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Development of a novel multi-adjuvanted vaccine using the synergistic effects of TLR4 agonists and NOD2 ligands in an *in vitro* influenza model

Jovial Praavaa
MSc candidate Laurentian University, Canada

Adjuvant development is crucial for the advancement of new and effective vaccines for those who are immunocompromised. Although the importance of adjuvants is indispensable, only minimal numbers of adjuvants had been included in licensed vaccines. The obstacles in adjuvant development primarily include preparation in suboptimal conditions, confinement in adjuvanticity, excess reactogenicity, and finding the right adjuvant that is relevant to the pathogen of interest. Influenza continues to disproportionately impact the elderly and the young and vaccination is the most effective way to provide not only prophylactic but also therapeutic protection to this ongoing situation, reduce the cost of care and improve the quality of life of the elderly. As a rule, multi-adjuvanted compounds provide a stronger immune response as opposed to the individual components on their own. We evaluated the safety and ability of the diethanolamine-based lipid A (A1), a synthetic Toll-like receptor (TLR) 4 agonist synthesized in-house, and muramyl dipeptide (MDP), agonist of Nucleotide-binding oligomerization domain (NOD)2 receptors to stimulate innate immune responses. We used human peripheral blood mononuclear cells (PBMCs) *in vitro* and primary and immortalized murine cells *in vitro*. We assessed the ability of the adjuvants to enhance antigen-specific memory responses in an *in vitro* influenza viral challenge model using PBMCs. The data suggests that simultaneous stimulation of TLR4 and NOD2, has a multifunctional immunomodulatory, synergistic effect leading to enhanced innate immune responses with increases in proinflammatory cytokines and decreased levels of IL-10. Furthermore, in the challenge model we demonstrate the induction of T-helper type 1 (Th1) responses based on IL-6, IFNγ, IL1β, TNFα and IL-2 production. We believe that this approach to harness a novel influenza vaccine formulation by combining a NOD2 ligand and a TLR4 agonist will ameliorate currently available influenza vaccines by stimulating and directing innate and qualitative adaptive immunity necessary for immune cells trafficking and successful influenza vaccine-specific responses. Comparison between TLR 2/4 and TLR 4 Agonists based on cytokine production. Levels of TNFα, IL-6, IL-10, IL12p40 and IL-1β produced by J774A.1. Cells were seeded at a concentration of 5×10^5 cells/mL/well in a 24 well plate and left untreated or treated with either 10 ng/mL TLR 2/4 and TLR4 agonist (LPS), MDP 1 and 10 µg/mL or both. Supernatants were collected after 48 hours of treatment. N = 6, ±SEM. an ANOVA determined a representative of two experiments and statistical significance with a Tukey HSD. $p<0.001$,$^{**}p<0.01$ compared to the LPS 10 ng/mL treatment group.

Biography

Jovial Praavaa attending the Laurentian University in an effort to obtain her M.Sc. in Biology (Immunology). She received her M.Sc. in microbiology from Dhaka University, Bangladesh with Honor. After graduation, she came to Canada to continue her studies. At LU, she is the member of "Balance Life Society" and work as a volunteer worker at "Healthy life style program for teens" in Sudbury local public school. Prior joining, Laurentian university she worked as a Quality Assurance personnel and Administrative assistant at Toronto, Ontario. After graduation, she is planning to continue to study and get the Ph.D. degree and pursue her carrier in research.

jpraavaa@laurentian.ca