PLA-PEG nanoparticles are suitable for effective maturation and uptake by dendritic cells for *Chlamydia trachomatis* outer membrane peptide-based vaccine

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ABSTRACT

Chlamydia trachomatis is considered an important human pathogen because it is the most reported sexually transmitted bacterial infection globally. We showed that M278 (a peptide derived from the major outer membrane protein of *C. trachomatis*) encapsulated within poly (lactic acid)-b-Poly (ethylene glycol) (PLA-PEG) nanoparticles (NPs) triggered enhanced systemic adaptive immune responses in mice. PLA-PEG can facilitate passive uptake of antigens by dendritic cells (DCs) by increasing the influx of DCs in to the injection site. Therefore, in this study, we investigated the potential of PLA-PEG-encapsulated M278 NPs to induce maturation and activation of DCs for effective antigen presentation to bolster immune responses. DCs were derived from mouse bone marrow cells and exposed to of 2.5 μg/mL of naked M278 or encapsulated M278 for 24 hours to determine the expressions of costimulatory molecules (CD80 and CD86) and the MHC complex. Results from flow cytometry and immunofluorescence microscopy revealed that encapsulated M278 enhanced the expression levels of CD80, CD86, CD40 and MHC complex on DCs as compared to naked M278, suggesting that NPs were more efficiently processed by DCs. Collectively, these data suggests that our nano-encapsulated M278 vaccine drives maturation of DCs and efficient antigen presentation for elicitation of enhanced Th1 adaptive immune responses.

Key words: *Chlamydia trachomatis*, bacteria, poly (lactic acid)-b-Poly (ethylene glycol), dendritic cells, antigen presentation

1.0 INTRODUCTION

In pursuit of a vaccine against *Chlamydia trachomatis*, the most reported bacterial sexually transmitted infection globally, we previously published the successful encapsulation and slow release of M278 (a peptide derivative of *C. trachomatis* major outer membrane protein) from PLA-PEG [poly (lactic acid)-poly (ethylene glycol), and its elicitation of enhanced adaptive immune responses in immunized mice [1]. However, it is important to investigate the mechanisms of induction of adaptive immune responses and the role of dendritic cells (DCs) in effective antigen presentation for bolstering these responses. PLA-PEG, a biodegradable copolymer, has the ability to delay release of protein over several weeks, a characteristics that makes it attractive for vaccine delivery [1]. PLA-PEG sustained release, sub-cellular size, and enhanced biocompatibility properties facilitate uptake of antigens by APCs as well as increasing APCs influx to the injection site. In this study we investigated the mechanisms of PLA-PEG-encapsulated M278 uptake and processing by DCs and focused on expressions of CD80 and CD86.

2.0 MATERIALS AND METHODS

2.1 Preparation of nanoparticles

M278 was encapsulated in PEG-b-PLA Diblock polymer nanoparticles by a modified water/oil/water double emulsion–evaporation technique essentially as described [2, 3]. Briefly 300 mg of PLA-PEG was emulsified in Ethyl acetate followed by addition of 1 mg of M278, homogenization and than addition of 1% Polyvinyl Alcohol (PVA). The resulting double emulsion was gently stirred overnight at room temperature (RT) to evaporate the organic solvents, harvested by ultracentrifugation, washed and lyophilized in the presence of 5% trehalose. Sterile PBS was used in the primary emulsion formation to prepare PLA-PEG-PBS nanoparticles to serve as a negative control.

2.2 Isolation and Culture of DCs

Bone-marrow cells were flushed from mouse femurs (five to six per group) with sterile RPMI media containing glutamax (Gibco Invitrogen, Carlsbad, CA), washed twice and RBCs were lysed using ACK lysing reagent (Invitrogen, Carlsbad, CA). Cells were grown for 7 days in tissue-culture petri dishes at 37^0C and 5% CO2 with change of media on days 3 and 6. The culture medium consisted of RPMI glutamax supplemented with, 10% heat-inactivated fetal bovine serum (Gibco), 50 μM β-merceptoethanol, 20 ng/mL granulocytes macrophage colony-stimulating factor (GM-CSF) (R&D Systems, Minneapolis, MN) and antibiotic/antimycotic (Invitrogen) [4]. Dendritic cells (DCs) were harvested and were plated at 0.50×10^6/culture tube.

2.3 Flow cytometery (FACS)

The expression of CD80, CD86, CD40 and MHC complex in DCs (0.5×10^6) was assessed after 24 h of stimulation with M278, PLA-PEG-PBS and PLA-PEG-M278. DCs (0.5×10^6/mL) were blocked with Fc blocking antibody (BD Bioscience) in fluorescent-activated cell sorting (FACS) buffer (phosphate-buffered saline, 0.1% NaN3, 1.0% fetal bovine serum) for 15 minutes at 4°C. DCs were washed and stained with fluorochrome-conjugated antibodies against DCs surface receptors, using anti-CD80, CD86, CD40 and MHC antibodies (MHCI A647 (BD: 562832), CD86 FITC (BD: 553691), CD80 PECy-7 (BD: 562504), CD40 BV421 (BD: 562846) each at 0.250 µg/100 µL. Unstimulated control cell cultures (medium) were kept as background controls. DCs were then washed and fixed with 1% paraformaldehyde solution for 20 minutes at 4°C. Data were acquired on a BD FACS Canto II flow cytometer (BD Bioscience) with at least 1×10^5 events for each sample and analyzed using FlowJo software (Tree Star Inc, Ashland, OR, USA).

2.4 Immunofluorescence staining (IFA)

We conducted IFA to confirm expression of CD86 and uptake of nanoparticles by DCs. DCs (2×10^4 cells/well) were seeded in 8-well chamber slides and were stimulated with 5 µg/mL of M278, PLA-PEG-PBS (PPP) and PLA-PEG-M278 (PPM) for 24 h. DCs were washed, fixed with 2% paraformaldehyde and blocked with 10% normal goat serum followed by staining with CD86 (5 µg/mL), DCs surface marker CD11c (3 µg/mL) in 0.5% PBS BSA for 2 h at room temperature. DCs were permabilized with 0.05% Triton X 100 for 10 minutes followed by staining with FITC-conjugated anti-*C. trachomatis* polyclonal antibody (1:500 dilution) for 1 h, washed, and counterstained with 4′,6-diamidino-2-phenylindole (DAPI) combined with an anti-fade mounting solution (Life Technologies). DCs were visualized using a Nikon Eclipse Ti Confocal Microscope (Nikon Instrument, Melville, NY).

3.0 RESULTS AND DISCUSSIONS

In the present study we employed flow cytometric analysis to investigate quantitative differences in the levels of antigen uptake and surface receptor expression on DCs as exposed to M278, PLA-PEG-PBS and PLA-PEG-M278. Upon stimulation, antigen presentation marker (CD80, CD86, CD40) were significantly ($P < 0.001$) upregulated in PLA-PEG-M278 in comparison to M278. These finding suggest that M278 encapsulated NPs are taken up by DCs resulting in elevated expression of costimulatory molecules for induction of Th1 type immune responses that are necessary for *Chlamydia* clearance and protection.

3.1 Flow cytometric analyses

DCs (0.5×10^6/mL) were stimulated with M278, PLA-PEG-PBS and PLA-PEG-M278. DCs were collected after stimulation and were used to determine the percentages of CD40+ and CD80+ and CD86+ DCs subsets by flow cytometry. Figure A-D show the percentages for CD80+ (Q1), CD80+CD86+ (Q2), CD86+ (Q3), and CD80-CD86- (Q4). Percentages of CD80 and CD86 postive population in PLA-PEG-M278, PLA-PEG-PBS and M278 are (0.79/70)%, (4.5/50.1)% and (8.93/22.5)%, respectively. The costimulatory molecule CD86+ population was significantly increased in PLA-PEG-M278 stimulated DCs, which is required for suitable antigen presentation and direct T cells activation. Herein the data is also represented in terms of meanflourcent intensity (MFI), further validaitng the magnitude of expression of these surface moelcules.

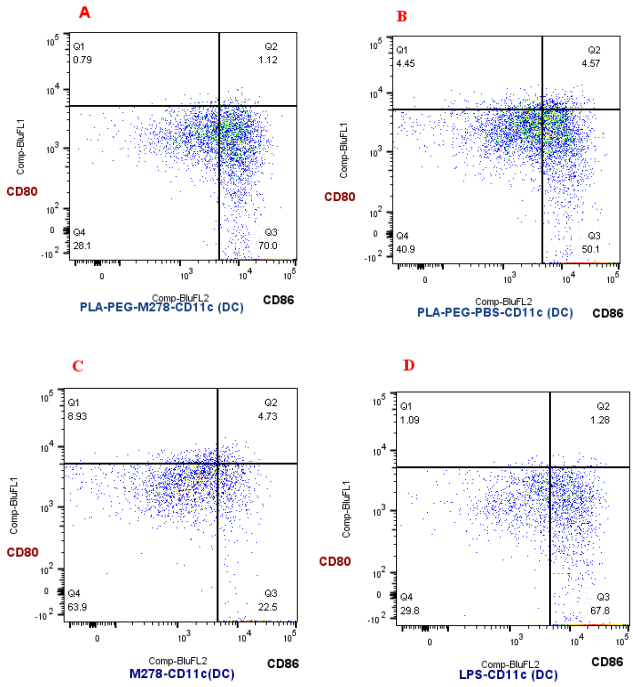
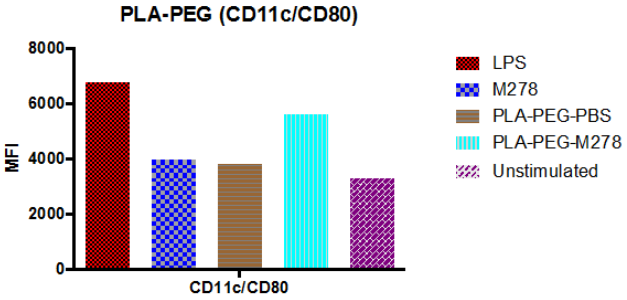


Figure 1. Expression profile of CD86 and CD80 surface receptors on DCs. DCs were exposed to 2.5 µg/mL of naked M278 or encapsulated M278 for 24 h and cells were gated on CD11c positive population.



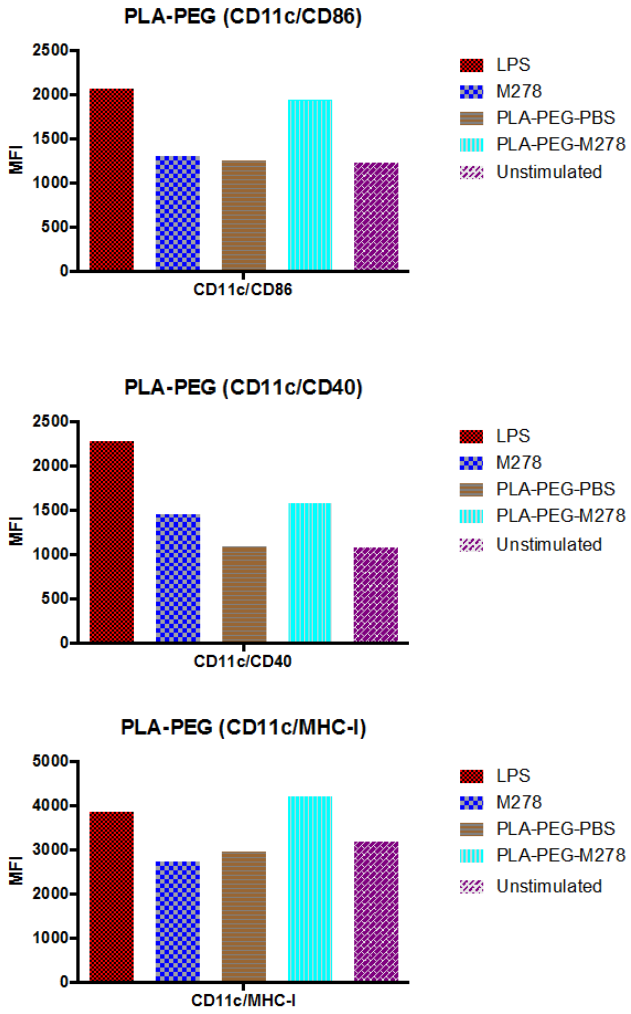


Figure 2. The mean fluorescence intensity (MFI) of CD80, CD86, CD40 and MHC I was markedly increased in PLA-PEG-M278 stimulated cells compared with that of M278. Cells were gated on CD11c marker for DCs. The cells were stained with anti-CD40 and anti-CD80 and CD86 antibody cocktail or with an isotype control cocktail antibody. Data were acquired on a BD FACS Canto II flow cytometer with at least 1×10^5 events for each sample and analyzed using FlowJo software.

3.2 Nanoparticle uptake and co-stimulatory molecule expression

To determine subcellular visual localization and trafficking of nanoparticles, DCs were exposed to PLA-PEG-PBS and PLA-PEG-M278 and M278. Our results show, by immunofluorescence microscopy, the subcellular localization of nanoparticles in the cytoplasm of DCs, thus confirming internalization of nanoparticles. Expression of CD86 is also evident in these images, which is in agreement with the flow cytometry findings. The endocytic internalization of nanoparticles in DCs is essential for activation of T-cell pathways that are involved in adaptive immune responses.

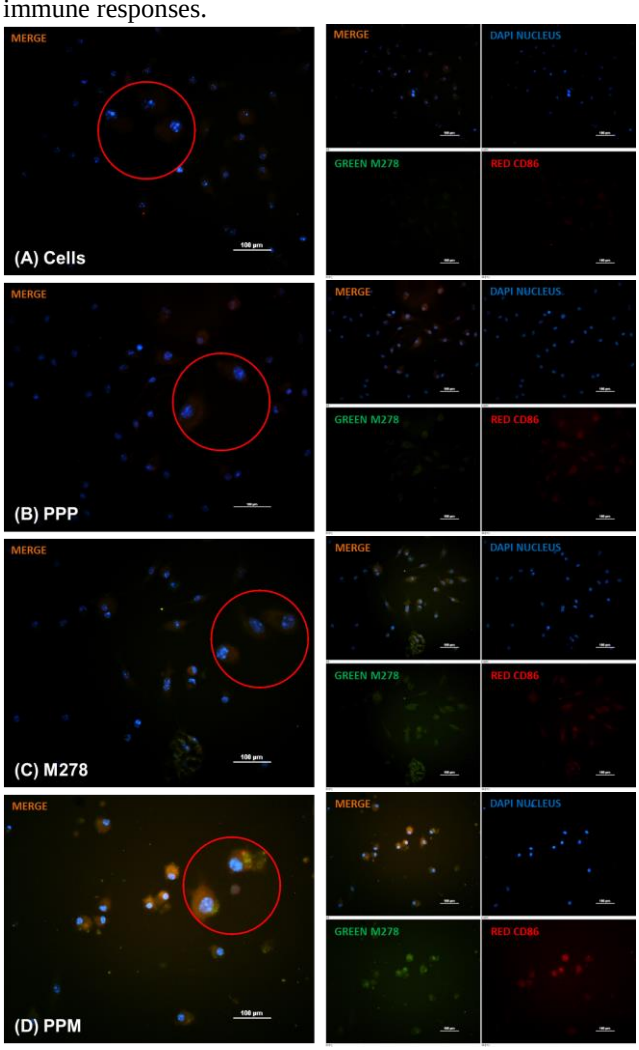


Figure 3. Immunoflourcent images (A) Cells (B) PLA-PEG-PBS(PPP), (C) M278 (D) PLA-PEG-M278 (PPM) showings uptake of nanoparticles and expression of the CD86 surface receptor. After 24 h of DCs stimulation (2×10^4/well) in 8-well chamber slides with M278, PPP and PPM, DCs were fixed, and stained with either a PE anti-mouse CD86 (shown in red) or FITC-anti-chlamydia (shown in green) antibody, and nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI), shown in blue). DCs were visualized using immunoflourcent microscopy at a magnification of 20X.

4.0 CONCLUSION

Results from flow cytometry and immunofluorescence microscopy data revealed that encapsulated M278 enhanced the expression levels of CD86, CD40 and the MHC complex on DCs as compared to naked M278, suggesting that NPs were more efficiently processed by DCs. CD86 expression, which is required for antigen presentation to T cells, was increased in PLA-PEG-M278 NPs stimulated DCs.

Therefore, we believe our NPs are also processed in a similar manner. Confocal images further confirmed the FACS data that PLA-PEG-M278 NPs are actively taken up by DCs. Collectively, these data suggests that our nano-encapsulated M278 vaccine drives maturation of DCs and efficient antigen presentation for elicitation of enhanced Th1 adaptive immune responses.

5.0 REFERENCES

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