Quantification and in vitro analysis of nanolipoproteins (NLPs) containing adjuvants

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Nanolipoprotein particles (NLPs) self-assemble into nanoscale structures that can be used as vaccines or drug delivery agents. Due to the nature of NLPs, a variety of immune-stimulating compounds or adjuvants can be readily incorporated into NLPs to enhance the immune response. In light of this, we developed and tested a variety of methods for quantifying these amounts of NLPs, with the aim of quantifying the amount of adjuvant actually incorporated into NLPs as a quality of high importance. The graph in Figure 1 shows the results of these experiments, with each point representing the absorbance of a sample taken from a different batch of NLPs. The absorbance values range from 0 to 200, with higher values indicating a higher concentration of NLPs.

The different components (lipids, protein, and adjuvants) are then separated using a detergent in a buffer solution. As the detergent is removed from the solution by dialysis, the particles self-assemble into disk-shaped structures (Figure 2). Adjuvants are molecules that initiate an immune response and, when added to NLPs, during the assembly process or to pre-assembled particles, the adjuvants are used in these studies shown below (Figures 4 and 5).

The traces above show NLPs analyzed using two methods: UV-Vis and ELISA. In contrast to UV-Vis absorbance measurements, the ELISA can detect the components of the NLP that have no intrinsic absorbance (e.g., MDP and DOPC lipid) can be visualized. The gain can be changed to detect both small and large amounts of material.

NLPs can be characterized using size exclusion chromatography, a technique used to separate the components of a mixture by their size. The graphs above (Figures 3-6) show that all NLPs elute at approximately 85.5 minutes, regardless of composition. When NLPs are analyzed by a spectrophotometer using size exclusion chromatography alone, there is little difference between NLPs composed of different molecules.

The immunological response of the NLPs were compared to that of the adjuvant alone using an enzyme-linked immunosorbent assay (ELISA). ELISAs were used to measure the response of macrophages exposed to adjuvant formulations by measuring the amount of cytokines produced.

The cartoon above depicts a cell’s response to an adjuvant. The adjuvant can be taken up by the cell and cause the cell to produce signals called cytokines. Cytokines then signal other cells, producing an immunological response.

Two different amounts of adjuvanted NLPs were used to elicit immune responses, measured by macrophage production of IL-6 (Figure 13). When NLPs contain a single adjuvant, the amount of cytokine production is either modest (MPLA) or below the limit of detection (MDP). However, when particles are made with a combination of MPLA and MDP, there is a significant amount of IL-6 production. At the lower dose (0.05 μg/mL), MPLA there is a significant increase in the amount of IL-6 produced as the adjuvant is incorporated into the NLP versus the adjuvant alone. At the higher dose (0.5 μg/mL), MPLA, the strong stimulation by MPLA appears to mask any contribution of the relatively low levels of MDP. These results are similar to the measured production of a second cytokine, TNF-α (Figure 14).

Conclusion/Future Research

Although there are many different types of adjuvants that can be incorporated into NLPs, there are many factors to consider in relation to adjuvant response; that these adjuvant or adjuvant combinations will elicit the chemical characteristics of the adjuvants, the adjuvant ratio, the type of combination of different adjuvants. The in vitro results presented here indicate that while only MPLA incorporated into NLPs individually elicits a significant response, the combination of MDP with MPLA within the NLP further enhances cytokine production. It can be concluded that the two adjuvants in concert, especially when incorporated into the NLPs, to increase the potency compared to each adjuvant alone.

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References


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