# The biodegradable polymeric PLA-PEG nanoparticles is an efficient delivery system for a chlamydial M278 mucosal nanovaccine that provides protective systemic and mucosal antibody responses in mice

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## ABSTRACT

We have developed a *Chlamydia* nanovaccine by encapsulating M278 [a peptide of the major outer membrane protein (MOMP)] within poly(lactic acid)-poly(ethylene glycol) nanoparticles. Here we assessed the systemic and mucosal antibody responses provided by PLA-PEG-M278 in immunized female mice challenged with live *C. muridarum.* Our results reveal that PLA-PEG-M278 mice produced higher serum M278- and MOMP-specific IgG, IgG1, IgG2b and vaginal wash IgA antibodies as compared to bare M278 mice. Moreover, IgG1 antibody remained high after 222 days post-immunization. Nanovaccine mice produced more Th2 (IgG1) than Th1 (IgG2b) antibodies, suggesting a dominant Th2 response. Notably, nanovaccine mice produced heightened mucosal MOMP-specific IgA antibodies after a live bacterial challenged infection. Collectively, PLA-PEG-M278 induced systemic and mucosal IgA antibody responses that are essential in protecting mice against a chlamydial vaginal infection as well as providing long-lived immunity.

***Keywords*****:** *Chlamydia muridarum,* nanovaccine, MOMP (Major Outer Membrane Protein), PLA-PEG [poly(lactic acid)-poly(ethylene glycol)], antibodies.

## 1 INTRODUCTION

*Chlamydia trachomatis* (Ct) is an obligate, Gram-negative bacterium residing in the human genital tract and is the most common causative agent of bacterial sexually transmitted diseases worldwide. Chlamydial infections are frequently asymptomatic and may lead to disease progression with severe sequelae of disease manifestations [1]. Antibiotics are effective but because of the asymptomatic nature of Ct, development of a vaccine remains a prime concern for researchers. Our lab has emphasized on biodegradable polymeric nanoparticles to provide efficient delivery of biomolecules. We have developed Ct nanovaccine employing PLA-PEG encapsulated with M278, a recombinant MOMP peptide and have shown its efficacy to enhance immune responses and provide partial protection in mice. Previously, we reported that PLA-PEG-M278 nanoparticles potentiated robust *Chlamydia*-specific antibody and cellular adaptive immune responses in immunized mice [2]. Our goal in the present study is to assess the protective systemic and mucosal antibody responses engendered by the PLA-PEG-M278 nanovaccine in immunized BALB/c mice challenged with live *C. muridarum* (Cm).

## 2 MATERIALS AND METHODS

### 2.1 Preparation of Nanoparticles

A recombinant peptide (M278) derived from the major outer membrane protein (MOMP) of *C. trachomatis* was cloned [3] and encapsulated in PLA-PEG biodegradable nanoparticles using a modified water/oil/water double emulsion evaporation technique and then lyophilized in the presence of 5% trehalose (as a stabilizer) to obtain PLA-PEG-M278 nanoparticles as reported [2].

### 2.2 Mice Immunization and Challenge

Female 4-6 weeks-old BALB/c mice were purchased from Charles River Laboratory (Raleigh, NC) and acclimatized for two-weeks prior to all experimental procedures. The animal studies were performed following a protocol approved by the Alabama State University Institutional Animal Care and Use Committee (IACUC). Mice were housed under standard pathogen-free and controlled environmental conditions and provided with food and water *ad libitum*. Mice were divided into two experimental groups (9 mice/group) for the immunization and challenge studies. Groups of mice received three subcutaneous immunizations at two-week intervals with bare M278 and nanoparticles (encapsulating M278). The PLA-PEG-M278 mice each received 50 µg/100 µL of encapsulated-M278 in PBS. Mice in the M278 group received 50 µg/100 µL of purified M278. For the challenge study, immunized mice (6 mice/group) were injected subcutaneously with 2.5 mg of medroxyprogesterone acetate (Depo-Provera) two-weeks after the last immunization, follow a week later by an intravaginal challenge with $10^5$ IFU of Cm in sucrose-phosphate-glutamic acid (SPG) buffer [4]. All groups of challenged mice were sacrificed three-week post-challenge to collect serum and vaginal wash samples for antibody analyses. For immunogenicity study, the

unchallenged mice (3 mice/group) were sacrificed on day 222 following the last immunization, to collect serum samples for antibody analyses.

## 2.3 Serum and Mucosal Antibody Responses

Serum and vaginal washed samples were collected from mice and pooled for each group for the detection of M278- and MOMP-specific antibody isotypes (IgG, IgG1, IgG2a IgG2b and IgA) by enzyme-linked immunosorbent assay (ELISA) as previously described [2, 5, 6]. Serum or cervico-vaginal wash samples were used at 1:4000 (for serum IgG, IgG1) and 1:500 (serum IgG2a, IgG2b) or 1:5 (wash IgA) and added to the plates followed by addition of horseradish peroxidase-conjugated goat antimouse immunoglobulin IgG, IgG1, IgG2a, IgG2b, or IgA (Southern Biotech, Birmingham, AL, USA) antibody. Plates were washed and developed using TMB substrate (KPL, Gaithersburg, MD, USA). Absorbance was read at 450 nm.

## 2.4 Statistical Analysis

Data were analyzed by one- or two-way analysis of variance (ANOVA) followed by Tukey's Post-test using GraphPad Prism 6 Software (GraphPad Software, Inc., CA, USA. *P* values ≤0.05 was considered statistically significant.

# 3 RESULTS AND DISCUSSIONS

## 3.1 Antigen-specific serum antibodies in immunized-challenged mice

To assess how a Cm challenged infection might impact pre-existing antibody responses in immunized mice, antibody ELISAs were conducted using pooled sera of immunized-challenged mice against the specific M278 peptide and the parent protein, MOMP. We observed that mice immunized with PLA-PEG-M278 produced significantly higher (*P* <0.001) post-challenge antigen-specific IgG, IgG1 (Th2) and IgG2b (Th1) antibodies as compared to bare M278 **(Figure 1).** But overall, levels of IgG1 antibodies, both M278- as well as MOMP-specific, remained higher than those of specific IgG2a and IgG2b **(Figure 1)** antibodies. However, antibody responses against MOMP **(Figure 1B)** remained lower than those against the specific M278 antigen **(Figure 1A)**.

Our previous studies have shown that IgG1 (Th2) was the dominant antibody isotype in mice immunized with PLA-PEG-M278 and the Th1 (IgG2a and IgG2b) antibody responses were comparatively lower than the observed Th2 responses when measured two weeks after the last immunization.

It is well-established that humoral immunity plays an important role in limiting the spread of chlamydial infections and clearance during reinfection [7, 8]. Antibody-mediated neutralization and opsonization as well as enhanced antigen presentation to T-cells followed by receptor-mediated uptake are some of the possible mechanisms through which B-cells engender protective immunity to re-infection [9, 10]. The enhanced immunopotentiation observed here is attributed to the self-adjuvanting, sustained release, and nanoparticulate properties of PLA-PEG being used as delivery vehicle for the immunogenic peptide, M278. Vaccines based on nanoparticlute adjuvants, being similar in size to intracellular pathogens like bacteria and viruses, mimic the properties of actual pathogens and show enhanced immunogenicity without causing disease. Thus the significantly high IgG1 and IgG2b levels observed in the post-challenge sera of PLA-PEG-M278 immunized mice indicate the immuno-protective role of these nanoparticles in limiting the bacterial burden in nanovaccinated mice.

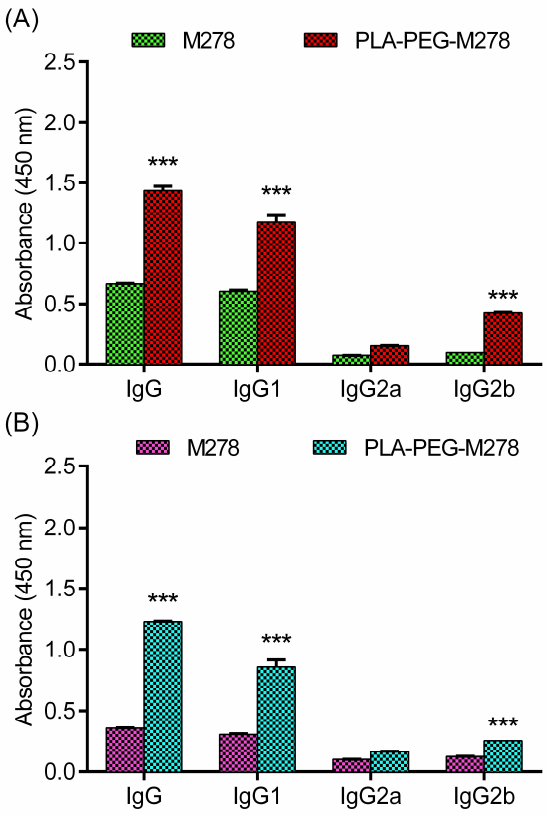


Figure 1: Antigen-specific serum antibodies production in immunized-challenged mice. M278-specific (A) and MOMP-specific (B) IgG, IgG1, IgG2a, IgG2b antibodies were measured by ELISA in pooled sera three-weeks post-challenge with *C. muridarum*.

## 3.2 Determination of long-term antigen-specific humoral immunity in immunized mice

Another set of experiments were performed to assess whether the antibody-mediated immunity elicited by PLA-PEG-M278 or bare M278 was long-lived or waned after a short time. The immunized mice were housed for a longer time period and sacrificed on day 222 post last immunization and antibody ELISAs were conducted using pooled sera as described earlier. The results clearly indicated that higher M278-specific ($P$ <0.01) **(Figure 2A)** as well as MOMP-specific ($P$ <0.001) **(Figure 2B)** antibody responses for IgG and IgG1 (Th2) were observed in sera of mice immunized with PLA-PEG-M278 as compared to M278 immunized group. But still the IgG and IgG1 responses against M278 remained higher as compared to those against MOMP **(Figure 2B)**. No significant differences were observed in levels of M278-specific or MOMP-specific IgG2b **(Figure 2)** antibodies as produced in PLA-PEG-M278 and bare M278 immunized mice. However, the overall pattern showed that the Th2 antibody response was predominant even after ~7 months (222 days) post last immunization.

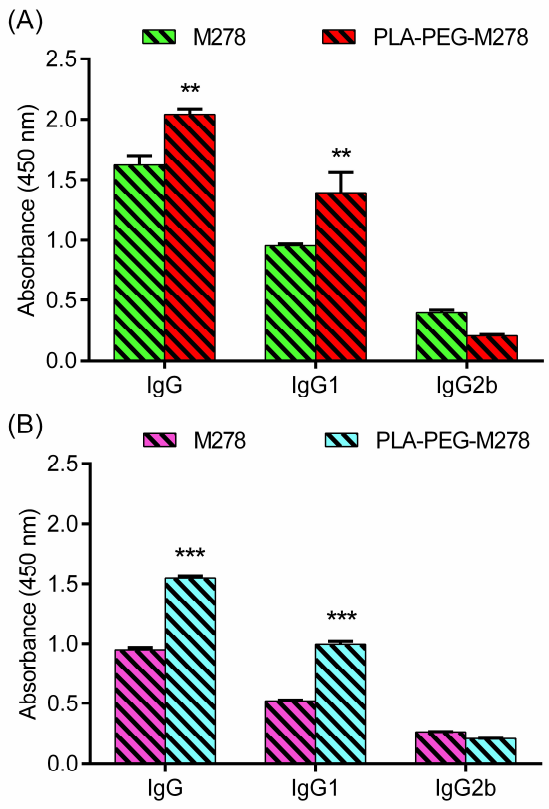


Figure 2: Antigen-specific serum antibodies production in immunized mice. M278-specific **(A)** and MOMP-specific **(B)** IgG, IgG1, and IgG2b antibodies were measured by ELISA in pooled sera 222 days after the last immunization.

The results clearly show that immunization with PLA-PEG-M278 potentiated long-lasting humoral immune responses. A short-lived immunity has, for long remained a challenge in developing an efficacious vaccine against *C. trachomatis*. It seems that PLA-PEG-M278 provided long-lived immunity in terms of elevated levels of antibody responses up to 222 days post-immunization, which could overcome the abovementioned challenge that has plagued the development of a *C. trachomatis* vaccine.

## 3.3 Antigen-specific mucosal IgA antibodies

Mucosal IgA antibody was also measured in the cervico-vaginal samples post-challenge. Both M278-specific ($P$ <0.01) and MOMP-specific ($P$ <0.001) IgA responses were upregulated post-challenge in mice immunized with PLA- PEG-M278 as compared to the bare M278 mice **(Figure 3)**. Of interst was the up-regulation of MOMP-specific IgA responses in both challenged groups of mice; nonetheless IgA was still higher in PLA-PEG-M278 immunized mice **(Figure 3)**.

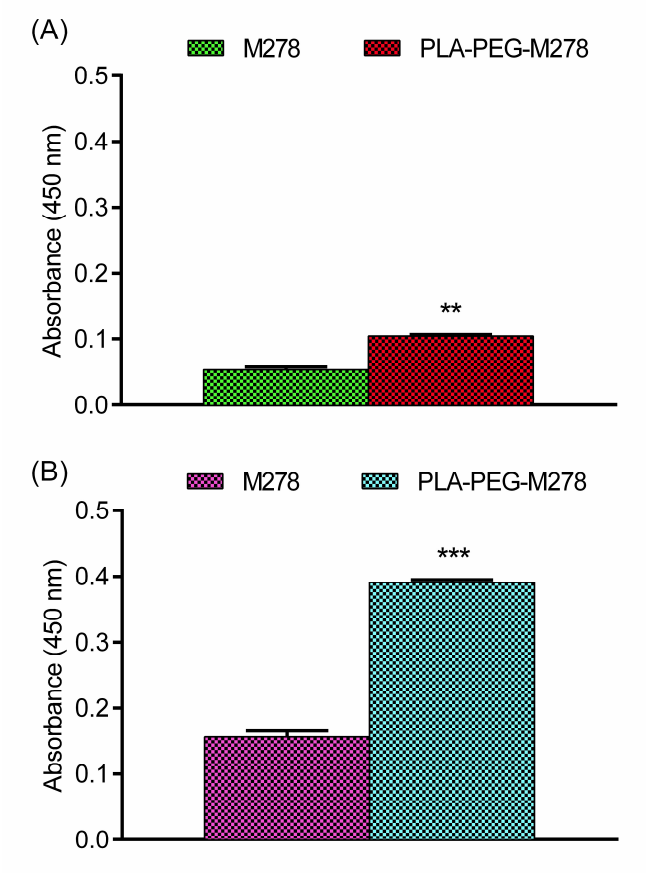


Figure 3: Antigen-specific mucosal IgA antibodies in immunized-challenged mice. M278-specific (A) and MOMP-specific (B) IgA antibodies were measured by ELISA in pooled cervico-vaginal washes three-weeks after the challenge infection (post-challenge) with *C. muridarum*.

Studies conducted on humans [11] as well as murine models of *Chlamydia* demonstrated that high levels of IgA are associated with decreased endocervical bacterial load [5, 8, 11] conferring protective immunity at mucosal surfaces. Our findings show that immunization with the PLA-PEG-M278 nanovaccine induced enhanced mucosal IgA, which neutralizes the bacteria by either preventing attachment to genital epithelial cells or inhibiting intracellular replication to afford significant protection against the pathogen.

## 4 CONCLUSION

*C. trachomatis* is an obligate intracellular pathogen, which enters the host through the epithelial mucosa of the genital tract and a vaccination strategy that could provide a sufficiently high mucosal immunity would be warranted to control this pathogen. The PLA-PEG-M278 nanovaccine stimulated significant systemic and mucosal antibody responses to protect mice against a *C. muridarium* challenged infection. The enhanced secretion of mucosal IgA suggests its protective role against chlamydial vaginal infections in mice. Our data provides additional evidence of the chlamydial PLA-PEG-M278 nanovaccine in providing short and long-term immunity in mice.

## REFERENCES


[1] Albritton HL, Kozlowski PA, Lillis RA, McGowin CL, Siren JD, Taylor SN, Ibana JA, Buckner LR, Shen L, Quayle AJ. PLoS One. 2017;12(8):e0183101.

[2] Dixit S, Singh SR, Yilma AN, Agee RD 2nd, Taha M, Dennis VA. Nanomedicine. 2014; 10(6) 1311-21.

[3] Taha M, Singh SR, Dennis VA. Nanotechnology. 2012; 23, 325101.

[4] Pal S, Tatarenkova OV, de la Maza LM. Immunology. 2015; 146(3), 432-43.

[5] Singh SR, Hulett K, Pillai SR, Dennis VA, Oh MK, Scissum-Gunn K. Vaccine. 2006; 24(8), 1213-24.

[6] Fairley SJ, Singh SR, Yilma AN, Waffo AB, Subbarayan P, Dixit S, Taha MA, Cambridge CD, Dennis VA. Int J Nanomedicine. 2013; 8, 2085-99.

[7] Yu H, Karunakaran KP, Jiang X, Brunham RC. Vaccine. 2014; 32(36), 4672-80.

[8] Wern JE, Sorensen MR, Olsen AW, Andersen P, Follmann F. Front Immunol. 2017; 17, 8:569

[9] Brunham RC, Rey-Ladino J. Nat Rev Immunol. 2005; 5(2), 149-61.

[10] Ekong EE, Okenu DN, Mania-Pramanik J, He Q, Igietseme JU, Ananaba GA, Lyn D, Black C, Eko FO. FEMS Immunol Med Microbiol. 2009; 55(2), 280-91.

[11] Brunham RC, Kuo CC, Cles L, Holmes KK. Infect Immun. 1983; 39(3), 1491-4.


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