**Understanding the Effect of TLR activation on mRNA Delivery**

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**Introduction:** mRNA lipid nanoparticles (LNPs) have emerged as a promising vaccine platform due to their ease of manufacture, versatility, and safety profile. These vaccines have broad therapeutic potential in protecting against viral infections, as any pathogenic protein can be produced by changing only the mRNA sequence. A current challenge in mRNA vaccination is mRNA clearance due to the innate immunogenicity of mRNA[1]. Modifications to the mRNA itself have been explored to improve the translation efficiency and reduce immune clearance of mRNA, however immune activation can also increase the utility of mRNA vaccines. Often, adjuvants are included in vaccine formulations to boost a productive immune response to the desired antigen. With increasing interest in developing mRNA vaccines against viral infections, there is a need to understand how immune activation and mRNA translation efficiency are related. Here, we study the impact of immune cell activation, in particular toll-like receptor (TLR) signaling, on mRNA translation to inform future vaccine development.

**Methods:** Lipid nanoparticles were prepared by combining an ethanol phase composed of an ionizable lipid (DOTAP), phospholipid (DOPC), cholesterol, and PEG-$NH_2$ with an aqueous phase containing citrate buffer (pH 3) and luciferase-encoding mRNA (mLUC). A human monocyte THP-1 cell line was stimulated with a variety of TLR agonists in the presence or absence of lipid nanoparticles containing mRNA encoding for luciferase. mRNA translation was then evaluated by luciferase reporter expression.

**Results:** Toll-like receptor (TLR) signaling elicited by pathogen associated molecular patterns (PAMPs) from viral and bacterial components leads to the production of pro-inflammatory cytokines (Figure 1a). Adjuvants that work as TLR agonists have been used in vaccines to boost the antigen-specific immune response.[2] To test the effect of TLR activation on mRNA translation, we co-delivered mRNA LNPs with a range of TLR agonists (Figure 1b). We show that the mRNA translation of luciferase is decreased in the presence of TLR agonists compared to an unstimulated control (Figure 1c). We show that this effect is ubiquitous among all TLR agonists tested both for viral and bacterial components, though to varied extents depending on the specific TLR agonist tested.

**Conclusions:** The co-administration of mRNA LNPs with TLR agonists decreases mRNA translation. This finding highlights the need to carefully design mRNA vaccines such that they balance a productive elicited immune response for antigen-specific recognition with an unproductive response that inhibits mRNA translation and decreases overall antigen expression.

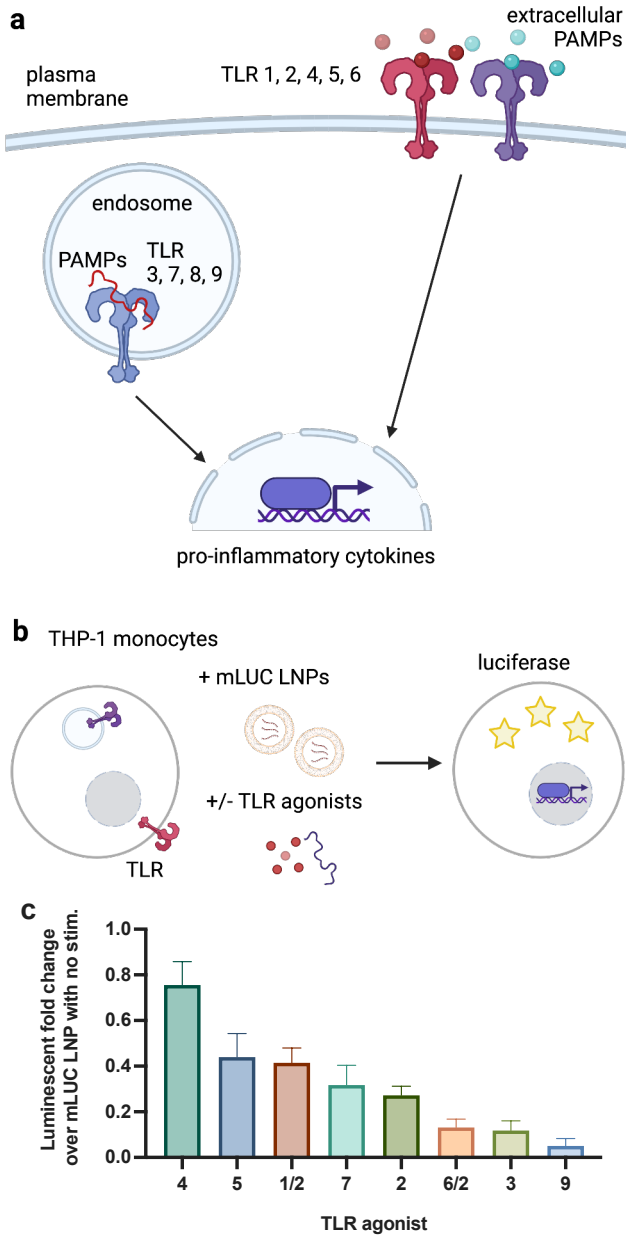


**Figure 1:** a) Schematic of TLR signaling leading to the production of an inflammatory response. b) Schematic of experimental set-up. c) Normalized fold change in luminescence in monocytes that were simultaneously stimulated with TLRs and transfected with luciferase mRNA LNPs compared to an unstimulated control. A value < 1 indicates that translation was impaired compared to the unstimulated control. Error bars represent SEM.

**References:** [1]Karikó et al., Mol Ther. (2008).
[2]Duthie et al., Immunol. Rev. (2011).