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Vaccination with the polymorphic membrane protein A reduces *Chlamydia muridarum* induced genital tract pathology ☆

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ABSTRACT

Chlamydia trachomatis serovars D-K are one of the most frequent causes of sexually transmitted infections of the female genital tract, with possible complications such as hydrosalpinx, pelvic inflammatory disease, extra-uterine gravidity or infertility. We used the murine genital tract infection model with *C. muridarum* for vaccination studies and found that more than 70% of the infected mice suffered from uterus dilatations and/or hydrosalpinx. Systemic consequences of the vaginal infection were apparent by splenomegaly ten to fifteen days post infection. While cultivable microorganisms were detectable for the first 23 days post infection, the first lesions of the genital tract developed at day 15, however, many lesions occurred later in the absence of cultivable bacteria. Lesions were not accompanied by pro-inflammatory cytokines such as IFNγ, TNF and IL-6, since these cytokines were almost undetectable in the genital tract 43 days post infection. To prevent genital tract lesions, we vaccinated mice with the polymorphic membrane protein (Pmp) A in combination with CpG-ODN 1826 as adjuvant. The vaccine lowered the chlamydial burden and the differences were significant at day 10 post infection but not later. More importantly the vaccine decreased the rate and severity of genital tract lesions. Interestingly, control vaccination with the protein ovalbumin plus CpG-ODN 1826 enhanced significantly the severity but not the rate of pathologic lesions, which was presumably caused by the activation of innate immune responses by the adjuvant in the absence of a *C. muridarum*-specific adaptive immune response. In summary, vaccination with recombinant PmpA plus CpG-ODN 1826 significantly reduced *C. muridarum*-induced tissue damage, however, CpG-ODN 1826 may aggravate *C. muridarum*-induced tissue injuries in the absence of a protective antigen.

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1. Introduction

Genital tract infections caused by *Chlamydia trachomatis* serovars D-K increase globally; they are one of the most frequent sexually transmitted diseases with over 100 million infections per year and most prevalent in young females aging between 19 and 24 years [1]. Unfortunately, the infections are asymptomatic in over 70% of cases and, thus, occur undetected. Consequently, chronic inflammation may result in organ damage of the female genital tract in the absence of antibiotic treatment [2]. In these cases, tissues of the uterus and oviduct are remodeled leading to hydrosalpinx of the latter and hence to extra-uterine gravidity or infertility. Additionally, spreading of the infection to the upper genital tract may lead to pelvic inflammatory disease.

These severe pathologic consequences can only be prevented by the availability of an efficient vaccine as screening programs were so far not successful to lower chlamydial infection rates [3]. The application of screening and treatment programs unexpectedly even increased infection rates. One among several hypotheses explaining this observation is that earlier antibiotic treatment may interrupt the development of immunity allowing higher reinfection rates [4].

Abbreviations: DDA/MPL, dimethyldioctadecylammonium bromide/monophosphoril lipid A; MOMP, major outer membrane protein; Mon-CpG, Montanide ISA 720/CpG-ODN 1826; ODN, oligodeoxynucleotide; Pmp, polymorphic membrane protein; TH1, T-helper 1.

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The infection with *C. trachomatis* induces a partially protective immunity but also immune-pathological consequences, which may as well be provoked by a vaccine. Earlier vaccination studies using an inactivated *C. trachomatis* strain to prevent trachoma, a frequent blinding disease caused by *C. trachomatis* serovars A-C, demonstrated that vaccinated individuals suffered from a more severe clinical picture [5]. Nevertheless, the vaccine also induced a short-lived protection, demonstrating that vaccination against the disease is, in principle, possible.

Attenuated living chlamydial strains were also used as experimental vaccines to prevent organ damage during genital infection in mice. Thus, infection of mice with a plasmid-cured *C. muridarum* strain did not lead to oviduct damage like hydrosalpinx indicating the importance of plasmid encoded genes for organ destruction [6]. Treatment of mice or nonhuman primates with the plasmid-cured strain and subsequent challenge infection with wild type *C. muridarum* or *C. trachomatis* reduced oviduct damage or trachoma, respectively [7]. Similarly, reduced ocular pathology was found in nonhuman primates upon vaccination with a plasmid-cured *C. trachomatis* strain and subsequent challenge with a wild type *C. trachomatis* strain [8].

Despite these at least partial successes with whole chlamydial vaccines, research efforts now focus on the development of a subunit vaccine. For this purpose different chlamydial proteins such as the major outer membrane protein (MOMP), the heat shock protein GroEL1, outer membrane complex protein B (OmcB), polymorphic membrane proteins (Pmps) and others have been identified [9]. For instance, native and recombinant MOMP combined with different adjuvants protected mice from a genital or pulmonary challenge infection with *C. muridarum*, respectively [10,11]. Pmps are surface exposed outer membrane proteins and described to be important for pathogenesis by mediating adhesion of chlamydial elementary bodies to the epithelial cell membrane [12,13]. They are characterized by multiple GGA(I, L, V) and FxxN motifs, which have been proven to be essential for adhesion to human cells [14,15]. Four of them, PmpE, PmpF, PmpG and PmpH, contain murine MHC class II epitopes and adoptively transferred dendritic cells pulsed with peptides derived from these Pmps partially protected mice from genital tract infection with *C. muridarum* [16]. A recent vaccination study using these recombinant Pmps plus MOMP and dimethyldioctadecylammonium bromide/monophosphoril lipid A (DDA/MPL) as adjuvant demonstrated that chlamydial burden was reduced by several orders of magnitude, although vaccination with living *C. muridarum* was more efficient [17]. Interestingly, Pmps were as potent as the MOMP protein to reduce chlamydial burden. However, the effect of the vaccines on genital tract tissue damage was not explored. This analysis is important, since a reduction in chlamydial burden may not be paralleled by a reduction in tissue damage. In a comparative study of sequence polymorphisms of all nine *pmp* genes from all *C. trachomatis* serovars, *pmpA* and *pmpI* displayed the lowest number of replacements and predicted amino acid substitutions among serovars [18]. Thus, PmpA and PmpI may represent promising vaccination candidates against different *C. trachomatis* serovars.

In the present study, we tested the ability of PmpA in combination with the adjuvant CpG-ODN 1826, which induces strong TH-1 responses, to lower chlamydial burden and more importantly severity of tissue damage in a murine genital tract infection model with *C. muridarum*.

2. Material and methods

2.1. Animals

Female C57Bl/6 mice (3 weeks old) were purchased from Harlan Winkelmann GmbH, Eystrup, Germany and allowed to acclimate for 7 days. All mice were housed under SOPF/SPF conditions in a climate controlled room with a cycle of 12 h light and 12 h darkness and were given rodent diet and water ad libitum. Co-housed sentinel mice were negatively controlled for a panel of murine pathogens according to FELASA guidelines.

2.2. Preparation of chlamydial recombinant proteins

A gene fragment of *pmpA* (aa 155-609) from *Chlamydia trachomatis*-E DK-20 was amplified by PCR and cloned for expression as described previously [12]. Plasmids containing *pmpA* were transformed into competent *Escherichia coli* strain XL-1-Blue (Stratagene, La Jolla, California). PmpA containing a C-terminal His-tag was expressed, subsequently purified using His-bind gravity flow purification system (Qiagen, Hilden, Germany) and stored at −80 °C.

2.3. Immunization

To evaluate the protective efficacy of our vaccines, four groups of C57Bl/6 mice (n = 5) were set up: (i) mice injected with PBS served as negative, (ii) mice only infected with *C. muridarum* as positive controls, (iii) mice immunized with ovalbumin (30 μg) + CpG-ODN 1826 (1 nmol) as vaccination control group and (iv) mice immunized with PmpA (30 μg) + CpG-ODN 1826 (1 nmol) as vaccine group. The adjuvant CpG-ODN 1826 (Coley Pharmaceutical, Düsseldorf, Germany) was pre-diluted in PBS. Mice were immunized two times subcutaneously at the base of the tail in 2-weeks-intervals. For evaluation of protection against infection, mice were intravaginally inoculated with 1×10^6 inclusion forming units (IFUs) of *C. muridarum* four weeks after the final immunization. This vaccination trial was three times independently repeated.

2.4. Infection

Four weeks after the final immunization all mice were pretreated with 2.5 mg medroxyprogesterone acetate (Depo-Provera, Pfizer, Germany) twice subcutaneously at day 10 and day 3 before infection and subsequently challenged intravaginally with 10 μl SPG buffer (250 mM sucrose, 10 mM sodium phosphate, 5 mM L-gluthamic acid, pH 7.2) containing 1×10^6 inclusion forming units of living *C. muridarum*. Control mice were inoculated with SPG buffer only.

2.5. Propagation of C. muridarum

C. muridarum was grown in HEp2-cell monolayers (ATCC CCL-23) in MEM alpha medium supplemented with 5% FCS (PAA, Pasching, Austria), 5 mg gentamicin, 25 mg vancomycin per 500 ml medium and 0.5 μg/ml cycloheximid (all Sigma Aldrich). *C. muridarum* infected cells were cultivated for 20-24 h at 37 °C with 5% CO_2, expanded, harvested and chlamydial elementary bodies were purified for storage at −80 °C in SPG buffer. *C. muridarum* aliquots were thawed immediately before use.

2.6. Assessment of gross/macroscopic pathology

Mice were euthanized by cervical dislocation and the genital tract was examined in situ for macroscopically visible changes. Subsequently the genital tract was completely excised and excessive fat was removed. The absence or presence of hydrosalpinx and uterine dilatations was recorded and scored (0 = no hydrosalpinx/uterine dilatation macroscopically visible; 1 = unilateral hydrosalpinx/uterine dilatation; 2 = bilateral hydrosalpinx/uterine dilatation; 3 = more than 2 lesions of hydrosalpinx/uterine

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