Investigating Adjuvant Potential of Astragaloside VII and Its Formulation with *Astragalus* Polysaccharide for Seasonal Influenza Vaccines

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Introduction

Highly purified recombinant proteins, or peptides, representing subunits of pathogens lack most of the features of the original pathogen and are often poorly immunogenic. Adjuvants will activate only the elements of the immune response required for protection to specific antigen and will not trigger a more generalized activation of the immune response. Several hundred natural and synthetic compounds have been identified to have adjuvant activity such as mineral salts, microorganism-derived fragments, emulsions, cytokines, polysaccharides, nucleic acid-based molecules, saponins etc [1]. Majority of them have not been approved for use in human vaccines because of their limitations including lack of efficacy, unacceptable local or systemic toxicity, difficulty of manufacture, poor stability, and high cost. For that reasons, novel adjuvants / adjuvant systems are under search [1,2].

Astragaloside VII (AST VII), a triterpenoid saponin isolated from a number of *Astragalus trojanus*, has been shown to induce cytokines such as IL-2, IFN-γ, TGF-β, antigen-specific IgG, IgG1, IgG2b antibody subsets and splenocyte proliferation. High solubility in water, low molecular weight, high stability, slight hemolytic effect and appropriateness to lyophilization are advantages of AST VII compared to QS-21, widely used saponin based adjuvant. *Astragalus* polysaccharides (APS) originating from the roots of the plant and used as a traditional medicine in China, have shown strong immunomodulatory properties [3,4]. Traditional *Astragalus* preparations, mainly water extracts, include both saponins and polysaccharides. We speculate that, in those preparations, polysaccharides act as encapsulation/carrier agents to deliver astragalosides. As it is known well, the combination of immunostimulants in a delivery system brings some advantages like targeting the antigen and adjuvant to a specific cell, preventing non-specific activation of the immune system, controlling biodistribution and facilitating uptake of the delivery system by the antigen presenting cell [1]. In the presented study, the immunomodulatory effects of AST VII and newly developed adjuvant nanocarrier system (ANS) in combination with AST VII and APS on seasonal influenza vaccine were investigated.

Materials &Methods

1. Antigen, Adjuvants and Adjuvant Nanocarrier System (ANS) Preparation

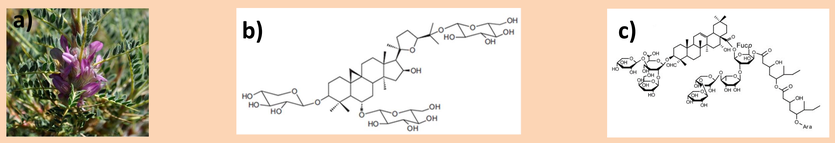


Figure 1: a) *Astragalus trojanus* b) Astragaloside VII c) QS-21

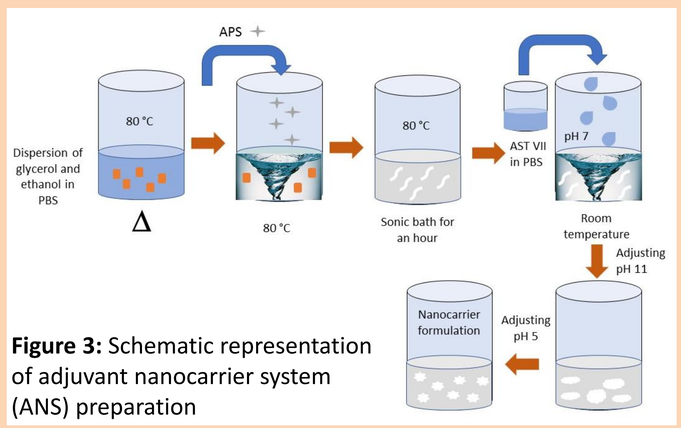


Figure 3: Schematic representation of adjuvant nanocarrier system (ANS) preparation

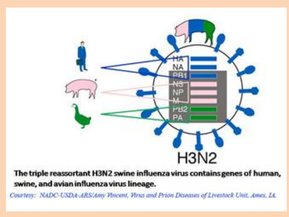


Figure 2: H3N2 swine influenza virus contains genes of human, swine and avian influenza lineage

A/duck/Hokkaido/5/77 Influenza A (H3N2) provided from Avian Influenza OIE /FAO Reference Lab., Hokkaido University, Japan as a gift. Inactivated Human influenza A (H3N2) virus with 8% BEI (binary ethylenimine) were used as an vaccine antigen. The combinations of inactivated influenza A virus (H3N2-256 HA) with adjuvants were prepared as a simple admixture before administration to the mice.

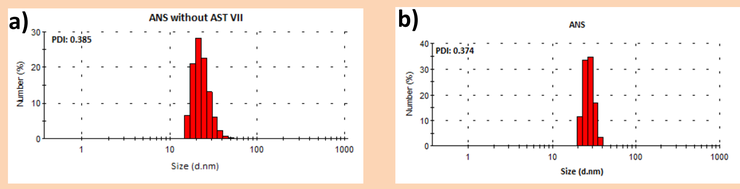
2. Immunization Studies

Inactivated Influenza A (H3N2-256 HA) and adjuvant, AST VII (10, 25, 75 µg/ml), QS-21 (10 µg/ml), Alum, ANS with or without AST VII, were immunized intramuscularly to Swiss albino mice (Ege University Ethical Approval Number: 2016-069). Mice were sacrificed 28 days post-challenge for analyses of immune response.

For this purpose, blood and spleen samples were collected for ELISA (antibody and cytokine determination) and splenocyte proliferation assays[4].

Results

1. Characterization of ANS



c)

Adjuvant Name	Surface Zeta Potential (mV)
ANS without AST VII	-22.9
ANS	-8.2

Figure 4: Particle size distributions a) ANS without AST VII b) ANS and c) surface zeta potentials of ANS without AST VII and ANS.

2. Evaluation of Animal Studies

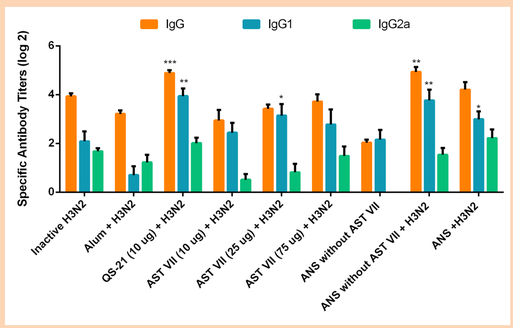


Figure 5: Effects of adjuvants on the production of IgG, IgG1 and IgG2a antibodies in sera

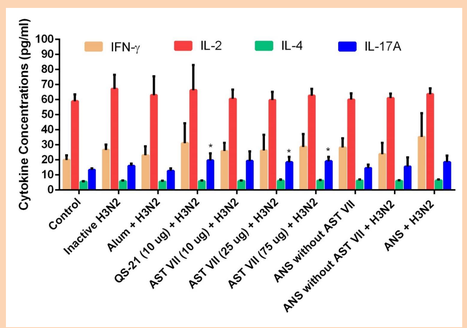


Figure 6: Effects of adjuvants on the production of IFN-γ, IL-2, IL-4 and IL-17A in sera

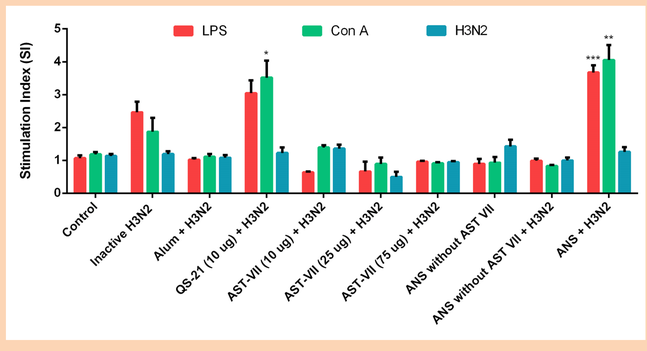


Figure 7: Stimulation indexes of LPS, Con A and H3N2 stimulated splenocytes *in vitro*

Conclusion

Most adjuvants induce humoral immunity, though cell-mediated immunity has a crucial role in combating viral diseases or cancer. Thus, vaccine formulations with appropriate antigens provide great promise to direct the immune system to the cell-mediated immune response in addition to the humoral immune response [1,2]. AST VII, ANS and ANS without AST VII increase the production of IFN-γ, IL-17A and IgG subsets. Moreover, ANS significantly enhances the stimulation of splenocyte proliferation. In this study, ANS and ANS without AST VII have a potency to be used as an adjuvant for human vaccines in terms of induction capacity for cell mediated and humoral immunity. Further studies will focus on optimization of the ANS formulation to increase the specific immune response to the antigen.

Acknowledgement This study was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK, Project No: 215S544).

References

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