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Protection against an intranasal challenge by vaccines formulated with native and recombinant preparations of the *Chlamydia trachomatis* major outer membrane protein

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Abstract

To compare the ability of a native and a recombinant preparation of the major outer membrane protein of *Chlamydia trachomatis* mouse pneumonitis (MoPn; Ct-nMOMP and Ct-rMOMP) to protect against an intranasal (i.n.) challenge, BALB/c mice were vaccinated by the intramuscular (i.m.) and subcutaneous (s.c.) routes using CpG-1826 and Montanide ISA 720 as adjuvants. Animals inoculated i.n. with live elementary bodies (EB) of *Chlamydia* served as a positive control. Negative control groups were immunized with either *Neisseria gonorrhoeae* recombinant porin B (Ng-rPorB) or with minimal essential medium (MEM-0). Mice immunized with Ct-rMOMP, Ct-nMOMP and EB developed a strong immune response as shown by high levels of *Chlamydia* specific antibodies in serum and a strong T-cell lymphoproliferative response. Following the i.n. challenge with 10^4 inclusion forming units (IFU) of *C. trachomatis*, mice immunized with Ct-nMOMP or Ct-rMOMP lost significantly less weight than the negative control animals immunized with Ng-rPorB or MEM-0 ($P<0.05$). However, mice vaccinated with the Ct-nMOMP lost less weight than those immunized with the Ct-rMOMP ($P<0.05$). Mice were euthanized at 10 days following the challenge, their lungs weighed and the number of IFU of *Chlamydia* determined. Based on the lung weight and number of IFU recovered, significant protection was observed in the groups of mice immunized with both Ct-nMOMP and the Ct-rMOMP ($P<0.05$). Nevertheless, significantly better protection was achieved with the Ct-nMOMP in comparison with the Ct-rMOMP ($P<0.05$). In conclusion, vaccination with a preparation of the nMOMP elicited a more robust protection than immunization with rMOMP suggesting that the conformational structure of MOMP is critical for inducing strong protection.

Keywords

Chlamydia trachomatis; vaccines; major outer membrane protein; mice

INTRODUCTION

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial pathogen in the World [1–3]. In the USA, 3–4 million individuals are infected annually. In females cervicitis

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and urethritis are the most common acute manifestations. Long-term sequelae include pelvic inflammatory disease, chronic abdominal pain, ectopic pregnancy and infertility [4–5]. In males urethritis is the most frequent clinical presentation. In newborns under six months of age, *C. trachomatis* is the most common cause of pneumonia and conjunctivitis [2,3]. In addition, in countries with limited sanitary resources, trachoma, the leading cause of preventable blindness in the World, and lymphogranuloma venereum (LGV) are frequent clinical presentations of a *C. trachomatis* infection [2,3].

Efforts to vaccinate individuals against trachoma were carried out several decades ago [2,6, 7]. Humans and monkeys were immunized with whole organisms. Although no vaccination programs were implemented several practical lessons were learned from those trials. Specifically, protection was found to be, for the most part, serovar specific, short lived, and in poorly protected individuals reexposure to *C. trachomatis* resulted in a more severe disease than the one observed in non-vaccinated controls [2,6,7]. As a result of these findings it was proposed that a subunit vaccine was needed in order to avoid the harmful effects of the preparations containing the whole organism [8–10].

Molecular characterization of the structure of the MOMP of *C. trachomatis* identified this protein as a potential immunogen [8,11,12]. MOMP was found to have variable domains unique for each serovar and therefore, most likely accounting for the serovar-specific protection observed during the trachoma trials [12]. This protein, like other similar porins from gram-negative bacteria, forms a homotrimer [13,14]. Pal et al. [15], using the nMOMP as a vaccine, elicited in mice a protective response against a genital challenge as effective as that elicited by live EB. Unfortunately, producing the nMOMP in sufficient quantity to vaccinate humans will be too costly. Therefore, there is a need to formulate a vaccine using a rMOMP that is at least as effective as the native preparation. Here, we produced a preparation of the Ct-rMOMP and compared it with the Ct-nMOMP for its ability to protect mice against an intranasal challenge.

MATERIALS AND METHODS

Bacterial stocks

The *C. trachomatis* MoPn (strain Nigg II; American Type Culture Collection (ATCC), Manassas, VA) was grown as described [16,17]. *N. gonorrhoeae* strain FA1090 was purchased from ATCC and was grown on chocolate agar plates.

Purification of the Ct-nMOMP

The purification of the Ct-nMOMP has been described [15]. The purified nMOMP was refolded by dialysis in 0.1 M phosphate buffer (pH 7.8), containing 2 mM reduced glutathione, 1 mM oxidized glutathione (Sigma, St. Louis, MO), 1 mM EDTA and 0.05% Z3–14. The protein was concentrated and fixed with 2% glutaraldehyde (Sigma) at room temperature for 2 min. Glycine (Bio-Rad Laboratories) was added to stop the reaction. The MOMP was concentrated and dialyzed before immunization against PBS (pH 7.4), containing 0.05% Z3–14.

Cloning of the Ct-rMOMP and Ng-rPorB

The gene of the *C. trachomatis* MoPn MOMP (GenBank, accession No. AE002272, X63409), without the leading sequence, was amplified by the PCR and cloned into the pET-45b vector (Novagen, Madison, WI). For expression, *Escherichia coli* BL21 (DE3) was transformed with the plasmid containing the MoPn MOMP sequence. The *N. gonorrhoeae* PorB gene, without the leading sequence, (GenBank ID: AAW90430) was amplified with the PCR and was cloned and expressed in the same vectors.

Expression and purification of Ct-rMOMP and Ng-rPorB

The recombinant proteins were extracted from the *E. coli* inclusion bodies as described by Marston [18]. The pellet of the Ct-rMOMP was solubilized in TEN buffer with 8 M urea, 0.1 mM PMSF and 0.02 mM DTT to a concentration of 10 mg/ml. Following solubilization the MOMP solution was loaded onto a Sephacryl-S-300 column (1× 50 cm; Sigma), which was pre-equilibrated with 100 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10 mM EDTA, 0.2 mM DTT, and 0.05% Z3-14 and the peak fractions were pooled [15,19,20]. The Ng-rPorB pellet was solubilized in 0.2M Tris, pH 8.2, 6M Gdn-HCl, 2 mM EDTA, 1 mM PMSF and 20 mM DTT and centrifuged at 12,000× g for 20 min. Before immunization, both recombinant proteins were dialyzed against PBS (pH 7.4) with 0.05% Z3-14 [15].

The apparent MW and purity of Ct-nMOMP, Ct-rMOMP and Ng-rPorB were determined by 10% tricine-SDS-PAGE [21]. Using the limulus amoebocyte assay (BioWhittaker, Inc., Walkersville, MD), both recombinant proteins and the native MOMP were found to have less than 0.05 EU of LPS /mg of protein.

Immunization protocol

Three-week-old female BALB/c (H-2^d) mice were purchased from Charles River Laboratories (Wilmington, MA). All experiments were repeated twice. The University of California Irvine, Animal Care and Use Committee approved the animal protocols. Animals were immunized by the i.m. (5 µg protein/dose/mouse) and s.c. (5 µg protein/dose/mouse) routes with MOMP or PorB. Adjuvants used were 10 µg/dose/mouse of CpG-1826 (5'-TCCATGACGTTTCCTGACGTT-3'; Coley Pharmaceutical Group, Ontario, Canada) and Montanide ISA 720 (Seppic Inc, Fairfield, NJ) at a 30:70 volume ratio [15,22]. The mice were boosted two times at 2-week intervals. A second negative control group of mice was inoculated i.n. with MEM-0. Positive control mice were immunized i.n. once with 10⁴ IFU of the *C. trachomatis* MoPn [23].

Immunoassays

Following immunization blood was collected to determine the antibody response using an enzyme linked immunosorbent assay (ELISA) [15]. 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.). In vitro neutralization assays were performed using HeLa 229 cell monolayers as previously described [24]. The neutralizing titer of a sample was the dilution that yielded 50% neutralization relative to the negative control serum from NG-rPorB-immunized mice. Western blots were performed using nitrocellulose membranes as described [15]. To measure the cell-mediated immune response we used a lymphoproliferative assay (LPA) [23]. In brief, the spleens of two mice from each group were harvested and enriched for T-cells using a nylon wool column. The lymphocytes were cultured and stimulated with UV-inactivated *C. trachomatis* MoPn EB. Concanavalin A was added to control wells as a positive stimulant. At the end of the fourth day of incubation, 1.0 µCi of [methyl-³H]thymidine (47 Ci/mmol; Amersham, Arlington Heights, IL) in 25 µl of RPMI 1640 was added to each well and the uptake of the [³H]thymidine was measured after 18 h.

Measurement of cytokines

Levels of IFN-γ and IL-6 were determined using commercial kits (BD Pharmingen, San Diego, CA) in supernatants from splenic T cells stimulated as described above [15].

Intranasal challenge

Four weeks after the last immunization, BALB/c mice, under ketamine/xylazine anesthesia, were challenged i.n. with 10⁴ IFU of *C. trachomatis* MoPn in 40 µl of MEM-0 [23,26].

Following the i.n. challenge the mice were weighed daily. On day 10 post challenge the animals were euthanized and their lungs harvested and weighed. After homogenization in 5 ml of SPG serial 10-fold dilutions of the tissues were inoculated onto HeLa cells grown in 48-well tissue culture plates. The plates were centrifuged for 1 h at $1,000 \times g$ at room temperature, and then incubated for 30 h at 37°C in a CO₂ incubator. The *Chlamydia* inclusions were stained with a pool of MAb as described [23]. The limit of detection was 50 IFU/lungs/mouse.

Statistics

Statistical analyses were performed with the SigmaStat software package on a McIntosh computer (Cupertino, CA.) The two-tailed unpaired Student's *t* test and the Mann-Whitney *U* test were employed to determine the significance of the differences between groups. Differences were considered significant for values of $P < 0.05$.

RESULTS

Characterization of the natural and recombinant proteins

Following extraction and purification of the Ct-nMOMP, Ct-rMOMP and the Ng-rPorB, the preparations were analyzed by SDS-PAGE. As shown in Fig. 1 a single band with a molecular mass of 39 kDa was observed on the gel when the Ct-rMOMP and the Ct-nMOMP preparations were heated before loading. When the Ct-nMOMP preparation was not heated before loading two bands, corresponding to the trimer (66 kDa) and to the monomer (39 kDa), were observed. Due to the formation of aggregates, the Ct-rMOMP did not enter the gel when the preparation was not boiled before loading. The boiled Ng-rPorB migrated as a single band with a molecular mass of 36 kDa.

Antibody response in serum

Groups of BALB/c mice were immunized with the Ct-nMOMP, the Ct-rMOMP, and the negative control Ng-rPorB, using CpG-1826 and Montanide 720 as adjuvants. As shown in Table 1, the day before the challenge, both groups of animals immunized with the Ct-rMOMP or Ct-nMOMP had high *Chlamydia*-specific IgG titers, 102,400 and 204,800, respectively. The control group immunized i.n. with 10^4 IFU of *C. trachomatis* MoPn had an IgG titer of 12,800. No IgM antibodies were detected in any of the groups. The two negative control groups immunized with either Ng-rPorB or MEM-0 had no chlamydial-specific antibodies in serum. In the groups of mice immunized with the Ct-rMOMP, the Ct-nMOMP and live EB, the ratios of IgG2a to IgG1 were >1 indicating a Th1 biased response.

High titers of neutralizing antibodies were measured in the serum from the mice vaccinated with the Ct-rMOMP (6,250), Ct-nMOMP (1,250) and live EB (6,250). Serum samples from the Ng-rPorB immunized group were used as controls.

Immunoblot analyses of the serum samples, using EB as the antigen, are shown in Fig 2. The mice immunized with the Ct-rMOMP and the Ct-nMOMP produced antibodies only against the MOMP. Mice immunized i.n. with *C. trachomatis* EB developed antibodies against several antigens above the 100-kDa range, the 60-kDa cysteine rich protein, the 60-kDa-heat shock protein, MOMP and the 18-kDa protein. No antibodies against chlamydial components were detected in the serum of the mice immunized with Ng-rPorB or MEM-0.

T-cell responses of immunized mice

To determine the cellular immune response T-cells were purified and stimulated with EB or medium, as a background control, and the amount of proliferation measured by determining the incorporation of ³H-thymidine. As shown in Table 2, in the two groups of animals immunized with Ct-rMOMP and Ct-nMOMP a significant proliferative T-cell immune

response was observed ($P<0.05$). Similarly, a strong T-cell proliferative response was determined in the mice immunized i.n. with live EB. The negative control groups, immunized with Ng-rPorB or MEM-0, did not elicit a proliferative T-cell response to EB.

Levels of IFN- γ and IL-6 from supernatants of splenocytes stimulated with EB were significantly higher in the mice vaccinated with nMOMP, rMOMP and live *C. trachomatis* when compared with the respective negative control groups.

Body weight changes following the intranasal challenge

Weight loss was used as a parameter of the general effect of a chlamydial infection. The weight of the mice was measured daily following the i.n. challenge. As shown in Fig. 3, the positive control mice immunized with live EB maintained their body weight. In contrast, the negative control mice immunized with Ng-rPorB and MEM-0 lost 17.7% and 26.8% of their body weight, respectively, by 10 days post infection ($P<0.05$). By the 4th day after the i.n. challenge, the group vaccinated with Ct-nMOMP had lost 7.6% of their body weight. This group of mice recovered most of their weight and by 10 days after the challenge the mean body weight was 1.8% below its initial value. The mice vaccinated with the Ct-rMOMP lost 12% of their body weight by 4 days after the challenge, then started to recover weight and by day 10-post challenge they were 7.6% below their initial body weight. In comparison to the groups immunized with the Ng-rPorB or MEM-0 the mice vaccinated with Ct-nMOMP or Ct-rMOMP had lost significantly less weight ($P<0.05$). However, the animals vaccinated with Ct-nMOMP had lost significantly less body weight than the group immunized with Ct-rMOMP ($P<0.05$).

Lung weight and recovery of *C. trachomatis* from the lungs

At 10 days following the i.n. challenge the mice were euthanized, their lungs harvested, weighed and the number of *Chlamydia* IFU determined (Table 3). The weight of the lungs was used as an indication of the local inflammatory response. The mean weight of the lungs from the positive control mice vaccinated with live EB was 0.24 g while the weight of the lungs from the negative controls immunized with Ng-rPorB or MEM-0 were 0.35 g and 0.36 g, respectively ($P<0.05$). The weight of the lungs from Ct-nMOMP (0.23 g) and Ct-rMOMP (0.29 g) vaccinated mice were significantly lower than those of the groups immunized with Ng-rPorB or MEM-0 ($P<0.05$). Significant differences were also found in the weight of the lungs from the mice immunized with Ct-nMOMP compared with those immunized with Ct-rMOMP ($P<0.05$).

The lungs were homogenized and the number of chlamydial IFU determined using monolayers of HeLa cells. In the positive control group immunized i.n. with live EB the median number of IFU was <50 and the range $(<50-0.0005) \times 10^6$ IFU (Table 3). For the two negative controls groups immunized with either Ng-rPorB or MEM-0 the median number of IFU recovered were 3.799×10^6 and 6.071×10^6 , respectively. A significant reduction in the number of IFU was observed in the groups of mice immunized with Ct-rMOMP, median 1.5×10^6 and range $0.0003-6.029 (\times 10^6)$, and Ct-nMOMP, median 0.02×10^6 and range $0.003-0.2 (\times 10^6)$, in comparison with the negative control groups ($P<0.05$). The animals immunized with the Ct-rMOMP had significantly higher yields of IFU from the lungs than the mice vaccinated with Ct-nMOMP ($P<0.05$).

DISCUSSION

In this study we have shown that a vaccine formulated with a recombinant preparation of the *C. trachomatis* MoPn MOMP can elicit in mice a protective immune response against an intranasal challenge. However, the degree of protection obtained with the rMOMP was not as robust as that achieved with a nMOMP preparation indicating that the structural conformation

of the MOMP is important for inducing protection. Based on the immunological parameters determined including, antibody ELISA and neutralizing titers, lymphoproliferative responses and levels of cytokines the vaccine formulations with nMOMP and rMOMP elicited similar immune responses. Although the Th1 response, as determined by the IgG2a/IgG1 ratio appears to be stronger for the rMOMP vaccine, the levels of IFN- γ in the supernatants from EB-stimulated splenocytes were equivalent for both formulations. A similar conclusion can be made in relation to the immune response elicited by live EB emphasizing the difficulty of establishing immunological parameters that correlate with protection against a *C. trachomatis* infection.

In spite of the evidence pointing to the MOMP as the most likely candidate for a protective antigen, early efforts using both natural and recombinant proteins as a vaccine were not very encouraging. For example, Taylor et al. [25] extracted the MOMP from EB and immunized monkeys to induce protection against an ocular challenge. They observed that, although there was a transient decrease in the inflammatory response, there was no reduction in the intensity or duration of the infection. Dong-Ji et al. [26] primed mice with a DNA vaccine expressing MOMP, boosted the animals with MOMP extracted from EB, and observed significant protection against an intranasal challenge. Unfortunately, Dong-Ji et al. [26] reported that this vaccination protocol elicited variable protection from experiment to experiment. It is not possible to ascertain if, in these experiments by Taylor et al. [25] and Dong-Ji et al. [26], the nMOMP, extracted from EB, maintained its native conformation since their molecular structure was not characterized.

Recombinant preparations of MOMP have also been used as a vaccine in several animal models. For instance, Tuffrey et al. [27] immunized mice with rMOMP and challenged them in the genital tract. They observed a decrease in the severity of the salpingitis and in shedding but they could not demonstrate protection against infertility. Berry et al. [28] immunized mice transcutaneously with a recombinant *C. trachomatis* MoPn MOMP preparation fused to the maltose binding protein (MOMP-MBP) and obtained very limited protection against a vaginal challenge with *C. trachomatis* MoPn.

Several investigators considered the possibility that conformational epitopes of the MOMP were required to induce optimal protection [8,29–32]. This possibility was supported by data using outer membrane preparations where the native conformation of the MOMP was assumed to be maintained. For example, Tan et al. [31] immunized sheep with the outer membrane of *C. psittaci* and obtained protection against abortion following a s.c. challenge. Su et al. [30] obtained highly significant protection against a vaginal challenge following immunization with dendritic cells pulsed ex vivo with non-viable *C. trachomatis* MoPn. In contrast, when the dendritic cells were pulsed with rMOMP no protection was observed [32].

Hansen et al. [33] have recently compared the ability of a nMOMP and a rMOMP for their ability to protect mice against a vaginal challenge with *C. trachomatis* MoPn. The nMOMP was extracted and purified following a method similar to the one we used here. Using CAF01, a cationic liposome Th1 adjuvant, both MOMP preparations decreased the number of IFU recovered from the vaginal cultures although there was no protection against hydrosalpinx formation. Based on their findings the authors concluded that the protection was not dependent on the conformation of the MOMP. The difference between their results and ours can be due to several factors. One possibility is that the rMOMP preparation tested by Hansen et al. [33] was refolded into the native-like structure when combined with CAF01. The opposite, that the nMOMP preparation was originally not correctly folded or was unfolded by CAF01, is also a possibility specially, if one considers the very limited protection observed by Hansen et al. [33] with both preparations. In addition, the animal model utilized may not have been able to

discriminate between the two preparations. For example, a higher challenge dose may have shown that the nMOMP preparation was more protective than the rMOMP.

Several immunological mechanisms could account for the differences in the levels of protection observed in our study between the nMOMP and the rMOMP. CD4⁺ Th1 cells appear to be necessary for resolving a chlamydial infection while the role that CD8⁺ T cells still remains controversial [7,10]. B cells and/or antibodies have also been found to play a part in controlling a chlamydial infection [34]. MAb have been described that recognize linear and conformational epitopes in MOMP [8,12,35,36]. MAb that recognize conformational epitopes, specifically the trimer of MOMP, are more effective at neutralizing the infectivity of *Chlamydia* than mAb to linear epitopes [35,36]. Antibodies to conformational epitopes are also thought to be critical for the protective immune response elicited by recombinant vaccines for HBV, HPV and *Yersinia pestis* [37–39].

In addition to the conformation of the MOMP modulating the antibody response it is also likely that the cellular immune response is affected. For example, Musson et al. [40] characterized the mechanisms of antigen presentation of CD4⁺ T cell epitopes of the Caf1 antigen of *Y. pestis*. They showed that the degree of antigen processing was dependent on the structural content of the epitopes. Epitopes located in the globular domain of the protein were presented by newly synthesized MHC class II after low pH-dependent lysosomal processing. On the other hand, epitopes in a flexible strand of the protein were presented by mature MHC class II, independent of low pH, and did not require proteolytic processing. Also, Hanada et al. [41] found that, cytotoxic T lymphocytes isolated from a human with renal cancer, recognized HLA-A3 MHC class I molecules presenting a non-contiguous peptide generated by protein splicing. Hanada et al. [41] proposed that the peptide was produced by post-translational excision in the cytosol followed by ligation.

Other than the overall conformation of the MOMP, specific components of the antigen can affect the CMI response [40–42]. The MoPn MOMP contains eight cysteine residues of which four form intramonomeric disulfide bonds in the nMOMP preparation used for these studies [43]. The presentation on a MHC II molecule can be influenced by the presence of a cysteine in a T cell epitope or when a disulfide bond, distant from the epitope, affects antigen processing [44]. This is not surprising since antigen processing and presentation occur in a tightly regulated cysteine rich environment [45,46]. Currently, we do not know if the cysteine residues in the rMOMP parallel the structural conformation of those in the nMOMP. However, it is likely that the structure of the disulfide bonds of the nMOMP and rMOMP are different and that may affect the immune response.

In conclusion, the data shows that the structure of the *C. trachomatis* MOMP plays a critical role in inducing protection. Therefore, in order to stimulate strong protection, the formulation of a rMOMP that closely mimics the structure of the nMOMP is required.

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Figure 1. Coomassie blue stained 10% SDS-PAGE of the *C. trachomatis* MoPn nMOMP and rMOMP and the *N. gonorrhoeae* rPorB

Lane 1) MW standards; Lanes 2 and 3): not-boiled and boiled Ct-nMOMP, respectively; Lanes 4 and 5): not-boiled and boiled CT-rMOMP, respectively; Lanes 6) boiled Ng-rPorB.

Figure 2. Western blots of EB probed with sera from immunized mice

Lane 1) MW standards; Lane 2) Pooled pre-infection serum; Lane 3) Serum from mice vaccinated with Ct-nMOMP; Lane 4) Serum from mice immunized with Ct-rMOMP; 5) Serum from mice vaccinated with live Ct-MoPn; 6) Serum from mice immunized with Ng-rPorB; 7) Serum from mice immunized with MEM-0; Lane 8) EB probed with a mAb to MOMP.

Figure 3. Percentage change in total body weight following the i.n. challenge
Immunized mice were challenged i.n. with 10^4 IFU of *C. trachomatis* MoPn and their body weight determined daily.

Table 1
Serum antibody ELISA titers of mice immunized the day before the challenge with *C. trachomatis* MoPn

Antigen	Adjuvant	Immunization route	Neutralizing titer	Anti- <i>C. trachomatis</i> MoPn ELISA titer					
				IgM	IgG	IgG1	IgG2a	IgG2b	IgG3
Ct-rMOMP	CpG+Montanide	i.m.+s.c	6,250	<100	102,400	25,600	204,800	51,200	1,600
Ct-nMOMP	CpG+Montanide	i.m.+s.c	1,250	<100	204,800	51,200	102,400	102,400	25,600
Ng-rPorB	CpG+Montanide	i.m.+s.c	<50	<100	<100	<100	<100	<100	<100
Live Ct-MoPn	-	i.n.	6,250	<100	12,800	3,200	12,800	12,800	12,800
MEM-0	-	i.n.	<50	<100	<100	<100	<100	<100	<100

Table 2
T-cell responses of immunized mice the day before the challenge with *C. trachomatis* MoPn^a

Antigen	Adjuvant	Immunization route	T-cell proliferative response (10 ³ cpm) to:		IFN- γ secretion (ng/ml)	IL-6 secretion (ng/ml)
			EB ^b	Medium	response to EB	Response to EB
Ct-rMOMP	CpG+Montanide	i.m.+s.c	4.2 $\pm$ 2.1 ^c	0.2 $\pm$ 0.1	21.2 $\pm$ 10.0 ^c	2.4 $\pm$ 0.6 ^c
Ct-nMOMP	CpG+Montanide	i.m.+s.c	5.9 $\pm$ 2.3 ^c	0.3 $\pm$ 0.05	23.4 $\pm$ 13.6 ^c	3.6 $\pm$ 1.0 ^c
Ng-rPorB	CpG+Montanide	i.m.+s.c	0.6 $\pm$ 0.2	0.3 $\pm$ 0.05	11.7 $\pm$ 3.0	1.6 $\pm$ 0.3
Live Ct-MoPn	-	i.n.	15.4 $\pm$ 0.7 ^{c,d}	0.01 $\pm$ 0.05	26.3 $\pm$ 17.3 ^c	3.2 $\pm$ 0.4 ^c
MEM-0	-	i.n.	0.7 $\pm$ 0.01	0.2 $\pm$ 0.01	5.1 $\pm$ 0.5	0.7 $\pm$ 0.2

^aThe values are means $\pm$ 1 SD for the triplicate cultures. The data are from one experiment representative of duplicate experiments.

^bThe ratio of UV-inactivated *C. trachomatis* MoPn EB to antigen presenting cells was 1:1.

^cP<0.05 as determined by the Student *t* test compared to the mice immunized with Ng-PorB or MEM-0.

^dP<0.05 as determined by the Student *t* test compared to the Ct-rMOMP or Ct-nMOMP immunized groups.

Table 3Recovery of *C. trachomatis* MoPn from the lungs of mice following the i.n. challenge

Antigen	Adjuvant	Immunization route	Weight of lungs (g) ^a	Median IFU (range) recovered from lungs × (10 ⁶)
Ct-rMOMP	CpG+Montanide	i.m.+s.c	0.29±0.06 ^b	1.5 (0.0003 – 6,029) ^d
Ct-nMOMP	CpG+Montanide	i.m.+s.c	0.23±0.03 ^{b,c}	0.02 (0.003 – 0.2) ^{d,e}
Ng-rPorB	CpG+Montanide	i.m.+s.c	0.35±0.04	3,799 (10 – 18,534)
Live Ct-MoPn	-	i.n.	0.24±0.03 ^b	0 (0 – 0.0005) ^{d,ef}
MEM-0	-	i.n.	0.36±0.03	6,071 (1,899 – 13,339)

^aThe values are means ± 1 SD.^bP<0.05 as determined by the Student *t* test compared to the mice immunized with Ng-rPorB or MEM-0.^cP<0.05 as determined by the Student *t* test compared to the Ct-rMOMP immunized group.^dP<0.05 as determined by the Mann-Whitney U test compared to the Ng-rPorB or MEM-0 immunized groups.^eP<0.05 as determined by the Mann-Whitney U test compared to the mice immunized with Ct-rMOMP.^fP<0.05 as determined by the Mann-Whitney U test compared to the Ct-nMOMP immunized group.