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Original article

Induction of protective immunity against *Chlamydia muridarum* intravaginal infection with the chlamydial immunodominant antigen macrophage infectivity potentiator

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Abstract

We previously reported that 5 *Chlamydia muridarum* antigens reacted with antisera from >90% mice urogenitally infected with *C. muridarum* and they are TC0660 (ABC transporter or ArtJ), TC0727 (outer membrane complex protein B or OmcB), TC0828 (macrophage infectivity potentiator or MIP), TC0726 (inclusion membrane protein or Inc) & TC0268 (hypothetical protein or HP). The orthologs of these antigens in *Chlamydia trachomatis* were also highly reactive with antisera from women urogenitally infected with *C. trachomatis*. In the current study, we evaluated these *C. muridarum* antigens for their ability to induce protection against a *C. muridarum* intravaginal challenge infection in mice. We found that only MIP induced the most pronounced protection against *C. muridarum* infection. The protection correlated well with robust *C. muridarum* MIP-specific antibody and Th1-dominant T cell responses. The MIP-immunized mice displayed significantly reduced live organism shedding from the lower genital tract and highly attenuated inflammatory pathologies in the upper genital tissues. These results demonstrate that MIP, an immunodominant antigen identified by both human and mouse antisera, may be considered a component of a multi-subunit chlamydial vaccine for inducing protective immunity.

Keywords: *Chlamydia muridarum*; Protective immunity; Macrophage infectivity potentiator; Vaccine

1. Introduction

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen and imposes serious health problems in humans. A major clinical challenge of *C. trachomatis* infection is that most acutely infected individuals do not seek treatment due to lack of obvious symptoms, thus potentially developing severe complications, such as pelvic inflammatory disease, ectopic pregnancy, and infertility [1−3]. Additionally, short-lasting natural immunity against chlamydial infections and non-treatment of affected sexual partners result in high reinfection rates [2]. Obviously, a most effective means to prevent *C. trachomatis*-induced complications is vaccination. However, there is still no licensed *C. trachomatis* vaccine [4].

To this end, multiple vaccination strategies for protection against genital *C. trachomatis* infection have been evaluated. The failed human trachoma trials more than 50 years ago [5,6] and the immunological studies in the past half-century [4,7−9] suggested that a subunit vaccine strategy is both necessary and feasible. So far, the major outer membrane protein (MOMP) in

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its native conformation appears to be the most effective antigen [10]. However, recombinant MOMP, MOMP synthetic peptides, DNA vaccines encoding MOMP, and the passive transfer of MOMP-specific monoclonal antibodies were only partially effective in protecting experimental animals from subsequent challenge [11−17]. Furthermore, immunization with chlamydial protease-like activity factor (CPAF) [18−20], translocated actin-recruiting phosphoprotein or Tarp [21], and other structural or secreted proteins all showed only partial protection against genital *C. trachomatis* challenge [22−26]. Given the complex chlamydial organism structures and chlamydial infection biology, it is likely that immunization with combinations of various antigens with each able to induce unique effector mechanisms to simultaneously attack multiple stages of the infection processes may be necessary to achieve a full protection against chlamydial infections and diseases. Therefore, more novel vaccine candidates are in urgent need.

Many previous studies on chlamydial pathogenic mechanisms and evaluating vaccine candidates have been carried out using mouse models intravaginally infected with *Chlamydia muridarum* (also called MoPn). Mice infected with *C. muridarum* can develop upper genital tract pathology that closely resembles the pathology in human fallopian tube following *C. trachomatis* infection [27,28]. In our previous studies with *C. trachomatis* whole genome array, we identified 38 ORFs dominantly recognized by >40% urogenital infected human antisera [29]. When their orthologs in *C. muridarum* were further profiled with antisera from mice intravaginally infected with *C. muridarum*, five antigens (ArtJ, OmcB, MIP, Inc & HP) were dominantly recognized by >90% of mice regardless of their strain background and H-2 haplotypes [30]. Thus, these five antigens were highly immunogenic in both human and mice, suggesting that chlamydial infection in both human and mice requires the expression of these antigens and these antigens may be important in chlamydial pathogenesis.

In the current study, we examined the protection efficacy of intramuscular (i.m.) immunization with these five antigens against genital *C. muridarum* challenge. We found that intramuscular vaccination with MIP plus CpG-IFA induced the most pronounced protective immunity that partially protected mice from chlamydial live organism infection and infection-induced upper genital tract pathology. This protection correlates with a robust MIP- and MoPn-specific antibody and Th1-dominant T cell responses. Taken together, these results have demonstrated that an immunodominant chlamydial protein, MIP, can be considered a component of a multi-subunit vaccine against chlamydial infection and diseases.

2. Materials and methods

2.1. Chlamydial organisms

C. muridarum Nigg strain (also called MoPn) stocks were prepared as described previously [31,32]. Confluent monolayers of HeLa cells were grown in Dulbecco's modification of Eagle's medium with 10% FBS and infected with *C. muridarum*. Cells were lysed using a sonicator and elementary bodies (EBs) were purified on Renograffin gradients as described previously. Stocks were stored at −80 °C in sucrose−phosphate−glutamine (SPG) buffer and diluted appropriately for the challenge.

2.2. Prokaryotic expression of chlamydial fusion proteins and protein purification

Five *C. muridarum* antigens, including ArtJ, OmcB, MIP, Inc & HP, were used for the current study (http://www.ncbi.nlm.nih.gov/Sites/entrez). These antigens are dominantly recognized by >90% of mice antisera regardless of their strain background and H-2 haplotypes and their counterparts in *C. trachomatis* were significantly recognized by *C. trachomatis*-infected women [29,30]. The 5 ORFs were cloned into pGEX vectors and expressed as fusion proteins with glutathione-s-transferase (GST) fused to the N-terminus. Expression of the fusion proteins was induced with isopropyl-beta-D-thiogalactoside (IPTG; Invitrogen, Carlsbad, CA) and the fusion proteins were extracted by lysing the bacteria via sonication in a Triton-X100 lysis buffer (1% Triton-X100, 1 mM PMSF, 75 units/ml of aprotinin, 20 mM leupeptin and 1.6 mM pepstatin). After a high-speed centrifugation to remove debris, the fusion protein-containing supernatants were further purified using glutathione-conjugated agarose beads (Pharmacia). Then the beads were used to purify soluble proteins by cleavage with a precision protease (Pharmacia). The freed chlamydial proteins were concentrated via centricon (Millipore, Billerica, MA) and used to immunize mice for evaluating protective immunity as described previously [21,32].

2.3. Mice

For the initial screening experiment, female Balb/c mice were purchased at the age of 3−4 weeks old from Charles River Laboratories, Inc. (Wilmington, MA) and divided into 7 different groups with 5 in each group. The MIP immunization experiment was repeated once with 15 mice per group. Mice were housed at the University of Texas Health Science Center at San Antonio and were given food and water ad libitum. Animal care and experimental procedures were approved by the University of Texas Health Science Center at San Antonio Animal Use Protocol committee.

2.4. Immunization procedure

All mice were immunized i.m. for a total of 3 times on day 0, day 20 and day 30 respectively. A total of 30 μg protein antigen or 10^6 IFUs of *C. muridarum* (or MoPn) EBs plus 10 μg of CpG in 50 μl PBS emulsified in 50 μl of IFA (incomplete Freund's Adjuvant, Sigma−Aldrich, St. Luis, MO) was given to each mouse each time. The first group was given GST protein as negative and the second group with EBs as positive control. The remaining 5 groups were each immunized with one of the 5 test antigens. The CpG with a sequence of 59-TCC.ATG.ACG.TTC.CTG.ACG.TT-39 (all nucleotides are phosphorothioate-modified at the 3′

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