Lipid nanoparticle SARS-CoV-2 vaccine-mediated immunopeptidome identification

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KEY FINDING

Lipid nanoparticle (LNP) components affect the presentation of class I and class II immunopeptides.

ABSTRACT

The expedient development of mRNA vaccines against SARS-CoV-2 was built upon several decades of work to better understand sub-micron-sized lipid-based particles as tools for drug delivery. Many aspects of these lipid nanoparticles (LNPs) make them ideal for delivery of mRNA in the context of vaccines, all of which can be tuned by lipid selection. Early work with any lipid formulation typically focuses on *in vitro* and *in vivo* expression of protein from the transcript cargo. However, it is also of vital importance how the protein resulting from LNP transfection is presented on MHC, as this will impact the type and magnitude of the immune response to the antigen. In this study, LNPs encoding SARS-CoV-2 Spike protein were formulated with several different lipids proposed to stimulate immune responses. These LNPs were used to transfect human monocyte-derived dendritic cells (MDDCs), and Spike-derived MHC class I and class II peptides were identified. Evaluation of immunopeptidomic differences between these formulations as well as understanding of resulting immune responses will help the design of vaccine LNPs in the future.

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 formulations can affect antigen presentation, we tested an established formulation, SM-102, and an experimental formulation, C12-200/αGC.[3]

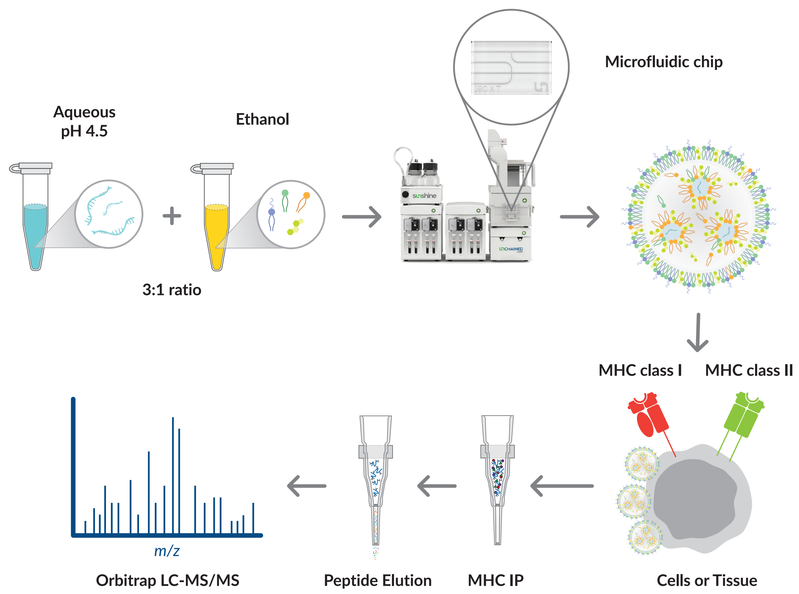

FIGURE 1 – LNP formulation, cell treatment, and immunopeptidome profiling.

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RESULTS

A.

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

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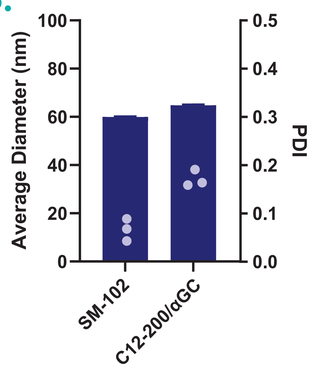

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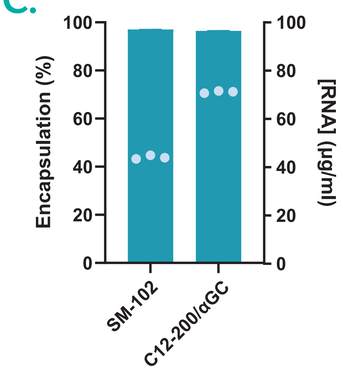

FIGURE 2 – Biophysical characterization of GFP-encoding LNPs.
LNPs based on the noted lipids were formulated first with eGFP mRNA and subsequently with SARS-CoV-2-Spike RBD mRNA. Percent molar ratios of lipid components are shown in the table **(A)**. Diameter and polydispersity index (PDI, **B**) were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

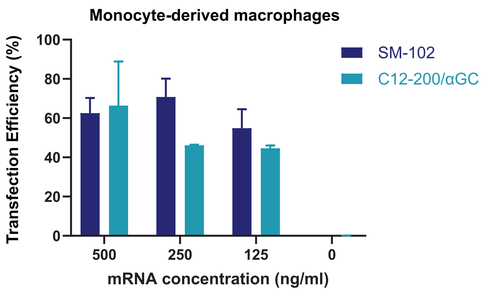

FIGURE 3 – Effective transfection of immune cells by LNPs
Primary human monocytes were differentiated into macrophages using M-CSF. LNPs encapsulating eGFP mRNA were incubated with macrophages and transfection efficiency was calculated by imaging, using Hoechst to stain nuclei and count GFP⁺ cells.

CONCLUSIONS

- mRNA LNPs used in vaccine formulations can successfully transfect antigen-presenting cells.
- Neither SM-102 nor C12-200/αGC-based LNPs affect the expression of MHC class I or II in MDDCs.
- LNP treatment does not affect overall peptide recovery from MHCs.
- Cargo peptide presentation requires both SM-102 and LPS maturation in MDDCs.

References

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2. Oberhardt, V., Luxenbuger,H., Kemming, J., *et al.* Rapid and stable mobilization of CD8+ T cells by SARS-CoV-2 mRNA vaccine. *Nature* 597, 268-273 (2021).
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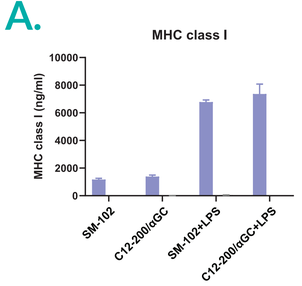
4. Kaabinejadian, S., Barra, C., Alvarez, B., et al. Accurate MHC motif deconvolution of immunopeptidomics data reveals a significant contribution of DRB3, 4 and 5 to the total DR immunopeptidome. Front. *Immunol.* 13, 835454.

A.



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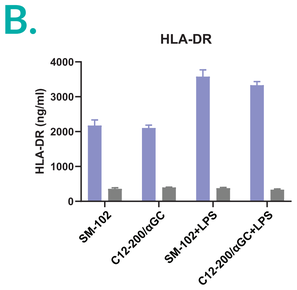

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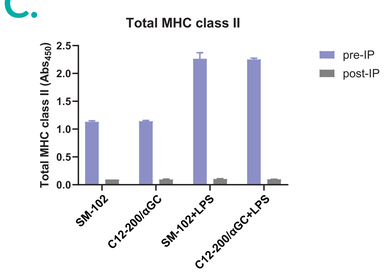
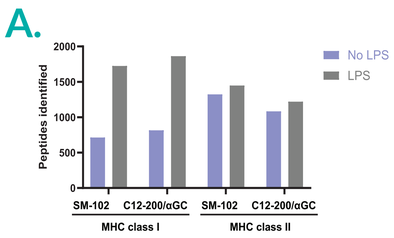


FIGURE 4 – MHC expression increases with LPS maturation and is completely captured by immunoprecipitation.
MDDCs were treated with LNPs encapsulating SARS-CoV-2 Spike RBD mRNA for 24 hours, then lysed. Lysates (after reserving a small amount for ELISA) were subjected to immunoaffinity enrichment of total class I, HLA-DR, and pan-class II in serial immunoprecipitations. The input (pre-IP) and flowthrough were subjected to ELISAs to quantify initial expression and depletion by the resin: MHC Class I (human) ELISA Kit **(A, Item No. 502060)**, HLA-DR (α and β chains) ELISA Kit **(B, Item No. 501810)** and a proprietary total human class II qualitative ELISA **(C)**.

A.



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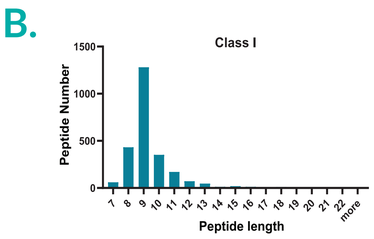

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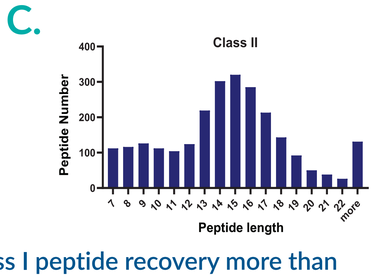

FIGURE 5 – LPS increases MHC class I peptide recovery more than MHC class II peptide recovery.
Peptides eluted from MHC class I and II complexes were submitted for mass spec sequencing (HLA-DR and remaining class II samples were combined for each treatment). Peptide numbers recovered from each sample are shown **(A)**. Peptide lengths correspond to expected lengths of class I (**B**, histogram centered around 9 AA) and class II (**C**, histogram centered around 15 AA).

DAVDCALDPLSETKCT

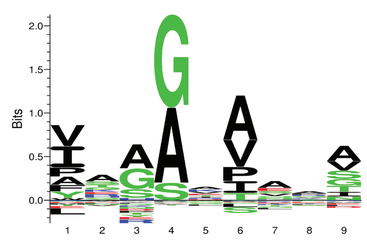

FIGURE 6 – Spike RBD peptide identified in MHC class II.
The identified 16-mer peptide has a high predicted binding score with DQA1*05:05/DQB1*03:01 (IEDB).[4] Core binding sequence is indicated in red. Eluted ligands of this haplotype form the motif to the right.