Lipid nanoparticle (LNP) components dictate the cellular immune response to vaccination

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Abstract No.

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KEY FINDING

LNP components can be tuned to achieve distinct immunological outcomes.

ABSTRACT

The type of cellular immune response elicited by a vaccination is key to determination of the success of that vaccination. Historical vaccines have been comprised largely of attenuated pathogen or large portions of the pathogen complexed with adjuvant, and most of the data around vaccines focuses on IgG antibody responses to the pathogen. Recent advances in mRNA and lipid nanoparticle (LNP) technology have been instrumental in controlling the worldwide SARS-CoV-2 pandemic and show promise for utility in developing vaccines for many common pathogens. A significant portion of the effort to develop new LNP vaccines uses protein expression levels as a surrogate or prerequisite for immune stimulation, but expression of target antigen is not the only factor to consider. In this study, we formulated LNPs with SARS-CoV-2 Spike mRNA as a model antigen. The LNPs were based on established ionizable lipids, and some included putative adjuvants as additional components of the LNPs. A cohort of mice was immunized, and immune responses were assessed by ELISA for total and neutralizing antibody production, flow cytometry for T cell subsets, and ELIspot for Spike peptide-specific immune responses. We found that the different LNP formulations stimulated different aspects of the immune response, which can inform future approaches to vaccine development.

BACKGROUND

During the COVID-19 pandemic, LNP vaccines protected well against severe consequences of infection, but incompletely against mild cases. Neutralizing antibody titer is a measure of immunity against virus, but wanes relatively quickly, within months, after vaccination. Beyond humoral immunity, cellular immunity may be key for inducing more durable resistance to virus and viral variants.[1] LNPs formulated for vaccine use against the COVID-19 pandemic demonstrated successful induction of humoral and cellular immunity.[2] Further, the modular nature of these vaccines suggested the possibility of optimization of formulations in a pathogen-specific manner. To better understand how addition of immune-stimulating moieties into vaccine formulations would affect the immune response, we tested established formulations (SM-102, C12-200) as well as C12-TLRa, containing a TLR agonistic component, and C12-200/αGC, containing an NKT cell agonist.[3,4]

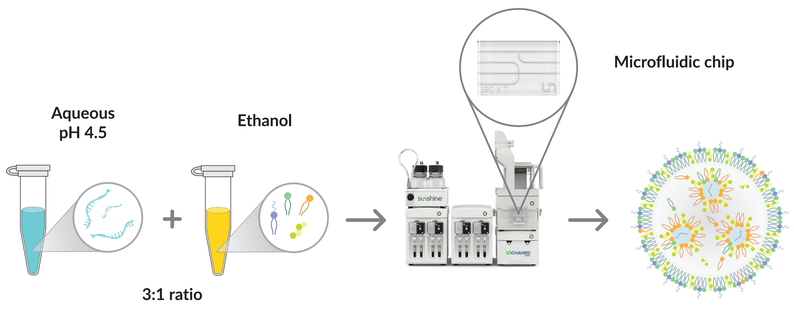

FIGURE 1 – LNP formulation method.
The aqueous (mRNA) and ethanolic (lipid) phases are mixed at a 3:1 ratio using the Sunshine microfluidics system from Unchained Labs. Laminar flow mixing takes place in the Sunny 190XT chip at a constant total flow rate to produce lipid nanoparticles. Resulting LNPs are dialyzed into PBS, pH 7.4 for downstream use.

RESULTS

	SM-102	C12-TLRa	c12-200	c12-200/αGC
Ionizable lipid	50	30	35	35
Cholesterol	38.5	46.5	46.5	46.5
DSPC	10	-	-	-
DOPE	-	16	16	16
DMG-PEG(2000)	1.5	2.5	2.5	2.5
C12-TLRa	-	5	-	-
KRN 7000	-	-	-	0.02

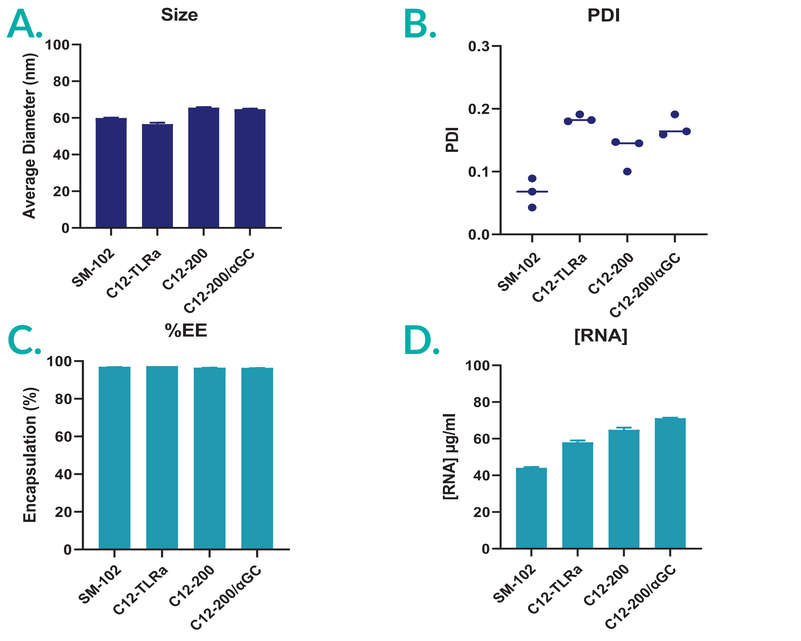

FIGURE 2 – Biophysical characterization of GFP-encoding LNPs.
LNPs based on the noted lipids were formulated first with eGFP mRNA and subsequently with SARS-CoV-2 Spike RBD mRNA. Percent molar ratios of lipid components are shown in the table. Diameter **(A)** and polydispersity index (PDI, **B**) were measured by dynamic light scattering. Encapsulation efficiency (%EE, **C**) and RNA concentration **(D)** were measured using a fluorescent assay.

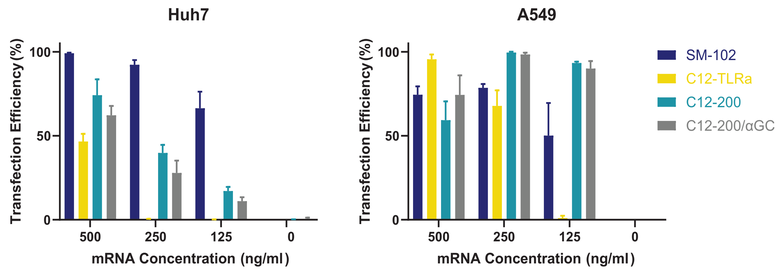

FIGURE 3 – Cell-type differences in transfection efficiency of GFP-encoding LNPs.
Each LNP was used to transfect hepatocytes (Huh7) and lung epithelial cells (A549) for 24 hours in a 96-well plate (n=3). Nuclei were stained with Hoescht and transfection efficiency was determined by percent of nuclei which were positive for GFP using an imaging plate reader.

CONCLUSIONS

- Addition of immunostimulatory components in LNPs does not compromise the integrity or transfection efficiency of LNPs.
- C12-200 induced the accumulation of all immune cells in the spleen, regardless of additional components.
- SM-102 LNPs induced the most potent neutralizing antibody response.
- Antigen-specific IL-2 production best correlated with neutralizing antibody, but C12-200 LNPs induced strong TNF and IFN-γ responses.

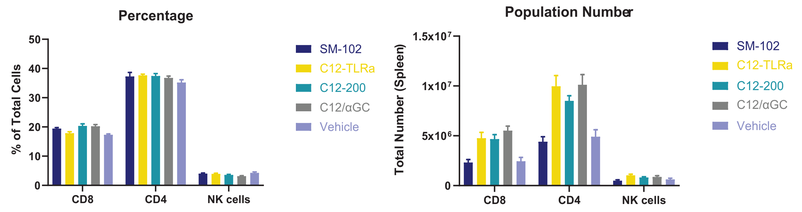

FIGURE 4 – Immunization with C12-based LNPs induced accumulation of immune cells in spleen.
LNPs encapsulating SARS-CoV-2 Spike mRNA were administered IM to mice (n=5 per group) at two timepoints, day 0 and day 21. Ten days after the second dose, spleens were harvested and splenocyte populations were stained for flow cytometric analysis of the indicated immune cell populations.

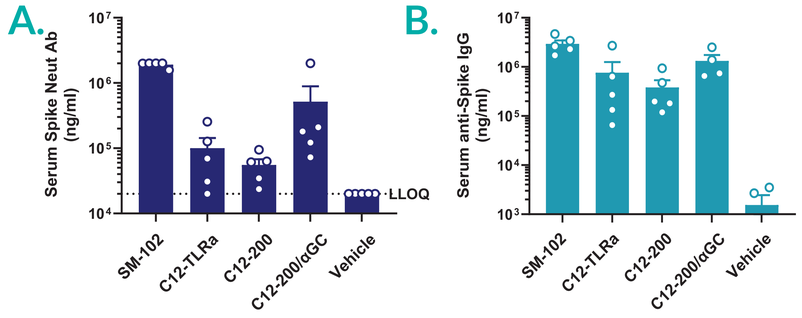

FIGURE 5 – Immunization with different formulations induced variable antibody response.
LNPs encapsulating SARS-CoV-2 Spike mRNA were administered IM to mice (n=5 per group) at two timepoints, day 0 and day 21. Ten days after the second dose, serum was tested for anti-Spike RBD neutralizing antibody **(A)** and total anti-Spike RBD mouse IgG **(B)**.

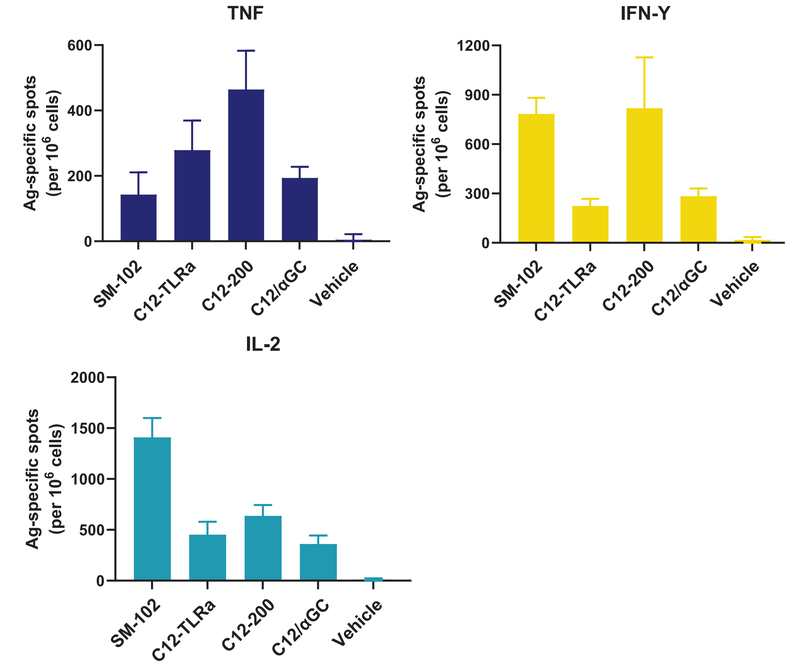

FIGURE 6 –Splenic IL-2 production best correlates with improved neutralizing antibody response.
Splenocytes harvested from immunized mice were plated with a Spike RBD peptide pool (MabTech) on multiplex FluoroSpot plates (MabTech), and TNF, IFN-γ and IL-2 antigen-specific responses were quantified.

References

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