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Vaccination of newborn mice induces a strong protective immune response against respiratory and genital challenges with *Chlamydia trachomatis*

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Abstract

Chlamydia trachomatis infections can occur early in life and may result in long-term sequelae. To assess the feasibility of implementing a vaccine in newborns, groups of 2-day-old BALB/c mice were immunized intranasally (i.n.) with 1×10^4 inclusion forming units (IFU) of *C. trachomatis* mouse pneumonitis (MoPn). As a control, newborn mice were sham-immunized i.n. with minimal essential medium. In the vaccinated animals, strong *Chlamydia*-specific humoral and cell-mediated immune responses were observed. Six weeks after immunization, mice were challenged with MoPn i.n. or intravaginally (i.vag.). For the i.n. challenge, mice were inoculated with 10^4 or 10^5 IFU of MoPn per mouse, and in the case of the i.vag. challenge, each animal received 10^6 IFU. By day 10 post-infection (p.i.), the vaccinated mice challenged i.n. with 10^4 IFU, had gained an average of $6.7 \pm 1\%$ of their body weight. In contrast, the sham-immunized mice had lost $14.9 \pm 1\%$ of their weight ($P < 0.05$). The mean number of IFU/lungs in the vaccinated animals was 800 ± 300, while for the sham-immunized mice was $211 \pm 49 \times 10^6$ ($P < 0.05$). Significant differences between the *Chlamydia*-vaccinated and the sham-immunized mice were also found in the groups challenged with 10^5 IFU. In the mice challenged i.vag., a significant decrease in the number of mice with positive cultures, and the intensity and duration of vaginal shedding was noted in the vaccinated mice compared to the sham-immunized mice ($P < 0.05$). In conclusion, these results indicate that vaccination of neonatal mice can result in a protective response against a subsequent pulmonary or genital challenge with *Chlamydia*.

Keywords: *Chlamydia trachomatis*; Neonatal vaccination; Mouse model; Newborn mice

1. Introduction

Infections due to *Chlamydia trachomatis* impose a significant medical and economical burden throughout the world [1–7]. In underdeveloped countries, children living under poor hygienic conditions are repeatedly infected with *C. trachomatis* from the time of birth. Following multiple exposures, they developed trachoma, the most common cause of preventable blindness in the world [5,6,8,9]. In young adults, *C. trachomatis* is the leading sexually transmitted bacterial pathogen in the world [2,3,5,6]. Symptomatic and asymptomatic infections may result in long-term sequelae, including chronic abdominal pain, ectopic pregnancy and infertility [5,10,11]. Furthermore, infected pregnant females frequently transmit *Chlamydia* to their newborns that may develop ocular and respiratory infections in the first 6 months of life [3,5,6,10].

The prevention of trachoma and sexually transmitted infections may be better accomplished by vaccinating young individuals. In an attempt to develop a vaccine against trachoma, several groups performed immunization trials in young children and in monkeys [6,12–16]. In these trials, live and killed vaccines were used in various combinations with adjuvants. Based on the results, it was concluded that protection could be achieved but it was short-lived and serovar-specific. Furthermore, certain individuals upon re-exposure developed a hypersensitivity reaction that persisted longer than the protective effect and was not strain-specific. In spite of this complication, the trials were considered to be

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encouraging enough that a commercial vaccine was produced for use in trachoma-endemic areas [6,12]. This vaccine was manufactured with the intent of preventing infection in young children and newly exposed individuals to avoid reinfection and to be used in conjunction with chemotherapy [12].

Non-human primates are ideal models to test vaccine candidates. However, the restriction and costs of using these animals limits the possibility of working with the large numbers required for initial testing of vaccine formulations. In addition to the human serovars, the *C. trachomatis* mouse pneumonitis (MoPn) serovar, initially isolated from mice inoculated with human respiratory specimens, has been widely used as an experimental model. In mature mice, this organism produces infections that closely parallel those occurring in humans [17–23]. Here, in order to assess the feasibility of vaccinating newborns, we established a mouse model. Based on the results obtained, we can conclude that, following immunization with viable *C. trachomatis* MoPn, newborn mice can develop an immune response that is protective against a respiratory and a genital challenge.

2. Materials and methods

2.1. Organisms

The strain Nigg II of *C. trachomatis* MoPn was obtained from the American Type Culture Collection (Manassas, Va.) and was grown in HeLa 229 cells using Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS) [19,20]. Elementary bodies (EB) were purified using Renografin (Squibb, Princeton, NJ), and were stored at −70 °C in SPG (0.2 M sucrose, 20 mM sodium phosphate pH 7.2 and 5 mM glutamic acid) [24].

2.2. Animals

Adult male and female BALB/c (H-2^d) mice were purchased from Charles River Laboratories (Wilmington, MA). Following mating, newborn mice were raised in the vivarium of the University of California, Irvine. Neonates were housed with their dams and were weaned at 4 weeks of age. Mice received normal diet and water ad libitum, and were kept in isolation cubicles with constant temperatures of 24 °C, and cycles of 12 h of fluorescence light and 12 h of darkness. All experiments were repeated twice. The animal protocols were approved by the University of California Irvine, Animal Care and Use Committee.

2.3. Immunization and challenge protocols

For immunization, groups of 2-day-old mice were immunized i.n. with 10^4 IFU of *C. trachomatis* MoPn in 5 μl of MEM without FCS (MEM-0) [20]. As a control, age-matched newborn mice were sham-immunized with 5 μl of MEM-0. The animals were inoculated i.n. without anesthesia.

Six weeks after immunization, mice were challenged i.n. or i.vag. [17,20]. For the i.n. challenge, mice were inoculated with 10^4 or 10^5 inclusion forming units (IFU) of *C. trachomatis* MoPn in 40 μl of MEM-0 under ketamine/xylazine anesthesia. Following the i.n. challenge, mice were weighed daily. On day 10 post challenge, the animals were euthanized, and their lungs were harvested and weighed. After homogenization in 5 ml of SPG serial 10-fold dilutions of the lungs were inoculated onto McCoy cells grown in 48-well tissue culture plates. The plates were centrifuged for 1 h at 1000 × *g* at room temperature, and then incubated for 30 h at 37 °C in a CO_2 incubator. The *Chlamydia* inclusions were stained with a pool of monoclonal antibodies (mAb) prepared in our laboratories. This pool included mAb to the major outer membrane protein (MOMP), the 60 kDa cysteine-rich protein (crp), the 150 kDa putative outer membrane protein and the lipopolysaccharide (LPS) of the *C. trachomatis* MoPn. The limit of detection was 50 IFU of *C. trachomatis* per pair of lungs.

For the i.vag. challenge, mice were inoculated with 10^6 IFU of *C. trachomatis* MoPn in 20 μl of SPG. The inoculations were made under xylazine/ketamine anesthesia. Vaginal swabs were collected at weekly intervals for 6 weeks to monitor shedding of *Chlamydia*.

2.4. Immunoassays

Following immunization blood and vaginal washes were collected to determine the antibody response using an enzyme-linked immunosorbent assay (ELISA). For ELISA, multi well plates (96 flat-bottom wells; Corning Glass works, Corning, NY) were coated overnight with 1 μg of purified *C. trachomatis* MoPn EB per well [20]. Serial dilutions of serum or vaginal samples were added to each well, and the plates were incubated for 2 h at 37 °C. The antigen–antibody reactions were detected by adding horseradish peroxidase (HRP)-conjugated goat anti-mouse antibodies. The following class- or subclass-specific antibodies were used: immunoglobulin G (IgG), IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM (Southern Biotechnology Associates Inc., Birmingham, Ala.). The substrate, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) was used for color development. The plates were read at 405 nm using an ELISA reader (Bio-Rad Corp.; CA).

In vitro neutralization assays were performed using HeLa 229 cell monolayers as previously described [20]. Briefly, 10^4 IFU of *C. trachomatis* MoPn were added to serial dilutions of the mouse serum made with Ca_2^+-, Mg_2^+-free PBS and supplemented with 5% guinea pig serum. After incubation for 45 min at 37 °C, the mixture was used to inoculate HeLa 229 cells by centrifugation. The monolayers were fixed after 36 h of incubation at 37 °C and stained with a pool of mAb as described above. The neutralization titer of a sample was the dilution that yielded 50% neutralization relative to the control serum from sham-immunized mice.