Enhancing transfection efficiency of primary immune cells through lipid nanoparticle-mediated delivery

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KEY FINDING

Transfection of primary immune cells with LNPs is efficient, non-toxic, and maintains immunogenicity.

INTRODUCTION

Modification of immune cells is critical for a diverse range of research endeavors, spanning disease modeling, therapeutic target discovery, immunotherapy, and vaccine development. Primary immune cells, in contrast to immortalized cell lines, more faithfully mimic *in vivo* targeting of nanoparticles, a pivotal aspect for advancing therapeutic strategies. However, genetic manipulation of primary immune cells is a challenging, costly, and time-consuming process. T cells, in particular, exhibit low transfection efficiency and poor viability when treated with common chemical transfection reagents. This study addresses these challenges by evaluating the potential of preformed, lyophilized/frozen, and ready-to-load lipid nanoparticles (LNPs) in transfecting primary cell cultures. Utilizing LNPs for the delivery of genetic cargo has several advantages over current viral transfection, such as reduced cytotoxicity. Transfection efficiency and immunogenicity of LNPs were assessed by monitoring the expression of mCherry/GFP mRNA cargo and by measuring cytokine levels using plate-based methods, respectively. Our study contributes valuable insights into the optimization of primary immune cell transfection methodologies, offering a new avenue for researchers to further explore the dynamic interactions of these cells.

METHODS

Primary Immune Cell Harvest

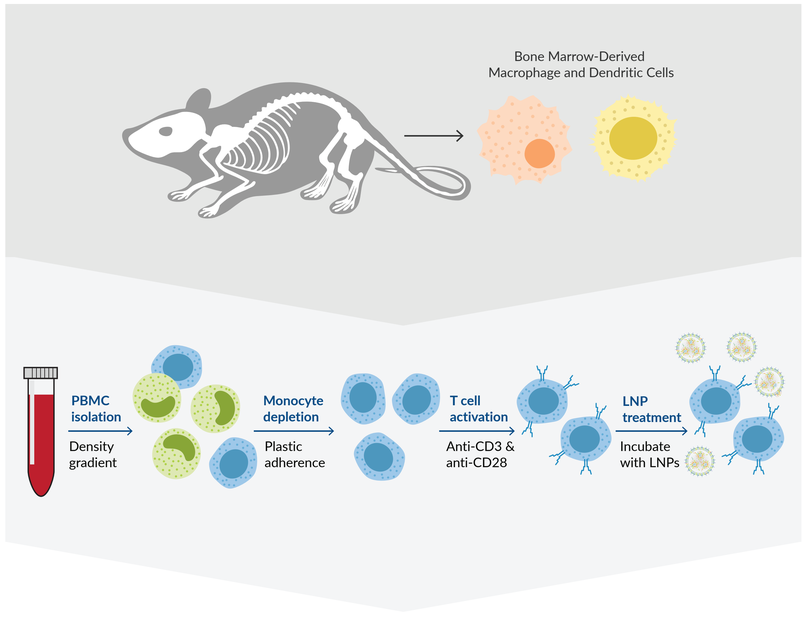

FIGURE 1 – Experiment Workflow
Mouse primary immune cells were harvested from bone marrow and human primary immune cells were harvested from peripheral blood. Once harvested, bone marrow and T cells were differentiated or activated, respectively, followed by transfection with 1, 2, or 4 µl per 100 µl media volume LipidLaunch™ Loadable LNPs (**Item Nos. 702620, 702750, 702780, 702860, and 502868**) carrying 12.5, 25, or 50 ng mCherry mRNA (**Item No. 41962**) or EGFP mRNA (**Item No. 39800**) cargo. Transfection efficiencies were determined after 48 hours *via* imaging or by flow cytometry. Cytotoxicity 48 hours post-transfection was determined using the Resazurin Cell Viability Assay Kit (**Item No. 702540**). Immunogenicity was measured by quantifying cell-specific cytokine release using ELISA 48 hours following stimulation by LPS.

RESULTS

Effective transfection of murine primary cells using LipidLaunch™ LNPs loaded with mCherry mRNA

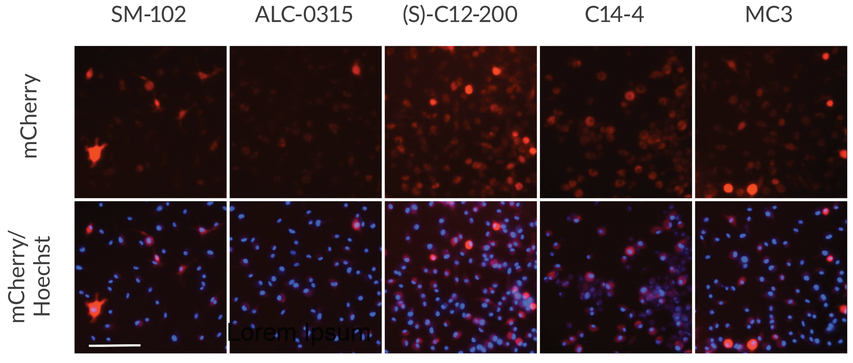

FIGURE 2 – Loadable LNPs can transfect murine primary cells.
Following harvest from 4-month-old BALB/c mice, bone marrow-derived macrophages/dendritic cells were transfected with LipidLaunch™ loadable SM-102, ALC-0315, (S)-C12-200, C14-4, and MC3 LNPs containing mCherry mRNA. 48 hours post transfection, nuclei were stained with Hoecsht 33258 (**Item No. 16756**). Scale bar represents 100 µm. N=4.

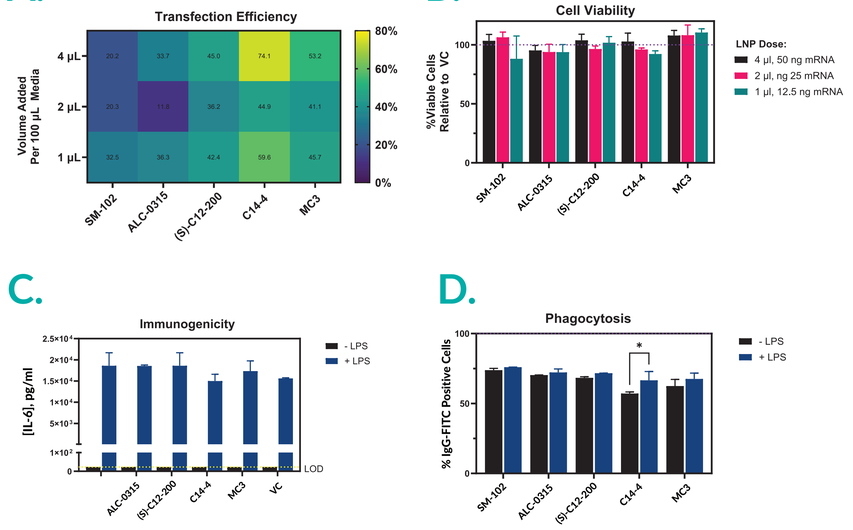

FIGURE 3 – Loadable LNP transfection is efficient and does not affect cell viability.
A) Transfection efficiency was calculated by counting the number of mCherry-positive cells compared to the total number of cells across multiple wells and expressed as a percent. **B)** Cell viability was measured by resazurin fluorescence. Dotted line represents no treatment control (NC). **C)** Immunogenicity was assessed by sandwich ELISA for the cytokine IL-6 (**Item No. 583371**). Limit of detection (LOD) is 23 pg/ml. **D)** The ability to phagocytose was determined using the Phagocytosis Assay Kit (IgG FITC) (**Item No. 500290**). **P < 0.01*

RESULTS *(CONTINUED)*

LipidLaunch™ LNPs can deliver mRNA cargo to human primary immune cells

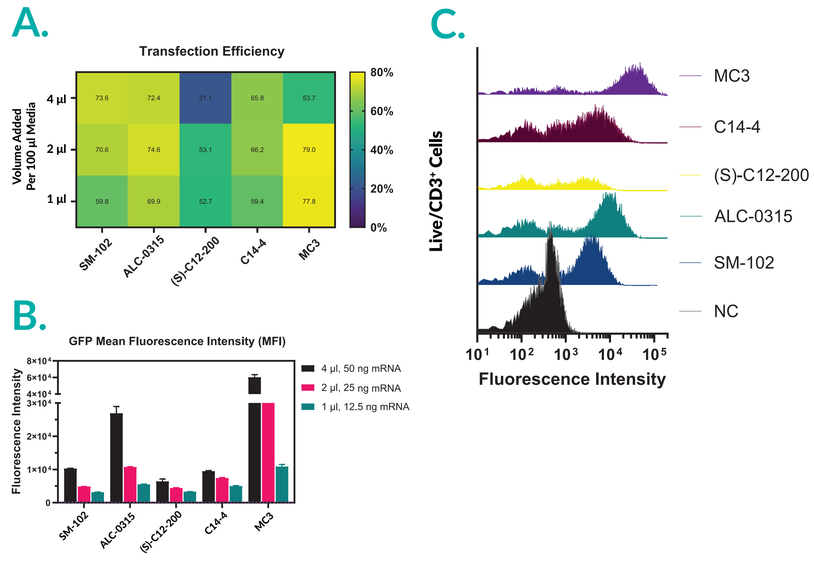

FIGURE 4 – Loadable LNPs can transfect human primary T cells.
A) Following treatment of T cells with LipidLaunch™ loadable SM-102, ALC-0315, (S)-C12-200, C14-4, and MC3 LNPs containing GFP mRNA LNPs, cells were stained with anti-CD3 antibody and DAPI. The number of GFP$^+$, CD3$^+$ cells was determined *via* flow cytometry and transfection efficiency was calculated by obtaining the percentage of GFP$^+$ cells relative to the total number of live CD3$^+$ cells. **B)** GFP mean fluorescence intensity **C)** Histogram of GFP fluorescence intensity *versus* live and CD3$^+$ T cells from flow cytometry analysis following treatment with 2 µl of GFP-loaded LNPs.

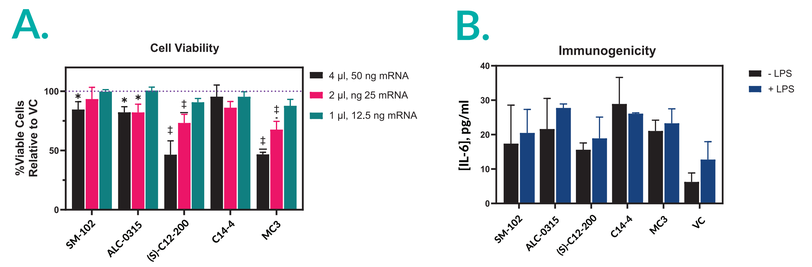

FIGURE 5 – Transfection of human PBMCs with LNP is non-toxic and does not alter immunogenicity.
A) Cell viability was measured by resazurin fluorescence. **B)** Immunogenicity was assessed by sandwich ELISA for the cytokine IL-6 (**Item No. 501030**). **P < 0.01, †P < 0.001, ‡P < 0.0001.*

CONCLUSION

- LipidLaunch™ loadable LNPs are an effective tool for transfecting both murine and human primary immune cells with mRNA of interest.
- Transfection of cells with cargo-loaded LipidLaunch™ LNPs is non-toxic and does not affect cellular viability.
- Cytokine production in response to stimulus is not impeded by LipidLaunch™ loadable LNPs.

Learn more about the LipidLaunch™ loadable LNPs

References

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