Synergistic Stimulation of Immune Cells by Combination of Adjuvant and Nanoparticles Composed of Amphiphilic Poly(γ-glutamic acid)

F. Shima\textsuperscript{1,2}, T. Uto\textsuperscript{1,2}, T. Akagi\textsuperscript{1,2}, and M. Akashi\textsuperscript{1,2}

\textsuperscript{1}Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan; \textsuperscript{2}Japan Science and Technology Agency, CREST, Kawaguchi, Saitama 332-0012, Japan

f-shima@chem.eng.osaka-u.ac.jp

ABSTRACT SUMMARY

CpG oligodeoxynucleotide (ODN) encapsulated poly(γ-glutamic acid)-graft-L-phenylalanine ethylester (γ-PGA-Phe) nanoparticles (NPs) were prepared to develop vaccine delivery and adjuvant systems. The CpG ODN-encapsulated γ-PGA-Phe NPs (CpG ODN-NPs) could synergistically activate macrophages. Moreover, co-encapsulated CpG OND and antigen in γ-PGA-Phe NPs induced potent antigen-specific cellular immunity at a higher level than the mixture of CpG ODN and antigen which is the conventional vaccine. These findings suggest that the combination of biologically-derived adjuvant and polymeric NPs will aid the development of a novel approach for safe and effective vaccine delivery and adjuvant systems.

INTRODUCTION

The drug delivery system which employs polymeric NPs has been widely studied to develop safe and effective vaccines. These NPs can deliver antigens selectively to antigen-presenting cells (APCs) and efficiently induce antigen specific immune responses. Recently, it has been revealed that the recognition of antigens by toll-like receptors (TLRs) induces innate and adaptive immune responses. To target and manipulate the immune responses, the vaccine adjuvants are co-administered with antigens and these adjuvants activate the APCs via TLRs. In particular, biologically-derived CpG ODNs are known as an immune adjuvant that effectively induces Th1 responses. The CpG ODN is recognized by TLR9 in endo/lysosomes. The recognition of CpG ODN induces Th1-biased immune responses. On the other hand, it has been revealed that the combination of multiple TLR ligands synergistically stimulated APCs and induced potent Th1 type immune responses.

In a previous study, we prepared γ-PGA-Phe NPs for the development of safe and effective nanoparticle-based vaccines. The antigen-encapsulated γ-PGA-Phe NPs could effectively induce the antigen specific immune responses. γ-PGA-Phe NPs themselves possess adjuvant effects and can stimulate APCs via TLR4 expressed on the cell surface.\textsuperscript{1} In this study, we determined the activation properties of CpG ODN-NPs. To determine their potential for vaccine delivery and adjuvant systems, CpG ODN- and antigen-encapsulated γ-PGA-Phe NPs were prepared and the induction of antigen-specific immune response was evaluated.

EXPERIMENTAL METHODS

In this study, we employed γ-PGA-Phe whose grafting degree of Phe was 50%. CpG ODN was mixed with protamine as a polycation to form a polyplex, then γ-PGA-Phe dissolved in DMSO was added to the same volume of polyplex solution and CpG ODN-NPs were prepared.\textsuperscript{2} To evaluate the activation properties, macrophages (RAW264 cells) were incubated with CpG ODN-NPs (CpG ODN; 15-150 ng/ml, NPs 100 µg/ml) for 24 h and the cytokine secretion was measured by ELISA. For determining the adjuvant effect of CpG ODN-NPs, CpG ODN- and antigen-encapsulated γ-PGA-Phe NPs were prepared. Ovalbumin (OVA) was employed as a model antigen. OVA was mixed with polyplex and they were encapsulated into the γ-PGA-Phe NPs (CpG-OVA-NPs). Then these NPs were subcutaneously injected to mice, and after 10 days, the OVA specific cellular immunity was measured by ELISPOT assay.
RESULTS AND DISCUSSION

The CpG ODN was stably encapsulated into the NPs when protamine was used as the polycation.\(^2\) The CpG ODN-NPs were taken up by macrophages and CpG ODN which was encapsulated into the NPs internalized into endo/lysosomes. Interestingly, CpG ODN-NPs synergistically activated macrophages more efficiently than the free CpG ODN or the mixture of CpG ODN and \(\gamma\)-PGA-Phe NPs (CpG + NPs) (Figure 1). This may be due to the multiple stimulation of TLRs by \(\gamma\)-PGA-Phe NPs (TLR4 ligand) and CpG ODN (TLR9 ligand). We previously reported that \(\gamma\)-PGA-Phe NPs are excellent vaccine adjuvants for inducing potent innate and adaptive immune responses via TLR4.\(^1\) The uptake amount of the encapsulated CpG ODN was almost same as the free one. When the CpG ODN-NPs were immersed into the endosomal environment for 1 h \textit{in vitro}, 40% of the encapsulated CpG ODN was released from the \(\gamma\)-PGA-Phe NPs, which suggested that the encapsulated CpG ODN may efficiently stimulate the cells via TLR9 after CpG ODN-NPs were taken up and localized into the endo/lysosomes. Moreover, the CpG-OVA-NPs induced potent antigen-specific cellular immunity at a higher level than the mixture of CpG ODN and OVA (OVA + CpG) which is the conventional vaccine system (Figure 2). This may be due to the more efficient delivery of both CpG ODN and OVA by \(\gamma\)-PGA-Phe NPs to the APCs \textit{in vivo}, and synergistic stimulation of them.\(^3\)

![Figure 1](image1.png)

**Figure 1.** Secretion of TNF-\(\alpha\) by RAW264 cells. The cells were stimulated with CpG ODN, CpG + NPs, polyplex, or CpG ODN-NPs. *P < 0.01.

![Figure 2](image2.png)

**Figure 2.** Antigen-specific IFN-\(\gamma\) producing T cells induced by CpG-OVA-NPs Spleen cells of the mice immunized with the indicated samples were stimulated with no peptide (PBS), control peptide, or the OVA\(_{257-264}\) peptide (10 \(\mu\)g/ml) and evaluated for their INF-\(\gamma\) production by ELISPOT. The data represent means \(\pm\) SD in each group (n = 3). *p < 0.01.

CONCLUSION

The different TLR ligands of CpG ODN and \(\gamma\)-PGA-Phe NPs cooperated in activation of APCs and induction of immune responses. The strategies of synergistic stimulation of APCs via selected TLRs and co-delivery of antigens and adjuvants to the same APCs by the conjugation of adjuvant and the NPs will aid in the development of novel approaches for safe and effective vaccine delivery and immune stimulating systems.

REFERENCES


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