20 min and suspended in 5 mL of Hanks’ balanced salt solution (HBSS). The EBs were initially separated from host cell material by centrifugation (18,000 rpm, SW28, 30 min) through 30% diatrizoate. The EBs were then washed with HBSS and purified using discontinuous gradients consisting of 30%, 40%, 45%, and 50% diatrizoate. After a wash to remove excess diatrizoate, EBs were suspended in SPG (200 mM sucrose, 10 mM sodium phosphate, 5 mM l-glutamate, pH 7.2), aliquoted, and stored frozen at −70°C.

**Neutralization assay.** The production of murine polyvalent sera was described previously [21]. Briefly, 6 groups of 30 mice were inoculated once with serovar A, B, C, D, G, or I. One year following the initial inoculation, each group of mice was divided into 6 groups of 5 mice each and challenged with each of the respective serovars. Sera were obtained 2 weeks after challenge, and aliquots obtained for the 5 mice in each group were pooled and used for neutralization.

The Hak neutralization assay was used as described [22], with the sera obtained following infection and challenge with homologous serovars. In some experiments, neutralization was evaluated for mice that were primed with either serovar C or I EBs and challenged with serovar I or C EBs, respectively. Likewise, neutralization was also tested for mice primed with either serovar B or D EBs and challenged with serovar D or B EBs, respectively. Unlike previously reported neutralization assays using polyvalent sera, the Hak system is not complicated by expression of antibody-binding Fc receptors [23]. Heat-inactivated sera were diluted by 2-fold dilutions from 1:8 to 1:131,072, and every other dilution was evaluated. Thus, each evaluation point represents two 2-fold dilutions of sera.

Diluted sera were mixed with an equal volume of a standardized inoculum (∼3 × 10⁴ inclusion-forming units [ifu]/mL) of serovar A, B, C, D, or I EBs and inoculated onto triplicate monolayers of Hak cells in wells of 96-well tissue culture plates. Inclusions were detected by immunofluorescence using a species-specific fluorescein isothiocyanate–conjugated monoclonal antibody [24] and ifu were determined using a ×5 objective (×50 magnification) of an inverted epifluorescence microscope and CCD camera imaging (Photometrics, Tucson, AZ) with computer-aided enumeration (IPLab Spectrum; Signal Analytics, Vienna, VA).

**Antisera absorption.** To evaluate the character of antibody present in polyvalent sera responsible for neutralization, antisera were diluted to a concentration that would yield ~1/e (~63%) neutralization of infectivity and absorbed with (1) synthetic peptides corresponding to the respective serovar-specific VS1, VS2, or VS4 antigens; (2) EBs that had been heat-treated at 4, 37, 40, 50, 60, or 100°C; or (3) recombinant *E. coli* expressing the entire chlamydial MOMP for serovars B or C. SPG was used for all dilutions. The synthetic peptides used have been described [21]. After absorption by addition of 100 μg/mL, antisera were tested by ELISA as described [21, 25] to verify that absorption had quantitatively removed peptide-specific reactivity previously described for these sera [21].

Following absorption with EBs for 30 min at 4 or 37°C, EBs were removed by centrifugation at 15,000 g for 10 min. No infectivity was detected in sera absorbed with EBs following centrifugation. Recombinant *E. coli* that express chlamydial MOMP were produced using the omp1 genes obtained from serovars B (TW-5/OT) and C (TW-3/OT) [26] as described [27]. MOMP expression of each recombinant was verified using serovar-specific and species-specific monoclonal antibodies [28] by immunofluorescence and immunoblot as described [27]. After induction of MOMP expression, *E. coli* were washed in PBS, suspended in SPG, and used unfixed for absorption of antisera. Aliquots of induced *E. coli* were evaluated concurrently by immunofluorescence and by immunoblot to assess the presence of MOMP.

**Results**

**Serovar specificity of neutralization.** Infectivity neutralization studies were conducted using murine sera obtained following infection, recovery, and challenge with *C. trachomatis*. On the basis of reactivity of polyvalent sera, *C. trachomatis* serovars are designated by letters A–L [29]. Three main serologic groups have been classified by close relationships of serovars within serogroups and distant relationships between serogroups: The B-complex serogroup includes serovars B, Ba, D, E, L1, and L2; the C-complex includes serovars C, A, H, I, J, K, and L3; and the F/G-complex includes serovars F and G [29]. Serovars A, B, C, D, G, and I were used for these studies, thus representing serovarvariants for each major serogroup: the B-complex (serovars B and D), the C-complex (serovars A, C, and I), and the F/G-complex (serovar G). These sera have been described and characterized for MOMP peptide serologic reactivity [21].

Each serum pool was evaluated for its ability to neutralize infectivity of EBs representing each of the six serovars. In every case, antibody-mediated concentration-dependent neutralization was obtained after exposure of chlamydial organisms to serial dilutions of immune serum, starting at a dilution of 1:16. Preliminary evaluations showed that preimmune sera did not have a significant effect on infectivity and thus were
not routinely evaluated. Data for neutralization of serovar A EBs using each of the 6 sera obtained from homologous challenge are shown in figure 1. At the lowest dilution (log₂ 16 = 2), sera derived from infection with C-complex serovars A, C, and I each neutralized infectivity of serovar A EBs by >95%, whereas sera obtained following infection with B-complex serovars (i.e., B and D) or serovar G only neutralized serovar A EBs by ~60%. Neutralization of serovar A EBs with sera derived following infection using serovar A was substantially more effective than neutralization with sera produced using the other C-complex serovars, C and I. These data illustrate the potential for a high level of serovar specificity of neutralization. We operationally attribute neutralization as specific for a given serovar(s) if the 50% neutralization titers for various chlamydial serovars differ by ≥2 log₂ dilutions.

Each of the other five serovar EBs was neutralized with the respective homologous sera at titers of ≥1:1024, except for serovar G sera (figure 2). The kinetics of neutralization were also similar for most serovars and homologous sera. Homologous neutralization typically began abruptly and showed a steep curve until maximal neutralization was obtained, usually within 2 log₂ dilutions (figure 2). At a log₂ dilution of 2, EB neutralization by homologous antisera reached at least 90%, except for serovars G and I, for which maximal neutralization only reached ~65% (figure 2). Differences in neutralization could be affected by antisera potency, either quantitative or qualitative, or by differences in serovar virulence for Hak cells. Previous serologic evaluation of these sera showed that they were remarkably similar in terms of antibody titers to another chlamydial protein (OMP2) and, with the exception of serovar G sera, each of the other 5 sera displayed similar reactivity to the respective homologous VS1 peptide [21]. Although serovar G sera had little reactivity to serovar G–specific VS1 peptide, it reacted strongly to circularized VS1 peptide [21]. These data suggest that differences in neutralization are probably not a function of antibody titer per se, but of qualitative differences in antibody specificity or differences in strain virulence for Hak cells. Evidence implicating increased virulence for Hak cells for serovars I and G was shown by the finding that sera elicited by serovars I and G neutralized at least one other serovar by >90% (data not shown), suggesting these sera are quantitatively and qualitatively potent.

The sensitivity of each serovar to be neutralized by different sera can be compared by the dilution at which 50% neutralization is obtained for each pair (figure 3); likewise, the ability of individual sera to neutralize different serovars can be discerned across categories in figure 3. For example, serovar A EBs were effectively neutralized by murine sera elicited following infection with serovar A EBs, although the serovar A–derived sera also effectively neutralized serovar C EBs. It is apparent from these data that neutralization using polyvalent sera is very sensitive and highly serovar-specific, even among serovars related within the B- or C-complex serogroups.

Serologic studies by Zhao et al. [21] showed that, when mice were primed with EBs from one serovar and challenged with EBs from a closely related serovar, there was an anamnestic serologic response elicited to the original priming serovar VS1 antigen in addition to production of antibodies recognizing

Figure 1. Neutralization of serovar A elementary bodies (EBs) by murine sera obtained following infection and challenge with EBs representing serovars A, B, C, D, G, and I. Mean ifu were determined after neutralization using 4-fold serial dilutions of sera from 1:16 to 1:262,144. Each integer of log₂ dilution represents two 2-fold dilutions of sera. Error bars show SE of ifu per field obtained from triplicate microtiter plate wells.

Figure 2. Neutralization of serovar A, B, C, D, G, and I elementary bodies (EBs) using 4-fold serial dilutions of corresponding homologous sera (e.g., A/A = A EBs neutralized by serovar A sera). % neutralization was determined based on no. of ifu without added antisera. For each determination, SE was <10% of total mean no. of counts.
cross-reacting VS1 determinants. We addressed how such sera would perform in the context of functional neutralization. Neutralization evaluations were conducted with serovar B and D EBs using sera derived from priming with serovar B EBs and challenging with serovar D EBs (BD sera) or vice versa (DB sera). Similar to the homologous B- and D-derived sera (figure 3), each heterologous pair neutralized both serovar B and D EBs and had high neutralization titers for the serovar that was used for priming (figure 4).

Sera produced using heterologous pairs that were more distantly related to one another and whose EBs were neutralized mutually exclusively, such as serovars C and I, were also tested. Both the CI- and IC-derived sera have been shown to recall antibodies to the original priming serovar in addition to developing cross-reactive antibodies [21]. While serovar C EBs could be effectively neutralized by serovar C sera but not by serovar I sera, and serovar I EBs were only neutralized by serovar I sera (figure 3), CI sera and IC sera each could neutralize both serovars (figure 4). As with the BD and DB heterologous pairs, there was a specificity preference for the original priming serovar.

**Role of VS region reactivity in neutralization.** Based upon the serovar specificity observed for neutralization and often similar specificity of the same sera for anti-VS1 reactivity [21], the serovar B- and C-derived murine sera were tested for an effect on neutralization following absorption of these sera with VS1, VS2, or VS4 synthetic peptides. Before and after absorption, the sera were tested by ELISA for seroreactivity to VS-specific peptides, and it was confirmed that the absorption had removed all detectable antibodies specific to the respective peptides (data not shown). Following absorption with peptides (100 µg/mL), there was no change in the ability of these sera to neutralize infectivity (data not shown), although by ELISA, <10 ng/mL was sufficient to reduce anti-VS1 reactivity by 50% [21].

It may be concluded from these experiments that the antibody specificities present in the murine polyvalent sera to antigens represented by VS-region peptides, although present as determined by ELISA [21], played little or no independent role in neutralization, even though high concentrations of monoclonal antibodies to these regions are capable of neutralization [8, 22]. This appears consistent with the observations that such synthetic peptides perform poorly at eliciting neutralizing antibodies when used as immunogens, although they produce high anti-peptide responses [17, 18]. These results demonstrate that serovar-specific neutralizing antibodies were present in the convalescent sera, independent of those defined exclusively by these VS-region synthetic peptides.

**Thermal lability of antigens.** One of the characteristics of potential conformational MOMP antigens is thermal lability following mild heat treatment [6, 20]; since antibodies responsible for neutralization were not absorbed by VS-region synthetic peptides, the potential role of conformation-dependent antibodies in neutralization was addressed by absorption of sera using heat-treated EBs. Serovar B and C EBs were treated at 4, 37, 40, 50, 60, and 100°C for 10 min and then used to absorb serovar B- and C-specific sera prior to neutralization of untreated EBs. Sera absorbed with EBs that had been heat-treated at 60 or 100°C retained the ability to neutralize, whereas sera absorbed with EBs treated at 4, 37, 40, or 50°C had lost the ability to neutralize infectivity (figure 5). Absorption losing serial dilutions of EBs pre-treated at different temperatures revealed that treatment at 50°C was intermediate in effect on the ability of EBs to absorb neutralizing antibodies (table 1).
The most straightforward interpretation of the data is that a serovar-specific heat-sensitive conformational determinant(s) accounts for virtually all of the ability of the murine sera to effectively neutralize infectivity. While it is formally possible that a different target antigen is lost or denatured by mild heat treatment, the observation that the neutralizing specificity is serovar-specific suggests the serovariant MOMP is the prime candidate.

**MOMP antigens are dominant targets of neutralization.** To establish that the putative serovar-specific antigens responsible for neutralization are defined by MOMP, the serovar B and C MOMP genes were cloned and expressed in *E. coli* as described [27]. It was confirmed that these clones produced substantial, and relatively equivalent, amounts of MOMP by immunoblot (figure 6). By immunofluorescence, unfixsed *E. coli* bound MOMP-specific monoclonal antibodies (data not shown), suggesting surface accessibility, as previously shown for serovar L2 MOMP [27]. Absorption of the murine serovar B- or C-derived sera with the homologous induced clone resulted in >98% reduction of the ability of these sera to neutralize infectivity (figure 7). The antigen specificity of absorption was verified by neutralization assessments following absorption of serovar B-specific sera with *E. coli* expressing serovar C MOMP (figure 7A) and serovar C-specific sera with *E. coli* expressing serovar B MOMP (figure 7B). The level of heterologous reduction of neutralization was relatively low. Whether this was due to cross-reacting MOMP antigens or nonspecific adsorption was unclear, as absorption of sera with *E. coli* lacking the MOMP gene reduced neutralization to levels similar to those of the heterologous MOMP adsorptions (data not shown).

These data demonstrate that B or C MOMP's expressed on the surface of *E. coli* absorb antibodies that recognize serovar-specific MOMP conformational determinants that on native EBs are primarily responsible for neutralization. The presence of heat-sensitive MOMP antigenic determinants was substantiated by finding that *E. coli* that expressed MOMP and were heat-treated at 100°C for 10 min lost the ability to absorb neutralizing antibodies (data not shown). It can be concluded from these data that the conformational serovar-specific antigen absorbed with heat-treated elementary bodies (EBs).

**Figure 5.** % neutralization of serovar B and C elementary bodies (EBs) with serovar B- and C-derived sera after absorption with EBs pretreated at indicated temperature for 10 min. Solid bars, serovar B-derived sera with serovar B EBs; open bars, serovar C-derived sera with serovar C EBs. For each determination, SE was <10% of total mean no. of counts.

**Table 1.** Relative effects on neutralization of infectivity by sera absorbed with heat-treated elementary bodies (EBs).

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<th>Temperature</th>
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<td>4°C</td>
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* Log4 dilution of EBs that absorbed 50% of ability of sera to neutralize infectivity.
Figure 6. Immunoblot demonstrating expression of chlamydial major outer membrane protein (MOMP) in *Escherichia coli*. Proteins in total lysates of induced *E. coli* used for antisera absorption were separated by SDS-PAGE, transferred to nitrocellulose sheets, and probed with MOMP-specific monoclonal antibody (2C5) as described [27]. Lane 1, *E. coli* (HMS 174) without MOMP gene; lane 2, *E. coli* containing serovar C MOMP gene; lane 3, *E. coli* containing serovar B MOMP gene. Bracket indicates MOMP that differs in mass for serovars C and B [26].

definitively is MOMP and expression of the chlamydial MOMP in *E. coli* produces a population of MOMP that is antigenically authentic in terms of conformation-dependent functional capabilities related to neutralization of chlamydial infectivity.

Discussion

Antigenic variation by microbial pathogens represents a fundamental virulence capability that promotes persistence by evading the host immune response. The primacy of conformational antigenic determinants that mediate chlamydial neutralization has implications for other bacterial pathogens, such as *Neisseria* and *Borrelia* species, that display antigenically variant surface proteins and have eluded understanding of structure-function activity because of structural complexity. The immune mechanisms that define protection or resolve *C. trachomatis* infection in humans are not conclusively known [30]. While antibody-mediated neutralization of chlamydiae has not been directly demonstrated to be an essential mechanism for immune protection or resolution of *C. trachomatis* infection, four factors support the conclusion that the antibody response to the only known variant surface antigen, MOMP, is an important mediator of immunity to *C. trachomatis* infection: the serovar-specificity of resistance to challenge [31], the presence of a polymorphic MOMP surface antigen [32], the selection of MOMP allelic variants in human populations with high prevalence of chlamydial infection [11, 12], and the demonstration that antibodies specific for serovariant-specific surface antigens can neutralize infection experimentally in vivo [6].

Defining the immunodominant neutralizing determinants is essential to understanding immunity, whether the mechanism of neutralization in vivo is by preventing EBs from infecting host cells [33] or by the presence of surface antibody that precipitates other effector mechanisms, such as complement-mediated killing or opsonization. The MOMP quantitatively dominates the surface architecture of the chlamydial EB and defines serovar specificity; however, its physical and antigenic structure is complex and not understood beyond the level of primary sequence [30, 32]. Although the mouse is not a natural host for *C. trachomatis* biovars that are natural pathogens of humans, mice can be infected with these strains and resolve infection by immune-specific mechanisms [34–37]. We sought to characterize the ability of sera to neutralize chlamydial infection in vitro using murine sera obtained following infection, recovery, and challenge, rather than using sera obtained by immunization.

Convalescent sera obtained from mice that had been inoculated and challenged with native EBs representing serovars A, B, C, D, G, and I [21] effectively neutralized infectivity, typically in a serovar-specific fashion. Whether the data are viewed by the ability of a given serovar to be neutralized by sera derived from different serovars or by the ability of a given serovar-elicted serum to neutralize different serovar EBs, they show that neutralization across the B and C serogroups is largely ineffective, and neutralization of serovars within a sero-
group is preferentially serovar-specific. It appears that sera derived from serologically junior serovars, such as serovars I and G, could mediate neutralization across a broader range of serovars than senior serovars, such as serovars C and B, whose sera were highly serovar-specific for neutralization.

Given that individuals may be reinfected following resolution or treatment of previous infections, the specificity of sera to neutralize EBs was evaluated for sera derived following a primary infection with one serovar and a challenge with a different serovar. Since challenge with serovars from different serogroups do not show serologic cross-reactivity [21], sera that reflect challenge with a closely related serovar were tested. In each heterologous challenge there was an antibody recall of the serospecificity of the priming serovar that neutralized infectivity. Neutralizing responses to the challenge serovar also were produced. These data suggest that boosting with closely related but distinct serovars will elicit the recall of antibodies that are capable of neutralizing both the priming serovar and the challenge serovar not previously encountered. The preference for higher neutralization titers for the priming serovar likely reflects the higher affinity of antibodies to the priming serovar for these sera [21].

The neutralization data obtained following absorption of antiserum strongly support a dominant role for antibodies that recognize conformational MOMP antigens in sera derived following infection. Absorbed sera were tested using dilutions above maximal neutralization to design the most sensitive measure of immunodominant antibodies that mediate neutralization [38]. Although monospecific [16] and monoclonal antibodies [6, 7, 39, 40] specific for single VS peptides are capable of mediating in vitro neutralization of chlamydiae and the murine polyvalent sera contained antibodies to VS-region peptides, absorption of the murine antiserum with VS-region peptides had no effect in diminishing the ability of these sera to neutralize infectivity. Absorption of sera with native EBs readily removed all ability of sera to neutralize infectivity; however, heating EBs for 10 min at temperatures >50°C resulted in loss of their ability to absorb neutralizing antibodies. These data demonstrate that in vitro neutralization is mediated by serovar-specific heat-sensitive antigenic determinants that are the immunodominant targets of antibodies elicited following resolution of infection in this murine model.

Koehler et al. [27] previously demonstrated that expression of the entire serovar L2 MOMP gene in E. coli results in surface localization of MOMP apparently inserted into the E. coli outer membrane. E. coli expressing chlamydial serovar B or C MOMP specifically absorbed the antibodies that mediated serovar-specific neutralization, unequivocally demonstrating that serovariant neutralization of EBs was mediated by MOMP antigens. That the ability of these sera to neutralize infectivity was dependent upon antibodies recognizing MOMP serovar-specific conformational determinants and that heat treatment of E. coli abolished the ability to absorb neutralizing antibodies additionally demonstrated that a significant proportion of MOMP expressed in E. coli was structurally and conformationally equivalent to native MOMP antigens in EBs.

We conclude from these studies using convalescent sera that the immunodominant and, hence, optimal targets for neutralization are represented by conformation-dependent and serovar-specific MOMP antigens. This is a surprising conclusion with profound implications for understanding antibody-mediated immune protection and vaccine design. The data suggest that an efficacious MOMP-based chlamydial vaccine must mimic conformational determinants. Monoclonal antibody studies using a variety of serovars have shown that MOMP VS1, VS2, VS4 [9, 10], and VS3 [41] are each surface-exposed, yet antibody reactivities to individual VS peptides played a minor role in neutralization compared with conformational antigens defined by the authentic structure of the entire protein or oligomers of protein. This suggests that each VS region structurally participates in delimiting the penultimate conformation-dependent neutralizing determinants. Presumably the VS2 region, having twice the sequence variation of the other VS regions, plays a defining role in conformation dependence. These findings may also be pertinent to the immunology of C. pneumoniae, in which antisera elicited by EBs neutralize infectivity but antisera elicited by MOMP peptides could not neutralize [42]. Given the findings of differences in relative efficacy of neutralization for MOMP-specific monoclonal antibodies that bind different VS-region peptides and their ability to compete for binding to EBs, Brunham and Peeling [8, 30] proposed that eliciting antibody responses to more conserved antigenic regions, such as VS4, actually inhibit neutralization by more effective serovar-specific neutralizing antibodies. This rationale may be expanded to consider that using single VS regions for vaccination, such as VS1 [15], would also be competitive and thus counter-productive for highly protective anti-MOMP antibody responses defined by conformation-dependent MOMP antigens.

The antigen conformation dependence of chlamydial neutralization and the sequence-variant character of MOMP suggest that minor changes in MOMP primary sequence, found even among strains of the same serovar designation [11], may affect the efficacy of conformation-dependent neutralization sufficiently to initially facilitate escape from rapid neutralization. This view is supported by the observation that sera obtained from several patients could neutralize a serovar D strain genetically similar to the serovar D isolate characterized as the infecting strain, but these sera could not neutralize one or more of very closely related serovar D variants that differ by only one or two amino acid substitutions [43]. However, our findings, that challenge with a different but related serovar elicits the recall of neutralizing antibody responses to the primary serovar as well as antibodies that neutralize the related serovar, suggest that a vaccine composed of relatively few serovars, especially junior serovars, may be sufficient to prime individu-
als for an effective immune response. Given the inability to genetically manipulate chlamydiae [32] and the ability of the MOMP expressed in E. coli to mimic conformation-dependent neutralizing antigenic determinants, the expression system developed by Koehler et al. [27] will provide a heretofore unavailable genetic experimental approach permitting manipulation of the MOMP gene to investigate the defining contributions to MOMP conformation-dependent neutralizing determinants.

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