





## Enhancing Immunogenic Responses through Synergistic Coformulations of TLR4 Agonists and Diverse Adjuvants

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## Abstract:

This study explores the synergistic potential of co-formulating Toll-like receptor 4 (TLR4) agonists with various adjuvants to enhance immune responses. Our research focuses on two primary areas: the combination of different TLR4 agonists to activate distinct downstream pathways, and the co-formulation of TLR4 agonists with other adjuvants.

Firstly, we examined the co-formulation of two TLR4 agonists, FP18 and FP20, which activate the MyD88 and TRAM-TRIF pathways, respectively. By experimenting with different ratios, we identified an optimal mixture (60% FP18 and 40% FP20) that produced a significantly higher cytokine response in THP1 cell lines, as determined by SEAP assay, compared to traditional MPLA and LPS controls. This synergistic effect suggests that combining TLR4 agonists with different pathway activations can yield a more potent immune response.

Secondly, we explored the formulation of FP20 with saponin, inspired by an FDA-approved adjuvant system incorporating MPLA and QS-21 with liposomes. Our approach involved using synthetic variants, namely FP20 for MPLA and QS-21 Echinocystic acid for QS-21. These co-formulations were characterized through differential scanning calorimetry, dynamic light scattering, zeta potential, and cryo-TEM analyses. Results from in vivo studies indicated that these co-formulations significantly enhanced the immune response beyond what was observed with the individual compounds.

In summary, our study demonstrates the effectiveness of co-formulating TLR4 agonists with various adjuvants, which can lead to more robust and diverse immune responses. These findings are particularly relevant for vaccine development against diseases like malaria, where a strong and varied immune response is critical. The synergistic effects observed in both areas of our study open new avenues for designing advanced vaccine adjuvants.