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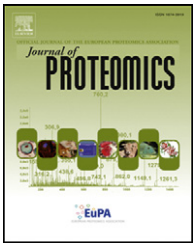

Proteomic identification of immunodominant chlamydial antigens in a mouse model

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ABSTRACT

Chlamydia trachomatis is the most common bacterial sexually transmitted pathogen in the world. To identify new vaccine candidates a protein microarray was constructed by expressing the open reading frames (ORFs) from *Chlamydia* mouse pneumonitis (MoPn). C57BL/6, C3H/HeN and BALB/c mice were immunized either intranasally or intravaginally with live MoPn elementary bodies (EB). Two additional groups were immunized by the intramuscular plus subcutaneous routes with UV-treated EB, using CpG and Montanide as adjuvants to favor a Th1 response, or Alum, to elicit a Th2 response. Serum samples collected from the three strains of mice were tested in the microarray. The array included the expression of 909 proteins from the 921 ORFs of the MoPn genome and plasmid. A total of 530 ORFs were recognized by at least one serum sample. Of these, 36 reacted with sera from the three strains of mice immunized with live EB. These antigens included proteins that were previously described as immunogenic such as MOMP and HSP60. In addition, we uncovered new immunogens, including 11 hypothetical proteins. In summary, we have identified new immunodominant chlamydial proteins that can be tested for their ability to induce protection in animal models and subsequently in humans.

1. Introduction

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen and the leading cause of preventable blindness in the world [1–3]. In the U.S.A. 1.2 million chlamydial infections were reported to CDC in 2009 [1]. In addition, the majority of the cases were not reported since most of these genital infections are asymptomatic [2,4,5]. Chlamydial infections can be treated with antibiotics however, due to its asymptomatic nature, most of them go untreated [4,6]. Untreated chlamydial infections can progress to serious reproductive and other health problems with both short-term and long-term consequences [7,8]. Furthermore, delayed or inadequate treatments fail to protect against long-term sequelae [7,8]. Therefore, a vaccine is the most effective way to control this disease [9–12].

Chlamydial vaccine development, to protect against trachoma, started in the 1960s using whole, inactivated, organisms [2,13,14]. Even though some of the vaccine formulations generated protection, the protection was short-lived and serovar, or serogroup specific [2,15]. Even in some instances, particularly if a low strength vaccine preparation was used, it

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appeared that vaccination might have enhanced the severity of ocular disease when individuals became re-infected, a problem that may also occur using live-attenuated vaccines [2,3,16]. Therefore, the need for a subunit vaccine is urgent.

Following the discovery of the major outer membrane protein (MOMP) of *C. trachomatis*, renewed attempts to develop a vaccine against genital infections were made in the 1980s [15,17]. Even though mice immunized with MOMP showed significant protection against chlamydial infection, some limitations were discovered [18–20]. For example, some of the protection generated by MOMP is dependent on the native 3-dimensional structure of the protein. Protection with recombinant MOMP was not as robust as that resulting from vaccination with the native MOMP [21,22]. Extraction of the native form of MOMP cannot be scaled up at a reasonable cost to manufacture for a human vaccine and therefore, alternative antigens need to be identified to formulate a vaccine.

Recent advances in generating whole proteome chips have led to a fast way to screen proteins that can generate an immune response [23,24]. Molina et al. [25] used sera from immunized mice and a chip containing approximately 25% of the *C. trachomatis* MoPn genome and identified seven immunodominant antigens that were recognized by immunized mouse sera. Cruz-Fisher et al. [26] generated a proteome chip of the *C. trachomatis* MoPn genome and identified 185 proteins that were recognized by sera of BALB/c female mice immunized by this bacterium. The great amount of variability in the human population will require that a *Chlamydia* vaccine includes antigens that can be recognized by individuals with multiple immunogenetic backgrounds. To address this issue, using three different strains of mice, we identified dominant novel antigens that can be further tested for their ability to induce a protective response against chlamydial infections in animal models and eventually in humans.

2. Materials and methods

2.1. *Preparation and titration of stocks of C. trachomatis MoPn*

The mouse *C. trachomatis* biovar (MoPn strain Nigg II), also called *Chlamydia muridarum*, was purchased from the American Type Culture Collection (Manassas, VA) and was grown in McCoy cells [27]. Eagle's minimal essential medium was supplemented with 5% fetal bovine serum (EMEM-FBS) and 1 μg/mL of cycloheximide. Purification of elementary bodies (EB) was done as described by Caldwell et al. [28]. The EB stock was stored at −70 °C in SPG buffer (0.2 M sucrose, 0.02 M sodium phosphate, pH 7.2, and 5 mM glutamic acid). The number of inclusion forming units (IFU) of the stock was determined by titration on HeLa 229 cells.

2.2. *Immunization of mice*

Three weeks old female BALB/c (H-2^d), C3H/HeN (H-2^k), and C57BL/6 (H-2^b) mice were purchased from Charles River Laboratories (Wilmington, MA). Groups of 12 mice were immunized as follows. For live intranasal (i.n.) immunization, BALB/c and C57BL/6 mice received 10^4 IFU of MoPn and C3H/HeN mice were inoculated with 10^1 IFU and for intravaginal (i.vag.) delivery mice received 10^5 IFU/mouse [29–31]. The animals immunized intravaginally were treated with 2.0 mg of medroxy-progesterone acetate (Greenstone, Peapack, NJ) subcutaneously (s.c.) 7 days before inoculation [32,33]. EB were inactivated by exposure to a UV transilluminator box (UV-EB) emitting at a wavelength of 302 nm (Spectroline, Westbury, NY) for 10 min as previously described [34]. For the combined intramuscular and subcutaneous (i.m.+s.c.) routes, the mice were vaccinated with 10^6 IFU of UV-EB per mouse three times 2 weeks apart [34]. To elicit a Th1 response, one of the groups immunized by the i.m.+s.c. routes was vaccinated using UV-EB with CpG oligodeoxynucleotide (ODN) 1826 (10 μg/mouse/immunization; Coley Pharmaceutical, Ottawa, Canada) and Montanide ISA 720 (Seppic, Inc., Fairfield, NJ) as adjuvants [18]. The Montanide was mixed at a 70:30 (vol/vol) ratio of the final preparation. To induce a Th2 response, a second group was immunized i.m.+s.c. using alum (250 μg/mouse/immunization; 0.3% aluminum hydroxide solution; Alhydrogel 85; Superfos, Denmark) as the adjuvant [35]. As a negative-control group, mice were immunized with ovalbumin and the three combined adjuvants. Another control group was not immunized. Serum samples were collected before immunization and then at two-weeks intervals up to 180 days post immunization. All the samples were tested with the microarray. In order to be able to perform all serological tests with the same sample, the sera from each group of mice were pooled. To identify antigens based on their ability to induce antibodies that are long term persistent after immunization, we selected those that gave a positive signal for at least five or more time points or were positive for three consecutive data points. The experiment was repeated once. All animal protocols were approved by the University of California, Irvine (Irvine, CA), IACUC.

2.3. *Microarray probing and data collection*

Mouse serum samples were probed on the full proteome *Chlamydia* MoPn protein chip [26,36,37]. Briefly, samples were diluted 1:100 with 1× protein array blocking buffer (Whatman, Piscataway, NJ) containing 10% *Escherichia coli* lysate (McLab, San Francisco, CA) and incubated at room temperature for 30 min with constant agitation. The microarrays were rehydrated in 1× protein array blocking buffer for 30 min and probed with the diluted serum samples for 2 h at room temperature with constant agitation [23]. The slides were then washed three times with Tris-buffered saline (TBS) containing 0.05% Tween 20 (TTBS) and incubated with biotin-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). After three washes with TTBS, the bound secondary antibodies were detected using streptavidin-conjugated Sensilight P3 (Columbia Biosciences, Columbia, MD), diluted according to the manufacturer's recommendations. The slides were washed three times with TTBS and three times with TBS, followed by a final wash with ultrapure water. The slides were air dried by centrifugation and scanned in a ScanArray Express HT microarray scanner (PerkinElmer, Waltham, MA), and the fluorescence signal was quantified using QuantArray software (PerkinElmer, Waltham, MA). All samples were tested in triplicate.