VACCINATION RESEARCH
Open Journal
Openventio PUBLISHERS

Published under Special edition: ***"Toll Like Receptors - Based Vaccines"***

Mini Review

Revolutionary Approach in the Application of Toll-Like Receptor Agonist in Nanoparticles Formulation for Peptide-Based Subunit Vaccine

Fazren Azmi, PhD*

Centre for Drug Delivery Research, Faculty of Pharmacy, The National University of Malaysia, Kuala Lumpur, Malaysia

Corresponding author
Fazren Azmi, PhD
Senior Lecturer, Faculty of Pharmacy, The National University of Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
E-mail: fazren.azmi@ukm.edu.my

Article information
Received: February 15th, 2018; **Accepted:** March 7th, 2018; **Published:** March 13th, 2018

Cite this article
Azmi F. Revolutionary approach in the application of toll-like receptor agonist in nanoparticles formulation for peptide-based subunit vaccine. *Vaccin Res Open J.* 2018; SE(1): Se1-Se3. doi: 10.17140/VROJ-SE-1-101

Subunit vaccines offer a safer alternative to the classical vaccine formulation that generally contains the whole pathogens. Subunit vaccines represent only a fraction of the immunogenic components of the pathogenic organism. Subunit vaccines can be composed of either proteins or peptides. Even in single proteins, there are hundreds of epitopes, in which not all of them are required to induce the desired immune responses. Some of the unnecessary epitopes may induce unwanted reactogenic or allergic responses. This situation has necessitated the use of peptides as antigens to create a safer and more immunologically defined vaccine than proteins.[1] Peptide vaccines represent a fragment of the protein, containing antigenic determinant that is capable to trigger specific T- or B-cells-mediated immune responses. Peptide vaccines can be produced synthetically, thus underscore the simplicity of production, possess a well-defined chemical structure and are entirely free of the biological hazards that are commonly encountered with inactivated or attenuated live pathogen vaccines. However, peptides are prone to enzymatic degradation and poorly immunogenic, thus require its formulation with a strong adjuvant to evoke a protective and long-lasting immunity. Various types of adjuvant and their recent advancement for the delivery of vaccine antigens have been extensively reviewed.[2] The development and selection of adjuvant is highly important, particularly in formulating peptide vaccines in order to make it a viable option for clinical use. Understanding the immune system and factors that can stimulate the presentation of the peptide antigens for further immunological processing should be of priority when developing a peptide-based vaccine.

The immune system has a unique mechanism for recognizing, screening and interacting with pathogens. Once the pathogens have been recognized, the immune system will react by releasing immune cells that are responsible to eliminate the invading pathogens and permit the development of immune memory. The immune system can identify the pathogens by detecting their structural features, thus enabling discrimination between pathogen and host cells for immunological reactions. These conserved structures are termed pathogen-associated molecular patterns (PAMPs), and are recognized by pattern-recognition receptors (PRRs). PRRs are mainly expressed on the surface of antigen-presenting cells (APCs), particularly dendritic cells and macrophages that are considered as the key players in the initial processing (innate immunity) of the pathogen antigens for further activation of T- and B-cells (adaptive immunity). Among the PRRs, toll-like receptors (TLRs) are the most extensively studied. Stimulation of TLRs by PAMP sleads to complex intracellular signalling pathways such as the release of pro-inflammatory cytokines and maturation of APCs that mediate the adaptive immune responses. Elaborated details regarding TLRs signalling pathways are well-established elsewhere.[3,4] Thus, by taking the advantage of this natural process of immune signalling pathways, the modern peptide-based vaccine formulation should aim to be capable of stimulating TLRs for efficient antigen presentation to the immune cells.

TLRs are expressed by various types of cells, including DCs, macrophages, B-cells and certain type of T-cells. Ten functional TLRs (TLRs 1-10) have been characterized in human populations.[5] Murine models, which have been extensively used in majority of experiments involving TLRs function, have the same

Vaccin Res Open J. 2018; SE(1): Se1-Se3. doi: 10.17140/VROJ-SE-1-101

TLRs as human, except for TLR10 and an additional three classes of TLRs.[6] Toll-like receptors function as either homodimers or heterodimers with other TLRs. For example, TLR2 forms dimers with TLR1 and TLR6 to recognize various lipid-containing PAMPs such as lipopeptides, lipomannan and lipoteichoic acids. TLR3 recognizes virus-derived double-stranded RNA, TLR4 is predominantly implicated in bacterially associated lipopolysaccharide (LPS) and TLR9 can be activated by unmethylated CpG DNA. In addition to the naturally derived TLRs ligand, its synthetic counterparts have also been developed. Several synthetic imidazoquinoline derivatives such as Imiquimod and Resiquimod have been identified to facilitate the activation of TLR7 while MPLA has been developed as a TLR4 ligand that possesses a more compromised toxicity than LPS. Therefore, the co-administration of peptide antigens and TLR agonist represents a promising strategy to 'delude' the immune system into believing that it is facing infectious foreign agent to trigger a robust immune response.

Nanoparticles have received considerable interest to be utilized as a vaccine delivery platform for peptide antigen and TLR agonist. The utilisation of nanoparticles in vaccine delivery technology is inspired by the fact that nanoparticles exhibit similar geometry and dimension to pathogens,[7] thus may mimic the pathogenic invasion and potentiate the uptake and processing of the antigen by professional antigen presenting cells (APC), such as dendritic cells (DCs). Nanoparticles also allow a high density and repetitive display of the peptide antigens, thus permitting a simultaneous binding to B-cell receptor for the induction of a more potent immune reactions compared to monovalent binding that is commonly attained by soluble peptide antigens.[8,9] Nanoparticles can be prepared from a various type of materials. The ranges of nanoparticles that have been extensively utilised as a delivery platform for peptide-based vaccine include virus-like particles, liposomes, solid-lipid nanoparticles and polymers.[1,10] The peptide antigens and TLR agonist can be encapsulated into or adsorbed to the surface of nanoparticles. Therefore, nanoparticles-inspired peptide-based vaccine design can simultaneously display the antigens of choice in combination with TLR agonist as a cargo to activate different innate immune signalling pathways and modulate the desired immune response raised by such particles.

A great number of advances have been made in the structural modification of peptide antigen in the vaccine formulation. Other than forming complexes within the molecules of nanoparticles component, peptide antigen can be covalently linked to TLR agonist to self-assemble into nanoparticles. One of the straightforward approaches include the conjugation of synthetic peptide antigens to lipidic moiety such as Pam2Cys/Pam3Cys,[11] Nepsilon-palmitoyl-lysine[12] and lipid core peptide (LCP)[13] that predominantly known stimulate the activation of TLR2. The experimental outcome demonstrated that the antigenic peptide-lipid conjugates induced a strong protective immunity against the respective pathogen of interest. The choice of peptide epitope (bearing cationic amino acid residues) and the lipidic moiety need to be taken into consideration to warrant balanced attractive/repulsive forces between the molecules to initiate the self-assembly process into nanoparticles. Another attractive approach of constructing engineered nanoparticles with TLR agonist is to functionalize the surface of nanoparticles with the respective TLR ligand. The surface functionalization of nanoparticles with TLR agonist can be achieved *via* covalent or non-covalent interaction. For example, a novel platform utilising nanoliposome as a peptide vaccine delivery platform against breast cancer has been developed recently.[14] Gp2 (antigenic peptide epitope derived from HER2 protein) was initially conjugated to Maleimide-Mpeg$_{2000}$DSPE-based micellar network.These micelles bearing Gp2 peptide network was further encapsulated into nanoliposomes that were fabricated from DMPC, DMPG phospholipids and fusogenic lipid dioleoylphosphatidylethanolamine (DOPE) containing monophosphoryl lipid A (MPL) adjuvant (DMPC-DMPG-DOPE-MPL-Gp2). MPL was incorporated as part of the lipidic moiety of the liposome structure which was displayed on the surface of the liposome. This formulation generated strong antitumour activity with the capability to halt the tumour growth and enhance the survival rate of the immunized mice. It is generally believed that linking the TLR ligand to the nanoparticles that bearing the peptide antigen or to the peptide antigen itself will ensure the same APC encounter the entire molecular structure for an efficient immunology processing.

Nanoparticles possessing multiple TLR agonists emerge as a viable strategy to induce a robust immune response as most pathogens present multiple TLR agonists to induce infections. The stimulation of various TLRs would lead to the upregulation of cytokines.[15] One of the examples of particulate peptide vaccine delivery system possessing multiple TLR agonists that have made progress in clinical trials is MelQbG10, as a treatment for advanced-stage melanoma.[16] MelQbG10 is a viral-like protein nanoparticle that is integrated with imiquimod and CpG TLR agonists. The peptide antigen (derived from Melan-A protein) was covalently linked to the surface of nanoparticles. In another study, Roy and co-workers have prepared various vaccine formulations based on PLGA nanoparticles harbouring single, dual and triple TLR agonists.[17] Their results showed that nanoparticles possessing multiple TLR agonists induced high titres of antigen-specific antibodies followed by dual and single TLR agonist formulation. They also demonstrated that combining either dual or triple TLR agonists induced antigen cross-presentation *in vitro*. This study underscoring the synergy effects of combining multiple TLR agonists in a single particulate platform to amplify the immune response production against the vaccine antigen.

CONCLUSION AND FUTURE PERSPECTIVES

Although we have witnessed much advanced research towards the development of the nanoparticulate-based delivery platform for peptide antigen, there is still no peptide-based vaccine available for clinical use. For this reason, there is an urgent need to further explore the adjuvant strategy that can translate the immunotherapy approach of using peptide vaccines for clinical application. A recurrent strategy in combining nanoparticulate carrier with TLR agonists has a profound contribution in augmenting the immune productions against the peptide antigens. However, a more sophisticated vaccine design is yet to be explored for peptide vaccine. The peptide-TLR agonist conjugates can be further incorporated, or can be utilized as part of the component to fabricate nanoparticles. These nanoparticles can be engineered to carry multiple TLR

agonists, either being encapsulated or decorated on the surface of nanoparticles. One of the attractive strategies to incorporate multiple TLR agonists on the surface of nanoparticles are by using the layer-by-layer assembly method.[18,19] Multiple TLR agonists can be sequentially deposited on the surface of nanoparticles *via* non-covalent binding such as electrostatic interaction and hydrophobic effects, depending on the physicochemical nature of nanoparticles and the TLR agonists. Altogether, as the immune system has rapidly evolved to protect the host from infectious viral nanoparticles or bacteria, developing a smart nanoparticles platform displaying multiple TLR agonists for the delivery of peptide antigen could recapitulate the pathogenic-like features in inducing the desired immune response with a tolerable cytotoxicity. Thus, it comes as no surprise that such nanoformulation could even be more potent than conventional vaccine formulations.

ACKNOWLEDGEMENT

The author would like to thank the National University of Malaysia (UKM) for providing financial support from the research grant (DCP-2017-003/2).

REFERENCES

1. Skwarczynski M, Toth I. Recent advances in peptide-based subunit nanovaccines. *Nanomedicine (Lond)*. 2014; 9(17): 2657-2669. doi: 10.2217/nnm.14.187

2. Azmi F, Ahmad Fuaad AA, Skwarczynski M, Toth I. Recent progress in adjuvant discovery for peptide-based subunit vaccines. *Hum Vaccin Immunother*. 2014. 10(3): 778-796. doi: 10.4161/hv.27332

3. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol*. 2004; 4(7): 499-511. doi: 10.1038/nri1391

4. O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol*. 2007; 7(5): 353-364. doi: 10.1038/nri2079

5. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol*. 2004; 5(10): 987-995. doi: 10.1038/ni1112

6. Kawai T, Akira S. TLR signaling. *Semin Immunol*. 2007. 19(1): 24-32. doi: 10.1016/j.smim.2006.12.004

7. Xiang SD, Scholzen A, Minigo G, et al. Pathogen recognition and development of particulate vaccines: Does size matter? *Methods*. 2006; 40(1): 1-9. doi: 10.1016/j.ymeth.2006.05.016

8. Bachmann MF, Jennings GT. Vaccine delivery: A matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol*. 2010; 10(11): 787-796. doi: 10.1038/nri2868

9. Minguet S, Dopfer EP, Schamel WW. Low-valency, but not monovalent, antigens trigger the B-cell antigen receptor (BCR). *Int Immunol*. 2010; 22(3): 205-212.

10. Zhao L, Seth A, Wibowo N, et al. Nanoparticle vaccines. *Vaccine*. 2014; 32(3): 327-337. doi: 10.1016/j.vaccine.2013.11.069

11. Jackson DC, Lau YF, Le T, et al. A totally synthetic vaccine of generic structure that targets Toll-like receptor 2 on dendritic cells and promotes antibody or cytotoxic T cell responses. *Proc Natl Acad Sci U S A*. 2004; 101(43): 15440-15445. doi: 10.1073/pnas.0406740101

12. Zhu X, Ramos TV, Gras-Masse H, Kaplan BE, BenMohamed L. Lipopeptide epitopes extended by an Nepsilon-palmitoyl-lysine moiety increase uptake and maturation of dendritic cells through a Toll-like receptor-2 pathway and trigger a Th1-dependent protective immunity. *Eur J Immunol*. 2004. 34(11): 3102-3114. doi: 10.1002/eji.200425166

13. Phillipps KSM, Wykes MN, Liu XQ, Brown M, Blanchfield J, Toth I. A novel synthetic adjuvant enhances dendritic cell function. *Immunology*. 2009; 128(Suppl 1): e582-e588. doi: 10.1111/j.1365-2567.2008.03038.x

14. Razazan A, Behravan J, Arab A, et al. Conjugated nanoliposome with the HER2/neu-derived peptide GP2 as an effective vaccine against breast cancer in mice xenograft model. *PLoS One*. 2017; 12(10): e0185099. doi: 10.1371/journal.pone.0185099

15. Zhu Q, Egelston C, Vivekanandhan A, et al. Toll-like receptor ligands synergize through distinct dendritic cell pathways to induce T cell responses: Implications for vaccines. *Proc Natl Acad Sci U S A*. 2008. 105(42): 16260-16265. doi: 10.1073/pnas.0805325105

16. Goldinger SM, Dummer R, Baumgaertner P, et al. Nano-particle vaccination combined with TLR-7 and -9 ligands triggers memory and effector CD8(+) T-cell responses in melanoma patients. *Eur J Immunol*. 2012. 42(11): 3049-3061. doi: 10.1002/eji.201142361

17. Madan-Lala R, Pradhan P, Roy K. Combinatorial delivery of dual and triple tlr agonists via polymeric pathogen-like particles synergistically enhances innate and adaptive immune responses. *Sci Rep*. 2017; 7(1): 2530. doi: 10.1038/s41598-017-02804-y

18. Richardson JJ, Cui J, Björnmalm M, Braunger JA, Ejima H, Caruso F. Innovation in layer-by-layer assembly. *Chem Rev*. 2016; 116(23): 14828-14867. doi: 10.1021/acs.chemrev.6b00627

19. De Geest BG, Willart MA, Lambrecht BN, et al. Surface-engineered polyelectrolyte multilayer capsules: Synthetic vaccines mimicking microbial structure and function. *Angew Chem Int Ed Engl*. 2012; 51(16): 3862-3866. doi: 10.1002/anie.201200048