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Maternal immunity partially protects newborn mice against a *Chlamydia trachomatis* intranasal challenge

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ABSTRACT

To determine the role of maternal immunity in protecting newborn mice against a *Chlamydia trachomatis* infection, female BALB/c mice were immunized intranasally (i.n.) with 10^4 inclusion forming units (IFU) of the *C. trachomatis* mouse pneumonitis biovar (MoPn). As a control, another group of female mice was sham-immunized i.n. with HeLa cell extracts. Immunized animals mounted strong immune responses as evidenced by high *Chlamydia*-specific antibody titers in serum and milk. Newborn mice born from immunized and sham-immunized dams were challenged i.n. with 10^3 IFU of MoPn at two post-natal days (PND). Following inoculation, newborn mice were euthanized at 7- and 18-PND and the lungs, spleen and intestine were cultured for *Chlamydia*. Overall, no significant differences were observed between the mice born from and fed by immunized dams and mice born from and fed by sham-immunized dams. Of the mice born from immunized dams, 75 and 25% had positive lung cultures at 7- and 18-PND, respectively. Of the mice born from sham-immunized dams, 82 and 50% had positive lung cultures for those same days. When the number of IFU recovered from the lungs and spleens was compared between the two groups no significant differences were observed. However, when the number of IFU recovered from the small intestine was compared, significant differences were observed between the two groups of newborn mice (2×10^5 versus 32×10^6 at 7-PND and 9.2×10^6 versus 85×10^6 at 18-PND). In conclusion, maternal immunity plays a limited role in protecting newborn mice against a *Chlamydia* infection.

1. Introduction

In the United States it is estimated that 5% of pregnant women are infected with *Chlamydia trachomatis,* and as a result, nearly 3.5 million newborns become infected at the time of delivery (Schachter and Caldwell, 1980). Approximately 30% of the infants exposed to *C. trachomatis* in the birth canal develop conjunctivitis and 10–20% show symptoms of pneumonia (Numazaki et al., 2003; Persson et al., 1986; Schachter and Caldwell, 1980). A total of 75,000 cases of inclusion conjunctivitis and 30,000 cases of *C. trachomatis* pneumonia are reported each year (Persson et al., 1986; Schachter and Caldwell, 1980). In addition, newborns may have vaginal and gastrointestinal tract infections with no known clinical consequences (Schachter and Caldwell, 1980). If diagnosed in time, most of the neonatal infections can be treated with antibiotics, and there are no long-term sequelae. However, some neonates develop persistent infections (Darville, 2005; Persson et al., 1986).

In general, neonates are highly susceptible to a variety of infectious diseases including *Chlamydia*. Several factors, such as the immaturity of antigen presenting cells, impaired IgG isotype switching and deficiencies in complement and Th1-type cytokines, are thought to be responsible for the high susceptibility to disease (Forsthuber et al., 1996; Levy, 2007; Ridge et al., 1996). Providing passive

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protection to infants by immunizing mothers could overcome some of these problems and therefore, bypass the limitations of the immature immune system of the neonates. However, during gestation, mainly components of the humoral immune response are transferred from the mother to the fetus (Seigrist, 2001, 2003).

Based on studies performed in the mouse model using live *C. trachomatis* for immunization, it appears that $CD4^+$ T cells play a predominant role in protective immunity. B cells and/or antibodies also have an important role, while $CD8^+$ T cells are not critical for protection (Morrison et al., 2000). Passive transfer of *C. trachomatis* MoPn antibodies to adult nu/nu and nu/+ mice has been shown to protect mice against intranasal infections (Williams et al., 1984). Similarly, Pal et al. (2008) have shown that passive immunization with monoclonal antibodies (mAb) recognizing a conformational epitope of the *C. trachomatis* MoPn MOMP resulted in significant protection in wild-type and C.B-17-SCID mice against an intranasal challenge.

Adult mice immunized with live *C. trachomatis* by the respiratory route develop robust cell-mediated and humoral immune responses and are protected against respiratory and genital challenges (Pal et al., 1994, 2002). Herein, we wanted to investigate whether maternal immunity could protect newborn mice against a respiratory chlamydial infection. To test this hypothesis, mice born from and fed by immunized or sham-immunized dams were challenged intranasally and the course of the infection was assessed.

2. Materials and methods

2.1. Organisms

The Nigg II strain of the *C. trachomatis* MoPn biovar (Ct-MoPn) (also called *C. muridarum*) was obtained from the American Type Culture Collection (Manassas, VA) and was grown in HeLa-229 cells using Eagle's minimal essential medium (Nigg, 1942; Pal et al., 1994). Elementary bodies (EB) were purified and stored at −70 °C in sucrose sodium phosphate glutamic acid (SPG) buffer (Caldwell et al., 1981).

2.2. Animals

Seven- to eight-week-old adult female and proven fertile breeder male BALB/c ($H\text{-}2^d$) mice were purchased from Charles River Laboratories (Wilmington, MA.). To determine the infectious dose $(ID)_{50}$ for newborn mice, newborn mice born from naïve dams were infected with 10^2, 10^3, and 10^4 *C. trachomatis* MoPn IFU per mouse at two postnatal day (PND). Inoculates were administered in 3 μl of MEM-0 onto the nostrils of newborn mice without anesthesia. Mice were euthanized at 11-PND, their lungs were harvested, and the number of IFU was determined (Pal et al., 2002).

For immunization, female mice were immunized with 10^4 IFU of Ct-MoPn or sham-immunized with HeLa cell extracts as described by Pal et al. (1994, 2002). Newborn mice born from immunized or sham-immunized dams were challenged with 10^3 IFU of Ct-MoPn at 2-PND. To examine the course of the infection, newborn mice were euthanized at 7- and 18-PND and their lungs, small intestines and spleens were harvested and homogenized for the isolation of *Chlamydia*. The University of California Irvine, Animal Care and Use Committee approved all animal protocols.

2.3. Collection of milk from mice

Milk was collected from lactating dams at various days PND to determine antibody titers as described by Parr et al. (1995). Briefly, dams were separated from newborns for 4–5 h in order to accumulate milk in the mammary glands. The dams were euthanized with a xylazine and ketamine solution and then inoculated i.p. with 1.5 U of oxytocin (Sigma Chemicals; St. Louis, MO) in 150 μl of PBS. Whey samples from the milk were prepared and stored at −70 °C.

2.4. Immunoassays

An enzyme linked immunosorbent assay (ELISA) was used to determine the antibody responses in serum and whey samples (Pal et al., 1994). At the time of euthanization, blood and milk were collected from dams and blood was collected from newborn mice by cardiac puncture. The following class or subclass-specific antibodies were used: immunoglobulin G (IgG), IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM (Southern Biotechnology Associates Inc., Birmingham, AL). T cell proliferation assays were performed using splenocytes as previously described (Pal et al., 1994).

In vitro neutralization assays were performed using HeLa-229 cell monolayers as previously described (Pal et al., 1994). Western blot analyses were performed using nitrocellulose membranes as described (Schagger and Von Jagow, 1987).

2.5. Organ culture

Following euthanasia the lungs, small intestines, and spleens were collected, homogenized in 2 ml of SPG and inoculated onto McCoy cell monolayers grown in 48-well tissue culture plates (Pal et al., 2002, 2008).

2.6. Statistics

All statistical analyses were performed with the SigmaStat software except multivariate analysis. A Multivariate regression analysis using generalized estimating equation (PROC GENMOD) was performed employing the SAS 9.2 software program.

3. Results

3.1. Determination of the ID_{50} for newborn mice

To determine the ID_{50} for newborn mice, animals born from naïve dams were inoculated at 2-PND with doses of Ct-MoPn ranging from 10^2 to 10^4 IFU per newborn mice. Table 1 shows the results of the lungs cultures at 11-PND. When newborn mice were inoculated with 10^2 Ct-MoPn IFU, four out of eight mice (50%) had positive cultures. Of