Chlamydial polymorphic membrane proteins: regulation, function and potential vaccine candidates

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Pmps (Polymorphic Membrane Proteins) are a group of membrane bound surface exposed chlamydial proteins that have been characterized as autotransporter adhesins and are important in the initial phase of chlamydial infection. These proteins all contain conserved GGA (I, L, V) and FxxN tetrapeptide motifs in the N-terminal portion of each protein. All chlamydial species express Pmps. Even in the chlamydia-related bacteria Waddlia chondrophila, a Pmp-like adhesin has been identified, demonstrating the importance of Pmps in Chlamydiaceae biology. Chlamydial species vary in the number of pmp genes and their differentially regulated expression during the infectious cycle or in response to stress. Studies have also demonstrated that Pmps are able to induce innate immune functional responses in infected cells, including production of IL-8, IL-6 and MCP-1, by activating the transcription factor NF-κB. Human serum studies have indicated that although anti-Pmp specific antibodies are produced in response to a chlamydial infection, the response is variable depending on the Pmp protein. In C. trachomatis, PmpB, PmpC, PmpD and PmpI were the proteins eliciting the strongest immune response among adolescents with and without pelvic inflammatory disease (PID). In contrast, PmpA and PmpE elicited the weakest antibody response. Interestingly, there seems to be a gender bias for Pmp recognition with a stronger anti-Pmp reactivity in male patients. Furthermore, anti-PmpA antibodies might contribute to adverse pregnancy outcomes, at least among women with PID. In vitro studies indicated that dendritic cells infected with C. muridarum were able to present PmpG and PmpF on their MHC class II receptors and T cells were able to recognize the MHC class-II bound peptides. In addition, vaccination with PmpEGF and Major Outer Membrane Protein (MOMP) significantly protected mice against a genital tract C. muridarum infection, suggesting that Pmps may be an important component of a multi-subunit chlamydial vaccine. Thus, Pmps might be important not only for the pathogenesis of chlamydial infection, but also as potential candidate vaccine proteins.

Introduction

The Chlamydia genus encompasses 11 species: C. trachomatis (human sexually transmitted disease and eye infection), C. muridarum (causes disease in mice and hamsters), C. suis (infects pigs), C. pneumoniae (responsible for human respiratory infections), C. pecorum (common pathogen in livestock), C. psittaci (prevalent in birds and causing pneumonia in humans), C. abortus (causes abortion in mammals), C. felis (species found in cats), C. caviae (species causing infection in guinea pigs), C. avium (comprising strains from pigeons and psittacine birds) and C. gallinacea (strains from poultry).1-3 C. trachomatis can cause sexually transmitted diseases, which can lead to ectopic pregnancies, pelvic inflammatory disease (PID), tubal infertility, and miscarriage.4-11 C. trachomatis is of particular importance to human health because the infection is mostly asymptomatic and induces inflammatory responses that can lead to immunopathological sequelae. The World Health Organization estimates that since 2008 there are over 100 million new sexually transmitted cases due to C. trachomatis infection.12 C. trachomatis can also cause trachoma (ocular disease) that can lead to scarring and blindness.13,14 In fact, trachoma is the leading cause of blindness worldwide.

Chlamydia exists as either the infectious, non-replicating extracellular elementary body (EB) or the reticulate body (RB), which is noninfectious, replicating and strictly intracellular.15 For such an obligate intracellular bacteria, one of the most important steps for infecting eukaryotic cells is the attachment to host’s cells mediated by adhesin proteins. Several adhesins have been identified, including OmcB,16 and polymorphic membrane proteins.

In this review we will discuss the main characteristics of polymorphic membrane proteins (Pmps), which are autotransporter-like immunogenic surface-exposed proteins that have been found to play an important role not only as adhesins, but also as potent antigenic proteins involved in the immunopathogenesis of chlamydial infections. We will review the regulation of Pmps and describe their diversity in the different chlamydial species with a particular focus on C. trachomatis and C. pneumoniae (Table 1). We will also discuss their functional properties as adhesins as well
as their role in pathogenesis, especially by triggering cytokine production and inducing inflammation and pathological lesions. Finally, their utilization as potential chlamydial vaccine candidates will also be presented.

Structure and Regulation of PMPs

Pmps are a group of membrane bound proteins present in all chlamydial species. These proteins are grouped together by the fact that all exhibit conserved GGA(I, L, V) and FxxN tetrapeptide motifs in their N-terminal portion.17 Pmps were first identified in *C. psittaci*18,19 and subsequent studies have shown that all the other members of the *Chlamydia* genus also encoded Pmp proteins.20-24 However, chlamydial species vary in the number of pmp genes. Figure 1 depicts the main characteristics of Pmp proteins in reference strains *C. abortus* S26/323, *C. avium* 10DC88,2 *C. caviae* GPIC,22 *C. felis* Fe/C-56,20 *C. gallinacea* 08-1274/3,2

Table 1. Summary of Pmp research

| Study | References |
|-------|------------|
| Genes regulation/Characterization | |
| C. trachomatis (pmpA-I) | 17,49,54,55 |
| C. trachomatis (pmpD) | 31,65,100 |
| C. trachomatis (pmpF) | 29 |
| C. trachomatis (pmpC, D, E) | 28 |
| C. pneumoniae (pmp1-13, pmp14, pmp15-18, pmp19, pmp20, pmp21) | 67,101 |
| C. pneumoniae (pmp1-21) | 45 |
| C. felis | 20 |
| C. psittaci (G family) | 24 |
| Protein Regulation/Characterization | |
| C. trachomatis (PmpA-I) | 17,53,54 |
| C. trachomatis (PmpE, G, H) | 52 |
| C. trachomatis (PmpG, H) | 102 |
| C. trachomatis (PmpD) | 31,47,58,100,103 |
| C. trachomatis (PmpB, D, F, H, G) | 51 |
| C. pneumoniae (Pmp2, 6, 7, 8, 10, 11, 13, 14, 20, 21) | 43 |
| C. pneumoniae (Pmp1-13, Pmp14, Pmp15-18, Pmp19, Pmp20, Pmp21) | 33 |
| C. pneumoniae (Pmp21) | 104 |
| C. abortus (Pmp18D) | 18,19 |
| Protection against Chlamydia infection in mice | |
| C. abortus (Pmp18D) | 96 |
| C. abortus (Pmp17) | 105 |
| C. muridarum (PmpG, PmpF) | 86,88,89,91 |
| C. muridarum (PmpG) | 92,94 |
| C. muridarum (PmpD) | 90 |
| C. trachomatis (PmpE, G, H) and C. muridarum (PmpG, G) | 93 |
| MHC class I/II molecules | |
| C. abortus (Pmp18D) | 96 |
| C. muridarum (PmpG, PmpF) | 86,88,89,91 |
| C. trachomatis (PmpE, G, H) and C. muridarum (PmpG, G) | 93 |
| CD4+ T cells | |
| C. abortus (Pmp18D) | 96 |
| C. muridarum (PmpG) | 92,94 |
| C. muridarum (PmpG, Pmp/E-F-2) | 86,89,91 |
| C. trachomatis (PmpE, G, H) and C. muridarum (PmpG, G) | 93 |
| C. trachomatis (Pmp/E-H) | 90 |
| C. trachomatis (PmpG) | 85,95 |
| C. trachomatis (PmpD) | 46 |
| C. pneumoniae (Pmp6, 8, 21) | 87 |
| C. psittaci (PmpD) | 98 |
| CD8+ T cells | |
| C. trachomatis (PmpG) | 85 |
| C. trachomatis (PmpI) | 76 |
| C. pneumoniae (Pmp6, 8, 21) | 67 |
| Cytokines | |
| C. abortus (Pmp18D) | 96 |
| C. muridarum (PmpG, PmpF) | 86,89,91 |
| C. muridarum (PmpG) | 92,94 |
| C. muridarum (PmpG, Pmp/E-F-2) | 86,89,91 |
| C. trachomatis (PmpE, G, H) and C. muridarum (PmpG, G) | 93 |
| C. trachomatis (Pmp/E-H) | 90 |
| C. trachomatis (PmpG) | 85,95 |
| C. trachomatis (Pmp6, 8, 21) | 46 |
| (continued) |

as their role in pathogenesis, especially by triggering cytokine production and inducing inflammation and pathological lesions. Finally, their utilization as potential chlamydial vaccine candidates will also be presented.
Figure 1. Schematic representation of Pmp proteins in *Chlamidiaceae*. The UniProt identifier, RefSeq identifier and the amino acid length are shown for each Pmp protein. Conserved domains were detected using MOTIF search (http://www.genome.jp/tools/motif/): FxxN motifs (yellow), GAA (I,L,V) motifs (blue), central PMP_M region (green) and autotransporter β-domain (red). Proteins containing frameshift mutations were omitted in the figure.
C. muridarum Nigg, C. pecorum DBDeUG, C. pneumoniae CWL029, C. psittaci ATCC VR-125/6BC, and C. trachomatis D/UW-3/Cx. Alternative names of Pmp in the Chlamydia genus are shown in Figure S1.

Studies have demonstrated that C. trachomatis (serovar A/HAR13 and D/UW-3) and C. muridarum (strain Nigg) both contain 9 pmp genes whereas 17 and 18 genes encode for Pmps in C. caviae and C. abortus, respectively. Of note, a pmp-like encoding gene has also been identified in the genome of the Chlamydia-related bacteria Waddlia chondrophila.

Numerous studies have indicated that Pmps function as autotransporters. Autotransporter proteins are characterized by 3 functional domains, which include a cleavable N-terminal signal for translocation to the membrane, a passenger domain for surface localization or secretion and a C-terminal β-barrel translocator sequence for outer membrane translocation. Pmp proteins share many of the characteristics of classical autotransporters including an N-terminal Sec-dependent leader sequence, a C-terminal β-barrel sequence and a passenger domain.

Autotransporter proteins are widespread in Gram-negative bacteria.
bacteria and contribute to virulence of many pathogens.\textsuperscript{34,36,39} These virulence factors include among others secreted proteases (\textit{Neisseria gonorrhoeae}, \textit{Haemophilus influenzae} and \textit{Shigella flexneri}), adhesins (\textit{Yersinia enterocolitica}, pathogenic \textit{Escherichia coli}, \textit{Salmonella enterica} and \textit{Bordetella pertussis}).\textsuperscript{38,40} Bacteria from the \textit{Chlamydia} genus possess a large number of autotransporter genes compared to other gram-negative bacteria, suggesting an important role of these chlamydial autotransporter proteins in pathogenesis. \textit{C. pneumoniae} contains 21 Pmps (5 of which contain frameshift mutations), which account for 17.5\% of its coding capacity.\textsuperscript{41} In addition to MOMP and outer membrane protein 2 (Omp2), Pmps represent the major proteins in \textit{C. pneumoniae} outer membrane complex.\textsuperscript{32} These Pmps are characterized by subtype G containing Pmp1-13 and subtype E encompassing Pmp15-18. The remaining 4 subtypes (Pmp14, 19, 20, 21) are represented by a single gene.\textsuperscript{32} Studies using 2D-PAGE and RT-qPCR have demonstrated that all \textit{pmp} genes are transcribed and expressed during the \textit{C. pneumoniae} developmental cycle\textsuperscript{43,44} and encode for proteins of various sizes.\textsuperscript{32} Pmp6, pmp20 and pmp21 genes encode for proteins that are between 1408 and 1724 amino acids long whereas \textit{pmp12}, \textit{pmp3}, \textit{pmp4}, \textit{pmp5}, \textit{pmp6}, \textit{pmp7}, \textit{pmp8}, \textit{pmp9}, \textit{pmp10}, \textit{pmp11}, \textit{pmp12}, \textit{pmp13}, \textit{pmp14}, \textit{pmp15}, \textit{pmp16}, \textit{pmp17}, \textit{pmp18}, \textit{pmp19}, \textit{pmp20}, \textit{pmp21}, \textit{pmp22}, \textit{pmpA}, \textit{pmpB}, \textit{pmpC}, \textit{pmpD}, \textit{pmpE}, \textit{pmpF}, \textit{pmpG}, \textit{pmpH} and \textit{pmpI} genes transcribe proteins that are shorter due to either truncation of the C-terminal (i.e. \textit{pmp12}) or frameshift mutation (i.e., \textit{pmp3}, \textit{pmp4}, \textit{pmp5}, \textit{pmp6}, \textit{pmp7}, \textit{pmp8}, \textit{pmp9}, \textit{pmp10}, \textit{pmp11}, \textit{pmp12}, \textit{pmp13}, \textit{pmp14}).\textsuperscript{45} Pmp proteins can also be processed into smaller fragments as in the case of Pmp6, 20 and the PmpD ortholog Pmp21.\textsuperscript{32} Studies investigating \textit{C. pneumoniae} Pmp processing suggest that the EBs produce at least 3 different forms of Pmp21 (N, M and C) and that the N-Pmp21 and M-Pmp21 forms are expressed on the chlamydial surface.\textsuperscript{32,33,46}

The \textit{C. trachomatis} genome encodes for 9 Pmp proteins termed PmpA-I that are further subdivided into A (PmpA), B (PmpB, C) D (PmpD), E (PmpE, F) G (PmpG, I) and H (PmpH) subtypes.\textsuperscript{42} Similar to \textit{C. pneumoniae}, a high percentage of coding capacity (13.6\%) is dedicated to \textit{C. trachomatis} \textit{pmp} genes\textsuperscript{28,41,42} suggesting that these genes play an important role in \textit{Chlamydia} biology and pathogenesis.\textsuperscript{47,48} Proteins between subtypes share variable similarities in terms of amino acid sequences. Indeed, \textit{C. trachomatis} (serovar E) PmpG and PmpI (subtype G) are 25\% similar and PmpB and PmpC (subtype B) share 43\% homology.\textsuperscript{42} There is also interspecies and inter-serovar Pmp homology. \textit{C. trachomatis} subtype PmpD and \textit{C. pneumoniae} Pmp21 share 33\% homology at the protein level\textsuperscript{42} and PmpD is the second highest conserved Pmp demonstrating 99.2\% homology among \textit{C. trachomatis} serovars.\textsuperscript{49} These similarities suggest common functional roles across chlamydial serovars and species for Pmps. However, there is some controversy on whether all 9 of the \textit{C. trachomatis} Pmps are expressed. Early investigations detected only PmpB, D, E, F, G and H,\textsuperscript{26,42,48} whereas later studies...
demonstrated that all *C. trachomatis* pmp genes were transcribed.17,53 *C. trachomatis* pmp genes are located in 2 clusters with pmpA-C and pmpDE-I comprising each cluster and pmpD being genetically isolated.58 Furthermore, pmpABC, pmpDE and pmpGH are co-transcribed in *vitro* indicating that these genes are organized in operons.54 Of all of the *C. trachomatis* Pmps, PmpA, D and I are the most conserved, with PmpA having 99.9%, PmpD 99.1% and PmpI 99.2% amino acid sequence similarity.54 All 9 pmps have been shown to be transcribed in *vitro* in various *C. trachomatis* strains including serovars D, E, and L217,31,55 and are all translocated to the bacterial surface.17 However, *C. trachomatis* pmp paralogues exhibit variable transcription patterns. Expression studies conducted by Kiselev and colleagues demonstrated that the pmpD gene from *C. trachomatis* serovar L2 was upregulated between 16 and 24 hours after infection, which is the time RBs differentiate into EBs.31 Studies by Carrasco et al. and Nunes and colleagues demonstrated that pmpA transcription peaked at 12 and 18 hours post infection.54,55 Similarly, pmpI was transcribed at 18 hours post infection. PmpBC, pmpEF, and pmpGH were all co-transcribed at later time points (32 and 48 hours post infection).54

There is evidence that Pmps are differently regulated in response to stress. Penicillin, a β-lactam antibiotic, is known to disrupt the growth of *Chlamydia* by blocking the conversion of RBs into EBs and induce so-called aberrant bodies.56,57 Further studies have demonstrated that penicillin also modulates the transcription and protein processing of several Pmps. Carrasco and colleagues demonstrated that when exposed to penicillin, transcription of pmpB, C, E, F, G, and H was downregulated, whereas expression of pmpA, D and I was mostly unaffected.54 The fact that pmpA, pmpD and pmpI were transcribed even under stress conditions such as penicillin suggests a critical role for these Pmps for the survival of the organism in a hostile environment and may be important for pathogenesis. Similarly, Kiselev et al. demonstrated that in the presence of penicillin, the cleavage and secretion of the passenger domain of PmpD was suppressed in *C. trachomatis* L2 serovar. This suppression was mediated by the inhibition of membrane proteases by penicillin, such as signal peptidases I, which cleave the autotransporter protein.31 These data suggest that penicillin may inhibit Pmp transcription and post-translation processing in different chlamydial serovars and strains.

Studies on PmpD have suggested that Pmps undergo an extensive processing before their translocation to the chlamydial surface and exist as oligomers with a flower-like structure.78 Werhl *et al.* has suggested a multistep process where the full length PmpD is exported to the periplasmic space via the Sec machinery. Then, after the signal sequence is cleaved, the C-terminal portion forms a β-barrel and the N-terminal passenger domain is exported and cleaved, resulting in an N-terminal surface exposed protein.35 Several investigators have generated antibodies against the N-terminal portion of all *C. trachomatis* Pmps including PmpD,17,47 further supporting the hypothesis that Pmps are present on the surface of *Chlamydia* and that the N-terminal portions of these proteins are accessible to antibodies. However, in a study by Kiselev *et al.*, anti-PmpD polyclonal antibodies only recognized PmpD on the surface of RBs and not EBs31 suggesting only RBs are able to express Pmps on their outer surface membrane. In addition, more recent studies showed that PmpD might also be expressed on EBs but at a lower level.59 Noteworthy, Molken and colleagues demonstrated that *C. pneumoniae* Pmp21 is located on the surface of both EBs and RBs during infection60 indicating that the preferential expression of PmpD of *C. trachomatis* on RBs is not a common feature shared by all chlamydial Pmps.

**Functional Properties as Adhesins**

In 2004, Wehrl *et al.* investigated the ability of a polyclonal rabbit serum raised against N-Pmp21 to inhibit *C. pneumoniae* infection in *vitro*.55 A year later, Finco and colleagues demonstrated a similar result using a mouse kidney cell line (LLC-MK2) and polyclonal mouse sera to Pmp2 and Pmp10.61 These studies highlighted the importance of Pmps in the initial phases of infection. Furthermore, their role as adhesins was suggested by the fact that *Anaplasma phagocytophilum* express adhesins that contain the GGA(I, L, V) and FxxN motifs62 which are also present in chlamydal Pmps.

Molken *et al.* demonstrated that the *C. pneumoniae* Pmp21 adhesion to cell surface receptors required at least 2 copies of the Pmp repeats: either FxxN+FxxN or FxxN+GGA(I, L, A). Thus, scrambling one of the Pmp21-derived synthetic peptide FxxN motifs did not modulate the adhesive property of Pmp21, whereas deleting the 2 tetrapeptide FxxN sequences resulted in significant reduction or complete loss of adhesion capacity.60 They proposed that these repeats either indirectly modulate the protein conformation implicated in adhesion or directly modulate adhesin interaction with the eukaryotic receptors. Moreover, a more recent study identified EGFR (epidermal growth factor receptor) as the receptor for Pmp21.63 *Figure 1* depicts the localization of GGA(I, L, V) and FxxN motifs of Pmp proteins in reference strains of the *Chlamydia* genus.

*C. trachomatis* PmpD has also been shown to be a surface exposed and neutralizing target for anti-PmpD antibodies. Neutralizing assays were performed on HaK (hamster kidney) cells using 3 major *C. trachomatis* serogroups representing ocular serovars (A, Ba, C), noninvasive genital serovars (D, E, F, G, K) and genital invasive serovar (L2). Results from this study indicated that serovars Ba, D, E and L2 were more efficiently neutralized than serovars A, C, F, G and K. The α-PmpD serum failed to neutralize *C. muridarum, C. pneumoniae* and *C. caviae* indicating that PmpD is a specific strain-dependent neutralizing target.37 Interestingly, this study also demonstrated that antibody to MOMP and LPS blocked the ability of PmpD anti-serum to neutralize the chlamydial infection in HaK cells. MOMP and LPS are 2 of the most abundant immunodominant antigens on the EB surface.64 This could imply that during an infection, MOMP and LPS may act as decoys for neutralizing antibodies by blocking their binding to Pmps, which are important in the initial phase of
infection. A 2014 study conducted by Becker et al. investigated the ability of all C. trachomatis serovar E Pmps to act as adhesins in 2 human cell lines. The study demonstrated that PmpA-I mediated adhesion to human epithelial (HeLa) and endothelial (Hep-2) cells suggesting all C. trachomatis Pmps are involved in the chlamydial infection process and implicating the Pmps as a group of virulence factors. There is also evidence that Pmps may exist as 2 distinct forms and are variably expressed depending on whether they are on EBs or RBs. Western blot analysis and scanning electron microscopy studies have indicated that PmpD is more abundant on RBs than EBs and the RBs have a more homogeneous distribution whereas the PmpD on the EB exhibited a more polarized localization. 

Furthermore, PmpD might not only function as a surface protein, but also undergoes multiple proteolytic processing resulting in a soluble protein containing 3 fragments (p111, p73 and p30) that is restricted to infected cells. The authors of this study hypothesized that since the PmpD fragments are similar to Neisseria secreted IgA protease H. pylori VacA secreted toxins, the p111, p73 or p30 proteins may interact with immune cells to induce inflammatory cascades that are important in pathogenicity. Recently, a study conducted by Kari and colleagues used a serovar D pmpD null mutant, to define the role of PmpD in the pathogenesis of chlamydial infection. Although the study demonstrated that pmpD is not an essential chlamydial gene, pmpD mutants did exhibit abnormal morphology or ultrastructural phenotype and were significantly deficient in host-cell attachment in human epithelial cells during early host-cell interactions (70% reduction of the infection ability). When they performed the same experiment using a mouse cell line, the pmpD mutant and wild-type C. trachomatis EBs were equally competent in their ability to infect mouse cells. Although mouse and human chlamydial strains share 72% PmpD sequence similarity, the possible subtle differences in the proteins structure and configuration between the mouse and human PmpD may account for different infection capabilities and tropism. Using a macaque model of ocular infection, the authors also demonstrated that chlamydial burden was significantly reduced in monkeys infected with the pmpD mutant compared to control monkeys infected with wild-type C. trachomatis during the first 14 days of infection. Interestingly, at later time points (2 weeks post-infection), there were no differences in terms of ocular bacterial burden. These data suggest that PmpD plays a critical role in chlamydial pathogenesis at the early stages of infection.

**Human Pathogenesis**

Human studies have demonstrated that although C. trachomatis-infected patients develop antibodies to Pmps, not all Pmps are recognized equally. Tan et al. showed that PmpB, C, D and I were the most frequently recognized and elicited a stronger humoral immune response, whereas PmpA and E were the lowest recognized in sera that included women with pelvic inflammatory disease (PID), adolescent young females and male patients. This finding suggests that these Pmps may be more frequently exposed or abundantly expressed on the C. trachomatis surface. Interestingly, there was a gender bias for Pmp recognition. Serum from male patients tended to have a stronger anti-Pmp reactivity compared with serum from female patients. Furthermore, PmpB was the most recognized Pmp in sera from female patients (82% of serum) whereas in male sera PmpD was the most recognized Pmp (92% of serum). There was also a difference between adolescent female patients and women that exhibited PID. Antibodies specific for PmpB and PmpI were more prevalent in the PID group suggesting that these Pmps might contribute to the development of chronic inflammation. Another study investigating human sera in women with PID suggested that PmpA might have a role in adverse pregnancy outcomes. Taylor et al. demonstrated that women who had PID and also tested positive for anti-PmpA specific antibodies had a significantly lower chance of getting pregnant, less likely chance to have a live birth, and were more likely to have an upper genital tract infection than women who did not test positive for anti-PmpA antibodies. Furthermore, women who were positive for PmpI also were more likely to have upper genital tract infections.

**C. trachomatis** includes 3 human biovars composed of different serovars. Serovars Ab, B, Ba cause trachoma. Serovar D-K cause urethritis, PID, ectopic pregnancy and neonatal pneumonia and serovars L1, L2 and L3 are able to cause lymphogranuloma venereum (LGV). Gomes et al. demonstrated that only sera from serovars D, E and G-infected patients reacted to recombinant PmpC whereas all infected patients reacted to MOMP. Nunes and colleagues performed a similar study where sera from 39 adolescents were tested for immunoreactivity to recombinant PmpD and PmpF. All sera from patients that were positive for chlamydial serovars Ba, E, F and K also reacted to PmpD protein. However, patients that were infected with strains D, Ia, J and G did not have immunoreactive sera to PmpD. Surprisingly, no sera from any of the infected patients reacted to recombinant PmpF. This suggests that the expression of Pmps in different chlamydial serovars is variably regulated and may induce differential immune responses with specific serovars. It may also indicate that PmpC and PmpD elicit an especially robust humoral immune response in adolescent females.

**Immunology**

Studies have also demonstrated that Pmps are able to activate innate immune functional responses in infected cells. A study by Niessen et al. tested the ability of 15 C. pneumoniae Pmps to induce cytokine production in a human cell line. The study revealed that only Pmp20 and Pmp21 were able to induce IL-6 and MCP-1 (monocyte chemoattractant protein-1) secretion in human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner. When the 2 Pmps were co-cultured in HUVECs there was no increase in cytokine production compared to a single Pmp HUVEC culture suggesting the 2 Pmps were not synergistic in their ability to elicit IL-6 and MCP-1 secretion. Furthermore, the study revealed that Pmp20 and
Pmp21 induce their effect on endothelial cell cytokine production by activation of NF-kB (nuclear factor kappa B) pathway, which is a major pathway for inflammatory activation. Another study investigating the ability of a *C. trachomatis* Pmp to induce cytokine production demonstrated that recombinant PmpD incubated with a human monocyte cell line (THP-1) induced robust IL-8 production in a dose dependent manner.

Vaccine Studies

The asymptomatic nature of a chlamydial infection and its subsequent long-term consequences such as ectopic pregnancy, preterm delivery and infertility makes a chlamydial vaccine paramount. The first chlamydial vaccine studies utilized avirulent intact live *C. trachomatis* as a prophylactic for trachoma infection. However, even though in some cases the vaccine provided considerable protection from infection and pathology, some vaccinated individuals developed more severe disease after subsequent *C. trachomatis* infection. Vaccines containing live organisms are optimal because they contain not only native antigens that are recognized by antigen presenting cells but also negate the need for adjuvants. However, the drawbacks include the need for cold storage and the possibility of avirulent strains converting back to a strain that is able to cause disease. Subsequent chlamydial vaccine studies have utilized multiple strategies including subunit antigenic determinants, recombinant proteins and plasmid DNA in conjunction with adjuvants or other delivery vehicles to increase immunogenicity. Even though chlamydial major outer membrane protein (MOMP) is the most investigated vaccine candidate in animal and nonhuman primate studies, the protection elicited has been suboptimal necessitating further research for more efficacious vaccine candidates.

Immunity against *Chlamydia* requires CD4+ T cells and to a lesser degree CD8+ T cells that recognize specific chlamydial antigens on MHC molecules and the production of INF-γ. One of the major difficulties in developing an effective chlamydial vaccine is identifying the MHC-bound chlamydial protein epitopes that are recognized by T cells. A 2008 study conducted by Grotenbreg *et al.*, investigated specific chlamydial epitopes that are able to activate CD8+ T cells. They discovered that MHC tetramers corresponding to PmpI-D were able to induce the expansion of purified CD8+ T cells from mice previously infected with *C. trachomatis* LGV serovar L2. A study conducted by Finco and colleagues demonstrated that chlamydial proteins including MOMP contain T cell and B cell epitopes or both. Other chlamydial proteins have also been proposed as candidates for a chlamydial vaccine including Omp2 (outer membrane protein 2), HPS60 (chlamydial shock protein 60), CPAF (chlamydial protease-like activity factor), Cap1 (class I accessible protein 1) and CrpA (cysteine-rich protein A).

Pmps generally contain only T cell epitopes and have also been proposed as vaccine candidates. Indeed, several studies have investigated the efficacy of various Pmps to protect against *Chlamydia* infection and activate T cells. Using affinity chromatography and tandem mass spectrometry, Karunakaran *et al.* demonstrated that dendritic cells infected with *C. muridarum* presented PmpG and F on their MHC class II receptors. Furthermore, purified *Chlamydia*-specific CD4+ T cells produced high levels of INF-γ after co-culture with the previously *C. muridarum*-infected dendritic cells suggesting T cell recognition of the MHC class-II bound Pmps. In another study, mice receiving adoptively transferred dendritic cells that were previously pulsed with PmpG and F were partially protected against intranasal and genital *C. muridarum* infection. A study conducted by Mygind and colleagues discovered that splenic cells from *C. pneumoniae* nasally infected mice produced significant amounts of INF-γ after incubation with recombinant Pmp8, Pmp20 and Pmp21 and the INF-γ production was shown to be mediated by CD4+ T cells. Recombinant Pmp6, 9, 10, 11 were recognized inconsistently over time but with low responses. Yu *et al.* showed that vaccination with PmpE/F and MOMP in conjunction with either CpG-ODN, AbISCO-100 or DDA/TDB adjuvants to protect mice from *C. muridarum* genital infection. The results demonstrated that a multisubunit vaccine containing PmpG + PmpE/F + MOMP + DDA/TDB exhibited the highest degree of protection. In addition, compared to CpG and AbISCO, DDA/TDB + PmpG induced the strongest INF-γ responses by CD4+ T cells. A later study by Yu *et al.* showed that vaccination with PmpE, F, G and H significantly protected mice against a genital tract *C. muridarum* infection as measured by cervicovaginal shedding. Furthermore, PmpG persisted on splenic antigen presenting cells for at least 6 months. Interestingly, splenocyte culture that were restimulated with PmpG, F, E and H from *C. muridarum* genetically infected mice demonstrated robust INF-γ production in response to PmpG and H, low production after PmpF restimulation and no production after PmpE restimulation. In fact, studies by various researchers have also demonstrated the immunodominance of PmpG in the murine model. A more recent study by Yu *et al.* investigated protection elicited by a multisubunit vaccine containing PmpEFGH + Th1 polarizing adjuvant DDA/MPL in a genital tract infection. They demonstrated that a vaccine containing PmpEFGH + MOMP + DDA/MPL was superior in protecting against a *C. muridarum* genital infection compared to PmpG, MOMP or PmpEFGH measured by cervicovaginal shedding. They also showed that PmpE and H have low stimulatory capacities to induce INF-γ production in CD4+ T cells and therefore may not be suited for use in a chlamydial multisubunit vaccine.

*C. trachomatis* PmpG has also been used as an epitope in conjunction with vault nanoparticles. These studies demonstrated that a PmpG-vault nanoparticle vaccine incubated with a human monocyte cell line (THP-1) activated the protease caspase-1 and IL-1β production. In addition, mice immunized with the PmpG/vault nanoparticle vaccine induced PmpG-specific CD4+ T cells that were able to be restimulated with PmpG in vitro. Whereas PmpEFGH + MOMP and adjuvants or PmpG + nanoparticles appear to be promising vaccine candidates for protection against *C. trachomatis*, PmpD may be an interesting candidate for protection against *C. abortus* which can cause ovine enzootic abortion and poses a risk to pregnant women. Mice
immunized with recombinant PmpD in a Vibrio cholerae ghost delivery system elicited a robust antigen-specific IFN-γ, IgA and IgG2c immune response after genital C. abortus challenge. Length of vaginal shedding and number of inclusion forming units recovered following C. abortus challenge was also significantly decreased.96 C. psittaci is an avian chlamydial strain that is capable of causing pneumonia, encephalitis and even death in humans.97 A recent study demonstrated that chickens inoculated with a recombinant herpesvirus of turkeys (HTV) expressing C. psittaci with a recombinant herpesvirus of turkeys (HTV) expressing a chlamydial infection.

A recent study utilizing nanoparticles demonstrated that robust protection against C. trachomatis was attained in the genital tract of mice after immunization with UV-inactivated C. trachomatis bound to nanoparticles carrying adjuvants. This study highlights the importance of the site of vaccination (vaginal mucosa) and the requirement of multiple vaccinations in order to develop an effective protection against the infection.99

**Conclusion**

Pmps are a family of membrane bound surface exposed proteins that are expressed in all chlamydial species and other Chlamydiaceae, including Waddlia chondrophila. Since divergence of Chlamydiaceae and Waddliaceae families occurred more than 1 billion years ago, Pmps have thus a long evolutionary history, assuming that pmp genes have not been transferred horizontally. Pmps have been characterized as autotransporter adhesin proteins that are important in the initial phase of infection and have been shown to induce cytokine production in infected cells. Furthermore, Pmps may be involved in human pathology and infertility. Utilized as a component of a subunit vaccine in conjunction with effective adjuvants and/or delivery systems, these outer membrane proteins may contribute to the development of an effective chlamydial vaccine that elicits a protective Th1-mediated immune response that does not induce adverse immunopathologies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**Supplemental Material**

Supplemental data for this article can be accessed on the publisher’s website.
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