Mechanism of T cell mediated protection in newborn mice against a *Chlamydia* infection

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Abstract

To determine the immune components needed for protection of newborn mice against *Chlamydia muridarum,* animals born to *Chlamydia*-immunized and to sham-immunized dams were infected intranasally with *C. muridarum* at 2 post-natal days. T-cells isolated from immunized or sham-immunized adult mice were adoptively transferred to newborn mice at the time of infection. Also, to establish what cytokines are involved in protection, IFN-γ TNF-α, IL-10, and IL-12 were passively transferred to newborn mice. To assess the *Chlamydia* burden in the lungs mice were euthanized at 12 post-natal days. When T-cells from immunized adult mice were transferred, mice born to and fed by immunized dams were significantly protected as evidenced by the reduced number of *Chlamydia* isolated from the lungs compared to mice born to and fed by sham-immunized dams. Transfer of IFN-γ and TNF-α also significantly reduced the number of *Chlamydia* in the lungs of mice born to immunized dams. Transfer of IL-10 or IL-12 did not result in a significant reduction of *Chlamydia*. In vitro T-cell proliferation data suggest that neonatal antigen presenting cells can present *Chlamydia* antigens to adult T-cells. In conclusion, maternal antibodies and *Chlamydia* specific T-cells or Th1 cytokines are required for protection of neonates against this pathogen.

Keywords

Neonatal immunity; Maternal antibodies; *Chlamydia muridarum*; Neonatal mouse model; Passive immunity

1. INTRODUCTION

Sexually transmitted infections are a major health problem in all countries. *Chlamydia trachomatis* is the most common sexually transmitted bacterial pathogen with approximately 100 million new cases reported each year [1]. Depending on the type of population studied, about 5 to 20 percent of women are positive for *C. trachomatis* and more than 50% of the

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genital infections are asymptomatic [1]. If *Chlamydia* is not diagnosed and treated during pregnancy the babies could acquire the infection from their mothers. About one in three of the exposed infants develop inclusion conjunctivitis while approximately one in six are affected with pneumonia [2-4]. Infections of the genitourinary and gastrointestinal tracts of newborns have also been reported [3]. In addition to maternal transmission, in endemic area with a high incidence of trachoma about 10% infants are infected with *C. trachomatis* [5].

In the United States, it is estimated that nearly 3.5 million newborns are infected at the time of delivery and a significant number of them are asymptomatic [6, 7]. *C. trachomatis* pneumonitis is characterized by its insidious onset within the first three months of life. The clinical manifestations in neonates with *Chlamydia* sometimes are indistinguishable from infections with other respiratory pathogens such as respiratory syncytial virus, *Ureaplasma urealyticum*, cytomegalovirus and *Pneumocystis carinii*. Coinfection with other viruses which contribute to the severity of the original infection have been reported [8]. Neonatal chlamydial infections can be treated with antibiotics. However, in spite of antibiotic treatment, some neonates develop persistent infections [4, 6].

Neonates are highly susceptible to infections. Several factors, such as the immaturity of antigen presenting cells, impaired IgG isotype switching, deficiencies in complement and Th1-type cytokines, are thought to be responsible for the high susceptibility to infection [9-11]. So far, very few studies have attempted to understand the protective immune components of neonates in the presence of chlamydial maternal antibodies. This is probably due, at least in part, to the lack of appropriate animal models. Recently, we developed a mouse model of neonatal infection [12]. In this model, mice born to and fed by *Chlamydia* immunized dams, were infected at 2 postnatal days (PND). Using this model we showed that maternal antibodies from previously immunized dams were not sufficient to protect newborn mice against an i.n. challenge with *Chlamydia* [12]. In adult mice Th1 immunity is necessary to protect against *C. trachomatis* genital infections [13]. In addition, the IL-12/IFN-γ axis has been found to induce protection in newborn mice against a pulmonary infection [14].

Maternal antibodies have the ability to protect neonates whereas maternal T-cells cannot, because of the differences in tissue antigens, particularly HLA, between the mother and the fetus [15]. These differences raise the possibility of an attack on the fetus by maternal T-cells, but this danger is avoided by the absence of HLA antigen in the areas of placental contact [15]. Similarly, the risk that fetal lymphocytes will attack the mother is likewise low, because of the incompetence of fetal T-cells [15]. Since, neonates are born with immature T- and antigen presenting-cells (APC), the question arises whether neonates can utilize *Chlamydia*-specific adult T-cells to clear an infection. If they can, then it will be important to determine what kind of Th1 cytokine controls the infection. Here, to address this question, T-cells from *Chlamydia* immunized and sham-immunized adult inbreed mice were passively transferred to newborn mice before an i.n. challenge. In addition, T-cell derived Th1 effector cytokines, IFN-γ and TNF-α, and modulating cytokines, IL-10 and IL-12, were passively transferred to newborn mice. Results of this study show that both T-cells, and T-cell derived Th1 effector cytokines, and maternal antibodies are required for protection against a chlamydial infection in neonates.

2. MATERIALS AND METHODS

2. 1. Organisms

The Nigg II strain of the *C. trachomatis* mouse pneumonitis MoPn (MoPn) biovar (now called *Chlamydia muridarum*) was obtained from the American Type Culture Collection (Manassas, VA) and was grown in HeLa-229 cells using Eagle's minimal essential medium [16-18]. Elementary bodies (EB) were purified and stored at −70°C in sucrose phosphate glutamic acid buffer (SPG) [18].

2. 2. Animals

Female and proven breeder male BALB/c (H-2^d) mice were purchased from Charles River Laboratories (Wilmington, MA.). Groups of 3-4 week-old female BALB/c mice were intranasally (i.n.) immunized with 10^4 inclusion forming units (IFU) of MoPn [17]. Control mice were sham-immunized with HeLa-229 cell extracts. For mating, six weeks after i.n. inoculation, four female mice were placed in the same cage with one male. Mice born from immunized or sham-immunized dams were inoculated i.n. with 10^3 MoPn IFU ($10 \times ID_{50}$) in 3 μl of MEM-0 within the first 48 h of birth (2 PND) [12]. Prior to infection, newborn mice received intraperitoneally (i.p.) nylon wool enriched T-cells from immunized or sham immunized adult mice (2.5×10^6, 2.5×10^4 or 2.5×10^4 cells per newborn mouse) [19]. To examine the role of cytokines, newborn animals were inoculated i.p. with one of the following in PBS (pH 7.2): IFN-γ (40 ng), TNF-α (10 ng) [20], IL-10 (10 ng) [21] or IL-12 (50 ng) [22]. As controls, a group of newborn mice only received PBS (pH 7.2). At 10 days post infection (p.i.; 12 PND), newborn mice were euthanized, their lungs collected, homogenized in SPG and the number of MoPn IFU determined by infecting HeLa-229 cell monolayers. The animal protocol was approved by, the University of California Irvine, IACUC. All experiments were repeated.

2. 3. Collection of milk from mice

Milk was collected from lactating dams at 12 PND as described [12, 23]. Briefly, to accumulate milk in the mammary glands, dams were separated from newborns mice for 4 to 5 h. The dams were anesthetized with xylazine and ketamine and then inoculated i.p. with 1.5 U of oxytocin (Sigma Chemicals; St. Louis, MO) in 150 μl of PBS (pH 7.2). Whey samples from the milk were prepared by centrifugation and stored at −70°C.

2. 4. Immunoassays

To determine the antibody levels in serum and whey samples an enzyme linked immunosorbent assay (ELISA) was performed [17]. At the time of euthanization, blood was collected from dams and newborn mice, while milk was collected from dams. The following class and subclass-specific antibodies were used: immunoglobulin G (IgG), IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM (Southern Biotechnology Associates, Inc., Birmingham, AL).

A T-cell lymphoproliferative assay (LPA) was performed using splenocytes as described [12]. Briefly, spleens from two to three newborn or adult mice were collected, teased, and enriched for T-cells by passing through nylon wool columns. After passage through a nylon wool column 90% of the cells were T-cells as determined with a fluorescence-labeled

monoclonal antibody to CD3+ (GIBCO BRL, Grand Island, N.Y.). Enriched T-cells were counted and 10^5 cells/well were placed in a 96-well plate. APC were prepared by treating splenocytes with 3300 rads using a ^{137}Cs irradiator. UV inactivated *Chlamydia* MoPn were added at a concentration of 10, 1 and 0.1 EB to 1 APC.

To study accessory cell function each well received irradiated splenocytes (1×10^5), nylon wool enriched T-cells (1×10^5), and Concanavalin A (5 μg/ml; Con A; Sigma). Negative control wells received medium instead Con A. Following 96 h of incubation, cell proliferation was measured by adding 1μCi of [methyl ^{3}H]] thymidine per well. The mean counts per minute (cpm) were determined from triplicate cultures.

2. 5. Organ culture

At 12 PND, lungs were collected, homogenized in 2 ml of SPG and dilutions inoculated in HeLa cell monolayers grown in 48-well plates. Plates were incubated at 37°C and fixed with methanol at 30 h p,i. Inclusion s were detected by immuno-peroxidase staining using a pool of monoclonal antibodies to *Chlamydia* MoPn [12, 24].

2. 6. Statistics

Statistical analyses were performed using the SigmaStat software. To determine the significance between the two treated groups on the yield of MoPn IFU, Mann-Whitney U test was employed. Two-tailed unpaired Student's *t* test was used to determine significances between two sets of in vitro stimulation counts. Differences were considered significant when P values were <0.05.

3. RESULTS

3. 1. Measurement of Chlamydia antibody responses in dams and newborn mice

Table 1 shows *Chlamydia* MoPn-specific antibody titers in dams and newborn mice at 12 PND. Overall, high IgG antibody titers in serum and milk were detected in the i.n. immunized dams. The IgG2a/IgG1 ratio in immunized dams sera was 4 (25,600/6,400) indicative of a Th1 response. High levels of IgA (6,400) were also present in the serum samples of immunized dams. Low levels of MoPn specific antibody IgG titers (1,600) were detected in the milk of immunized dams. Interestingly, no IgG1 and low levels of IgG2a antibody (800) were measured in the milk of immunized dams. As expected, high levels of IgA (12,800) were found in the milk. At 12 PND sham immunized dams, whose newborn mice had been infected, had low IgG antibody titers (100 to 400) in serum suggesting that their *Chlamydia*-inoculated newborn mice may have infected the dams.

High levels of antibody were detected in mice born to and fed by immunized dams (Table 1). At day 12 PND (D10 p. i.) newborn mice born to immunized dams had an IgG2a/IgG1 ratio of 4 (25,600/6,400) and their serum IgA titer was 6,400. In contrast, mice born to sham immunized dams, and infected at 2 PND, had low IgG antibody titers (<100 to 400) in sera.

3. 2. Adoptive transfer of T-cells to newborn mice

To characterize the mechanisms of protection against a *Chlamydia* infection in newborn mice, T-cells from immunized, or sham immunized adult BALB/c mice, were adoptively transferred to 2 day-old newborn mice by i.p inoculation. Three different quantities of T-cells 2.5×10^6, 2.5×10^5, and 2.5×10^4 per newborn, were adoptively transferred to mice born to immunized or sham immunized dams. A control group of newborn mice received RPMI-0. Following the adoptive transfer of T-cells, newborn mice were challenged i.n. with 10^3 MoPn IFU and were euthanized at 12 PND.

Serum IgG titers for each group of newborn mice at 12 PND are shown at the bottom of Figure 1. Mice born to immunized dams, that received 2.5×10^6, 2.5×10^5 or 2.5×10^4 T-cells from immunized adult mice, were significantly protected when compared to mice born to sham immunized dams receiving sham immune T-cells (P<0.05). Higher doses of T-cells (2.5×10^6 or 2.5×10^5) from MoPn immunized mice also protected newborn mice born to sham immune dams. This may be due to the over-reactive function of transferred T-cells from immunized adult mice. The role of T-cells in adoptive immunity was best observed with the 2.5×10^4 T-cells dose. Significant level of protection (P<0.05) was seen in mice born to and fed by immunized dams receiving 2.5×10^4 T-cells isolated from adult immunized. However, the same number of T-cells was not protective in mice born to sham-immunized dams. For example, the median (range) number of MoPn IFU recovered from the lungs of mice born to immunized dams, receiving 2.5×10^4 T-cells from immunized mice, was 120 (0-123,900). On the other hand, in mice born to sham immunized dams, receiving 2.5×10^4 T-cells from immunized or sham-immunized adult mice, the median number of MoPn IFU was 12,000 (0-1,156,400) and 18,998 (80-4,284,400), respectively. Control newborn mice, born to immunized dams or sham immunized dams, receiving RPMI-0 yielded a median number of 79,709 (0-1,428,000) and 176,000 (0-23,255,200) MoPn IFU from the lungs, respectively. Therefore, protection was associated with the presence of high levels of antibodies in newborn mice and transferred T-cells from immune adult mice.

3. 3. In vitro proliferative responses of T-cells from newborn mice

To characterize the ability of newborn mice to present antigens to T-cells, uninfected mice born to immunized or sham-immunized dams were euthanized at 6 PND and their splenocytes were employed in an in vitro T-cell proliferation assay [12]. At the same time, MoPn immunized and sham immunized adult mice were euthanized and their splenocytes collected. T-cells from newborn or adult mice were stimulated with various concentration of MoPn EB in the presence of irradiated splenocytes used as APC [12]. T-cell proliferation was measured by [^{3}Hthymidine] uptake after culturing the cells for four days (Figure 2).

When T-cells isolated from newborn mice were stimulated with MoPn EB in the presence of neonatal APC, no proliferation was observed. However, T-cells isolated from adult immunized mice and stimulated the same way, proliferated significantly when compared to T-cells isolated from adult sham immunized mice (P<0.05). This proliferation was *Chlamydia*-specific as there was no significant proliferation observed when T-cells isolated from adult sham immunized mice were stimulated with MoPn EB in the presence of APC from neonatal or sham immune adult splenocytes (Figure 2, plots f, g, h). For example, the

average cpm (at 1:1 EB to APC) for APC from newborn mice and T-cells from adult immunized and sham immunized mice were $11,852 \pm 2,258$ and 243 ± 87, respectively (P>0.05). In comparison the average cpm at the same antigen concentration, for adult mice APC and T-cells from adult immunized and sham-immunized mice were $9,865 \pm 4,071$ and 200 ± 100, respectively (P<0.05; Figure 2, plots b, f, d, h). These results suggest that neonatal APC can present *Chlamydia* antigens to T-cells from adult MoPn immunized mice.

To analyze the ability of newborn splenocytes to support T cell growth, newborn mice were euthanized at 6 PND and accessory cells were prepared by irradiating splenocytes as described above. Irradiated splenocytes were tested for their ability to function as accessory cells, in an in vitro T-cell proliferation assay, using Con A as a T-cell stimulant (Figure 3). These experiments were conducted based on the previous observation that Con A stimulated T-cells needed accessory cells for sustaining in vitro growth [25]. When T-cells from newborn mice were stimulated with Con A in the presence of newborn mice accessory cells, a low level of proliferation was observed. This indicates that either splenocyes from newborn mice could not function as accessory cells or that Con A could not stimulate newborn mice T-cells (Figure 3a).

When T-cells from newborn or adult mice were stimulated with Con A in the presence of accessory cells prepared from either newborn or adult mice significant proliferation was observed in all groups (Figure 3, plots b, c, d). The highest level of proliferation was observed with T- and accessory cells from adult mice. For example, when T- and accessory cells from newborn mice were stimulated with Con A the mean cpm was 2,304+494 (Figure 3a). In contrast, when T-cells from newborn mice and accessory cells from adult mice, or the reverse (T-cells from adult mice and accessory cells from newborn mice), were stimulated with Con A, the mean cpm increased from 12,295+1,799 to 20,449+3,048 (Figure 3 b, c). In addition, when both T- and accessory cells from adult mice were used, the mean cpm was 36,064+10,518 (Figure 3d). These results imply that neonatal splenocytes can function as accessory cells and support T-cell growth.

3. 4. Passive transfer of cytokines to newborn mice

Next, we asked whether T-cell derived effectors or modulating cytokines can help to clear a respiratory *Chlamydia* infection in newborn mice (Figure 4). IL-10 and IL-12 were tested as modulating cytokines and IFN-γ and TNF-α as effector cytokines. Following a passive transfer of the cytokine to mice born to immunized or sham immunized dams, newborn mice were infected with MoPn at 2 PND. As a negative control, newborn mice received PBS. The antibody titers of each group of newborn mice at 12 PND are shown at the bottom of Figure 4. Mice born to immunized dams receiving IFN-γ or TNF-α had significantly reduced *Chlamydia* burdens in their lungs compared to the control newborn mice receiving PBS (P<0.05; Figure 4). The median (range) number of IFU recovered from the lungs of mice born to immunized dams receiving IFN-γ or TNF-α, were 2,189 (0-520,380), and 22,000 (0-338,660), respectively. In contrast the mice born to immunized dams inoculated with PBS yielded 79,709 (0-1,428,000) IFU (P<0.05). Furthermore, the median number of IFU recovered from the lungs of mice born to sham immunized dams receiving IFN-γ TNF-α, and PBS were 64,240 (0-1,670,400), 488,400 (0-8,518,600), and 85,904 (0-40,129,400),

respectively. Therefore, Th1 cytokines can help newborn mice to control a chlamydial respiratory infection.

To assess if modulating cytokines were involved in protection, IL-10 and IL-12 were passively transferred to newborn mice. IL-10 and IL-12 did not reduced the *Chlamydia* burden in the lungs of mice born to immunized or sham immunized dams (Figure 4). In all passive cytokine transfer experiments a reduced *Chlamydia* burden was noticed in the lungs of newborn mice that had high levels of *Chlamydia*-specific antibodies and received IFN-γ or TNF-α.

4. DISCUSSION

Previously we had shown that newborn mice born to and fed by *C. muridarum* immunized dams were not protected against an i.n. challenge with MoPn [12]. Although dams had high levels of *Chlamydia*-specific antibodies in serum and milk, newborn mice could not clear the *Chlamydia* infection following an i.n. challenge. Thus, we postulated that, in addition to maternal antibodies, newborn mice needed T-cells or T-cell secreting cytokine(s) to clear the infection [12]. Here, we showed that in addition to *Chlamydia*-specific maternal antibodies, T-cells from MoPn immunized adult mice, or Th1 cytokines such as IFN-γ or TNF-α, are capable of reducing the *Chlamydia* burden in the lungs of newborn mice. In vitro T cell proliferation assays support the notion that newborn mice APC are capable of presenting antigen as well as functioning as accessory cells to T-cells from adult mice.

Several investigators have shown that in naïve adult female mice, passive transfer of *Chlamydia*-specific T-cells, or Th1 lymphocyte clones, can clear chlamydial genital infections [26-28]. Here we showed that mice, born to and fed by immunized or sham immunized dams, that received 2.5×10^6 or 2.5×10^5 T-cells from immunized adult mice, have a significantly reduced *Chlamydia* burden in their lungs. However, protection was not observed in newborns of sham-immunized mice that received less T-cells (2.5×10^4) from immunized adult mice. In this group, the protection was only observed in newborn mice born to and fed by immunized dams. These results are not surprising considering that CD4+ T-cells and B-cells or antibodies, were shown to be important for the clearance of chlamydial genital infections in adult immunized mice [29-31]. Since at risk babies are born from an infected mother, they will have maternal antibodies in their sera. Thus, neonates born from an infected mother need limited help from T-cells to synergistically work to reduce the *Chlamydia* burden. The importance of both maternal antibodies and T-cells has also been established for HSV infections in newborn mice. Kohl et al. (1982) showed that neonate mice can survive a lethal HSV infection when interferon, antibody to HSV, and leukocytes were, simultaneously, passively transferred [32].

Antibody dependent cellular cytotoxicity (ADCC) has been shown to be an effector mechanism for killing *Chlamydia* in mature adult hosts [33]. Since neonates are born with immature cellular immune system, ADCC may not function well in the presence of maternal antibodies. However, this condition changes in an experimental condition when newborn mice adoptively receive immune cells from adult mice. Adoptively transferred adult T-cells and maternal antibodies may work together to clear a *Chlamydia* infection by ADCC. This

may explain why significant protection was observed in newborn mice, born to and fed by immune dams that received mature T-cells from MoPn-immunized or sham-immunized mice. Similar level of protection by ADCC was reported in an experimental HSV infection. Newborn mice receiving both anti-HSV antibodies and leukocytes from humans were able to clear a lethal HSV infection [34, 35].

Passive transfer of Th1 effector cytokines such as, IFN-γ or TNF-α resulted in significant protection in newborn mice. These observations are in agreement with previous findings in which passively transferred IFN-γ or TNF-α cleared a chlamydial infection in adult mice [36-38]. Between the two effector-cytokines, IFN-γ is more desirable since excessive TNF-α production may deter neonatal development [39]. It is not well understood how TNF-α clears a *Chlamydia* infection, although several mechanisms such as, up regulation of IFN-β, NK cells or neutrophils, have been proposed [38]. Clearance of *Chlamydia* by IFN-γ has been well studied and three mechanisms have been proposed: 1) inhibition of indoleamine 2, 3-dioxygenase (IDO), 2) up-regulation of nitric oxide synthase, and 3) down-regulation of transferrin receptors for iron transport [40]. Recently, in mouse epithelial cells, p47 GTPases but not IDO, were shown to be involved in killing MoPn [41]. It will be important to know what kind of killing mechanisms against *Chlamydia* are active in the neonatal environment in the presence of IFN-γ.

In these studies, Th modulating cytokines, specifically IL-12 and IL-10, were not able to induce significant protection in newborn mice suggesting that they were not capable of inducing Th1 cytokines in a neonatal environment. However, Jupelli et al. [14], using IL-12p35−/− knockout mice, showed that IL-12 played an important role in the clearance of *Chlamydia* in newborn mice. In their study 100% mortality was observed in IL-12p35−/− newborn mice by day 17 p.i. following an intranasal challenge at 1 PND. The differences with our findings may be due to: 1) the mouse strain used (BALB/c versus C57BL/6); 2) length of time animals were studied (12 PND versus 18 PND), and 3) nature of the experiment (passive transfer of recombinant IL-12 versus IL-12/p35−/− mice). Although IL-10 has been shown to be a protective cytokine in neonatal mice against a lethal group B streptococcal infection [42, 43], in our study no protection was observed in mice. This observation is not surprising considering that adult IL-10 knockout mice showed enhanced Th1-like protective immunity following a MoPn lung infection [44]. These results suggest that modulating cytokines may not be effective in reducing *Chlamydia* burden in newborn mice.

When APC and T-cells from newborn mice were used in a LPA, no proliferation was observed probably because neonatal splenocytes are immature. Other investigators have observed this phenomenon [45]. However, when APC from newborn mice were cultured with T-cells from adult immunized mice in the presence of *Chlamydia* EB, APC from newborn mice were able to present antigen to *Chlamydia*-specific adult mice T-cells. This suggests that newborn APC are not as immunological immature as originally thought. This in vitro observation also supports our in vivo T cell transfer experiments where T-cells from immunized adult mice were passively transferred to newborn mice. Thus, it is possible that, following the passive transfer of T-cells, APC of newborn mice were able to present *Chlamydia* antigen(s) to *Chlamydia*-specific adult T-cells and hence, reduce the *Chlamydia*

burden from the lungs. Similarly, in vitro accessory cell function observations support the in vivo T cell transfer data. In both cases, APC from newborn mice were able to sustain adult T cell growth in vitro and in vivo when mixed with adult T-cells. Alternatively, it is possible that adult T-cells, when they are together in vivo or in vitro, can induce neonatal APC from a non-responsive to a responsive state. Further studies are needed to elucidate the role of adult T-cells in maturing neonatal cells.

In conclusion, our in vivo results confirm that, in addition to maternal antibodies, Th1 effector cytokines such as IFN-γ or TNF-α are required to clear a chlamydial infection in neonates. Furthermore, this is the first time that neonatal APC were shown to be capable of an adult-like function in the presence of adult T-cells. Thus, it seems that, under certain conditions, neonatal APC can be switched from immature to functional immunological mature cells.

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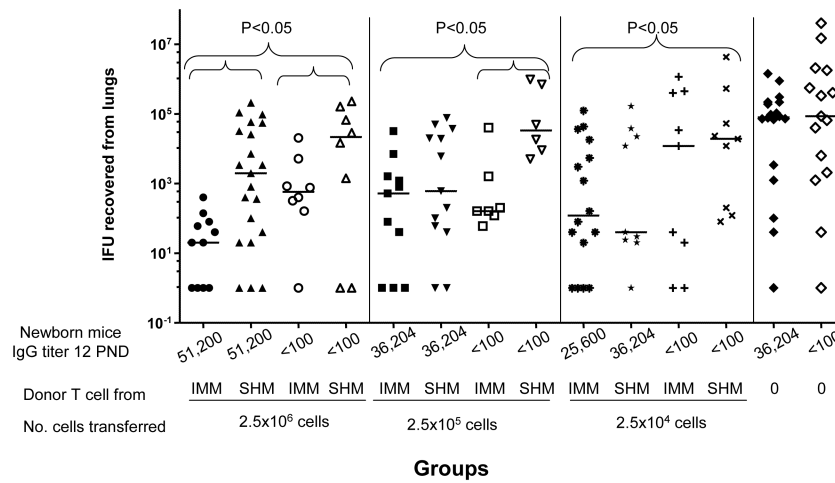
Figure 1. Adoptive transfer of T-cells from immunized or sham-immunized adult mice to 2 day-old newborn mice

Newborn mice received i.p. various amounts of adult T-cells (2.5×10^6, 2.5×10^5, and 2.5×10^4) from immunized adult (IMM) or sham immunized adult mice (SHM) at 2 PND before an i.n. challenge with MoPn. Newborn mice were euthanized at 12 PND. Horizontal bars represent median number of *Chlamydia* IFU recovered from each group. Antibody titers, for each group of newborn mice at 12 PND, are shown at the bottom of the figure.
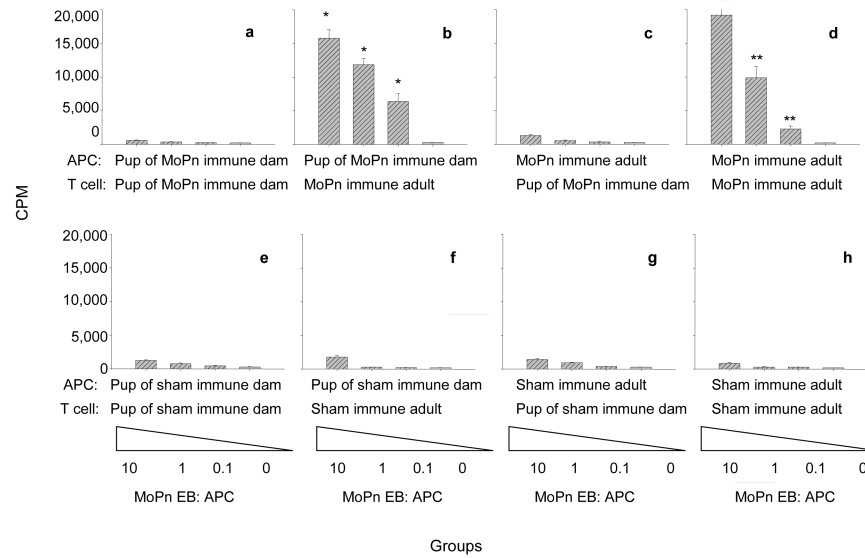
Figure 2. Antigen presentation by newborn splenocytes to adult T-cells

T-cells from newborn and adult mice were stimulated with MoPn EB in the presence of adult or newborn APC. The results are expressed as mean cpm derived from triplicate cultures. Plots 2a to 2e represent newborn mice born to MoPn immunized dams and plots 2e to 2g correspond to newborn mice born to sham-immunized dams. * Significant by the Student's *t* test (P<0.05) compared to T cell cultures of sham immunized adult mice with APC from newborn mice born to sham immunized dams. ** Significant by the Student's *t* test (P<0.05) compared to T cell cultures of sham immunized adult mice with APC from sham immunized adult mice.
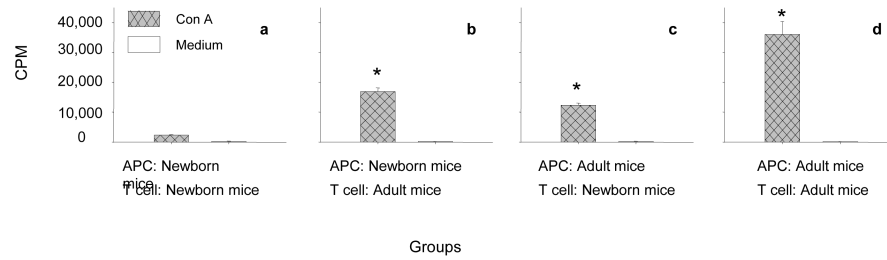
Figure 3. Accessory cell function of newborn splenocytes to adult T-cells
T-cells from newborn and adult mice were stimulated with Con A in the presence of adult or newborn APC. The results are expressed as mean cpm from triplicate cultures. * Significant by the Student's *t* (P<0.05) test when compared to the T-cell cultures with newborn T cells and accessory cells from newborn mice.
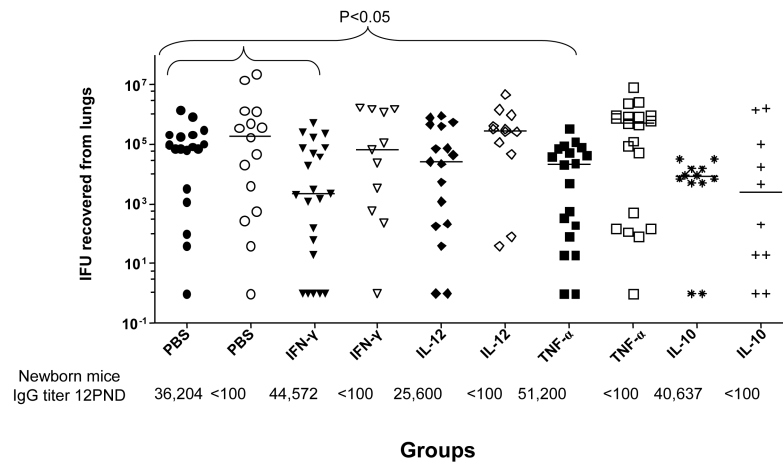
Figure 4. Passive transfer of cytokines to 2 day-old newborn mice

Newborn mice received various cytokines i.p. at 2 PND before an i.n. challenge. Following the challenge the newborn mice were euthanized at 12 PND. Filled symbols represent newborn mice born to immunized dams while open symbols represent newborn mice born to sham immunized dams. Horizontal bars represent median number of *Chlamydia* IFU recovered from each group. Antibody titers for each group of newborn mice at 12 PND are shown at the bottom of the figure.

Table 1

Antibody titer of dams and newborn mice at 12 PND following an i.n. infection.

Mouse type	Sample	Anti-MoPn ELISA titer						
		IgG	IgG1	IgG2a	IgG2b	IgG3	IgA	IgM
MoPn immunized dam	Sera	51,200	6,400	25,600	25,600	1,600	6,400	100
	Whey	1,600	<100	800	800	100	12,800	<100
Sham immunized dam	Sera	200	100	<100	100	<100	400	100
	Whey	<100	<100	<100	<100	<100	200	100
Newborn mice born to MoPn immunized dam	Sera	51,200	6,400	25,600	25,600	1,600	6,400	100
Newborn mice born to sham immunized dam	Sera	200	100	<100	100	<100	400	200