When carriers are not inert: exploring formulation-dependent immune responses to LNPs in THP-1 cells

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Introduction

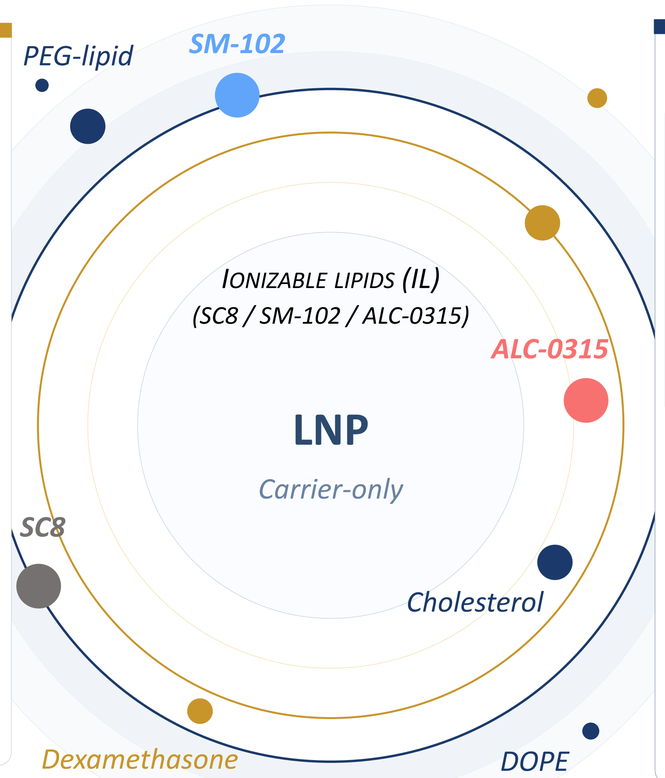
Lipid nanoparticles (LNPs) are increasingly recognized as biologically active systems whose carrier composition can directly **influence immune cell responses**[1].

However, the **intrinsic immunomodulatory effects of LNP formulations** remain incompletely understood, particularly independent of nucleic acid cargo[2].

[1] Karmaker S. et al., Nanoscale 17 (2025) 11864–11893.
[2] Zelkoski AE. et al., npj Vaccines 10 (2025) 73.

Aim

To **explore formulation-dependent immunomodulatory effects of cargo-free LNPs** (ionizable lipid identity ± Dx) in **THP-1-derived macrophages** under basal (M0) and pro-inflammatory (M1) conditions.



Study Workflow & Key Message

LNP Formulation — SC8, SM, ALC (Dx); *Variable IL*

Macrophage Exposure — *THP-1 derived macrophages*

Intracellular Readouts — DiD Uptake; Dx-associated Fluorescence

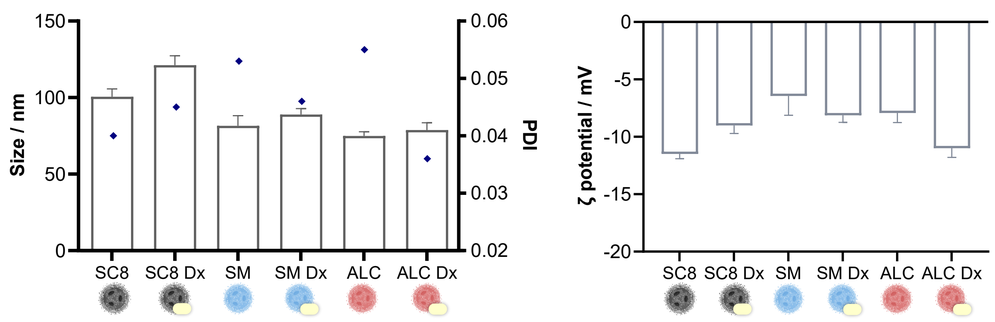
Immune-Marker Profile — M1-like: CD86, HLA-DR; M2-like: CD163, CD206; *Phenotypic changes over time (24h & 72h)*

Comparable physicochemical properties across all formulations → Similar Uptake Differential Dx-associated Fluorescence → ***Early Response*** *24h* *CD86-associated pattern differs* → ***Late Response*** *72h* *CD163/CD206 shift becomes clearer*

Formulation-dependent LNP effects evolve over time, with **ALC-Dx** showing the **clearest macrophage marker shift at 72 h**.

Particle Characterization

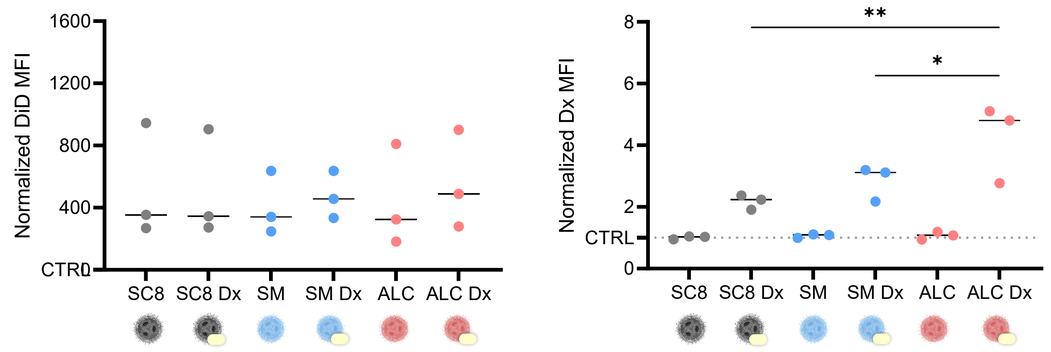
Size · PDI · ζ-potential



LNPs showed comparable size and low PDI across formulations

Cellular Uptake

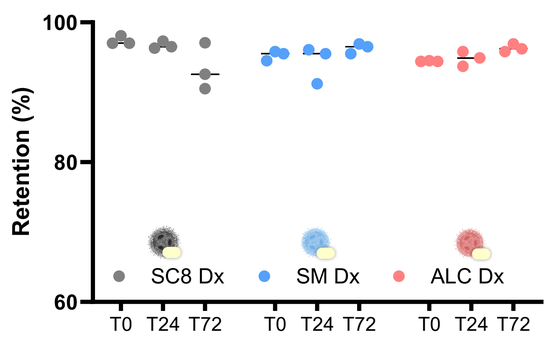
DiD signal · Dx-associated fluorescence



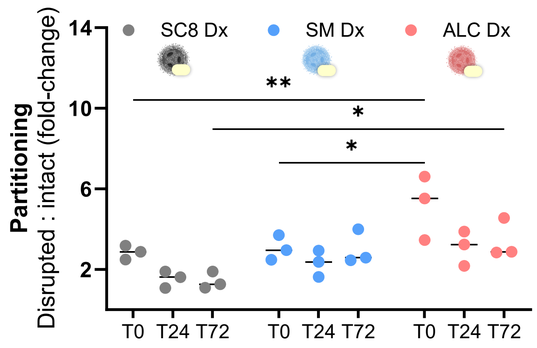
Comparable particle uptake (DiD signal) but differential Dx-associated fluorescence

Dx Retention/ Partitioning

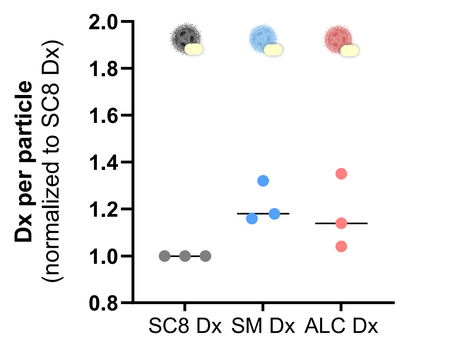
Particle-association over time · Formulation-dependent intracellular Dx exposure



Dx remained particle-associated (T0 / 24h / 72h)



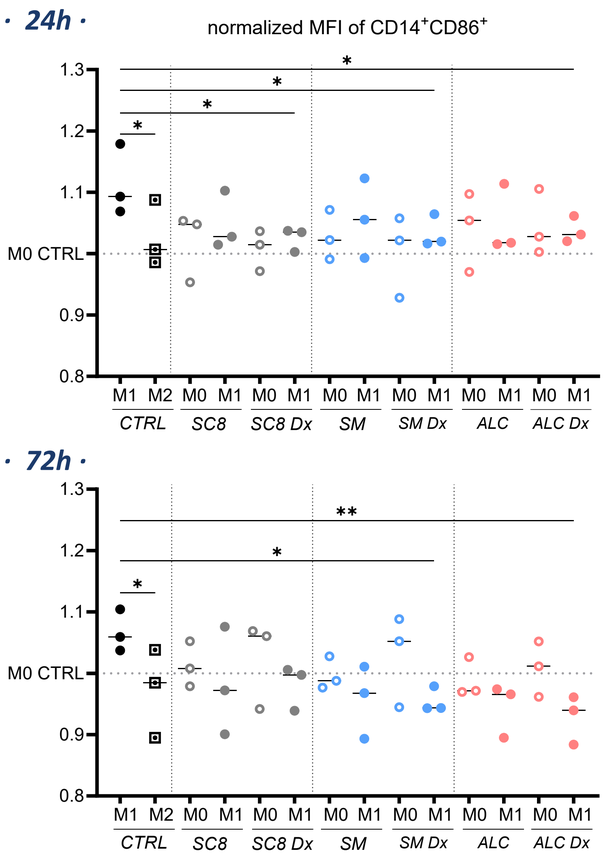
ALC-Dx showed the highest disruption-revealed Dx signal



Comparable loading across formulations

CD86

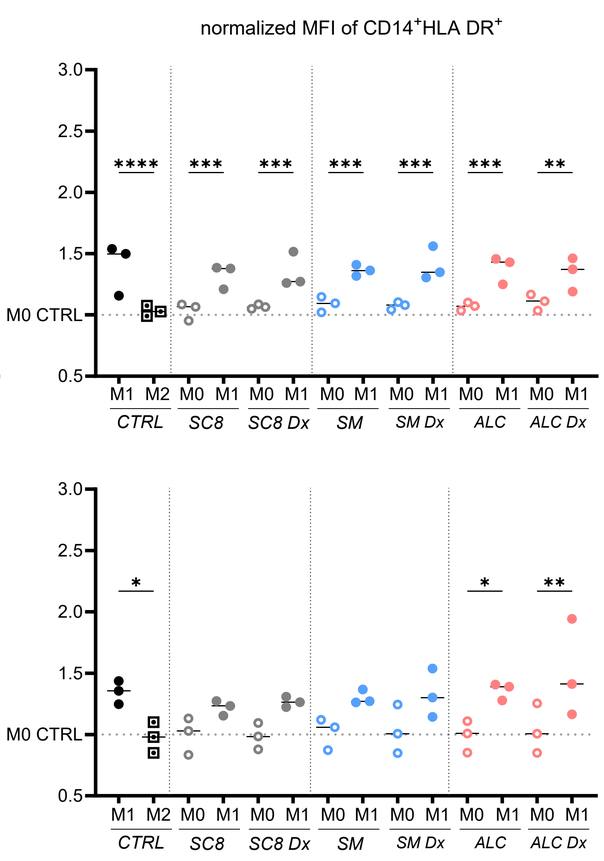
M1 activation · 24h & 72h



Dx-containing LNPs tended to reduce CD86, most consistently with ALC-Dx

HLA-DR

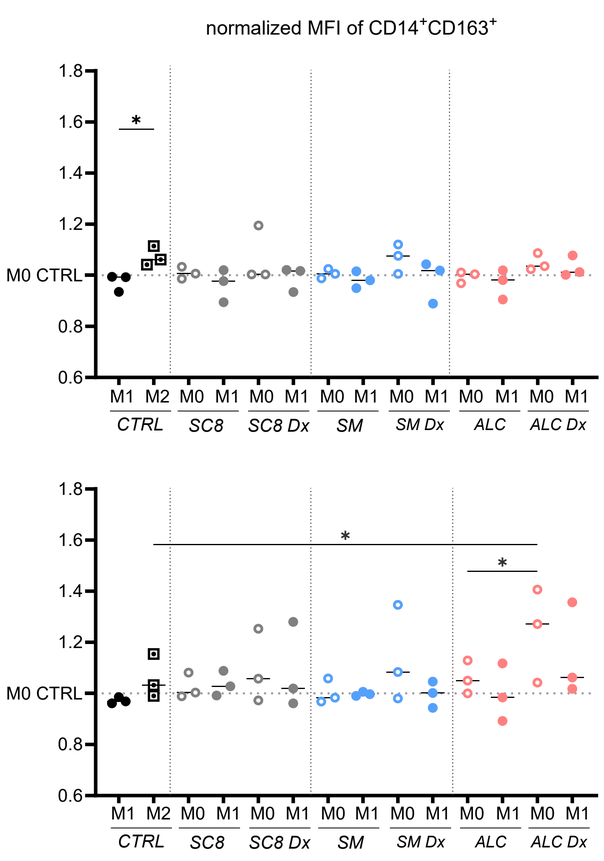
Antigen presentation · 24h & 72h



HLA-DR increased in several LNP-treated groups, with a late increase most apparent for ALC-Dx

CD163

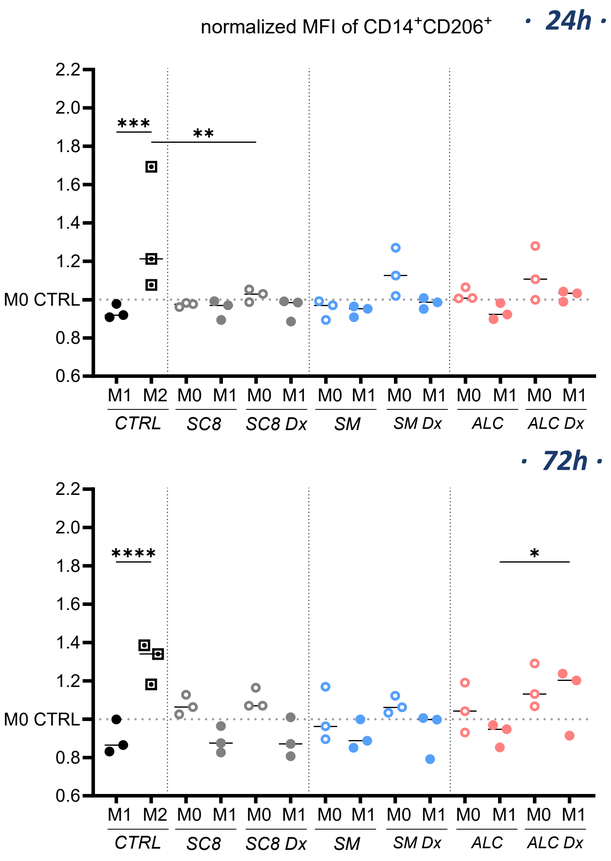
M2-assoc. receptor · 24h & 72h



CD163 increased at 72h, most notably with ALC and ALC-Dx

CD206

M2-assoc. receptor · 24h & 72h

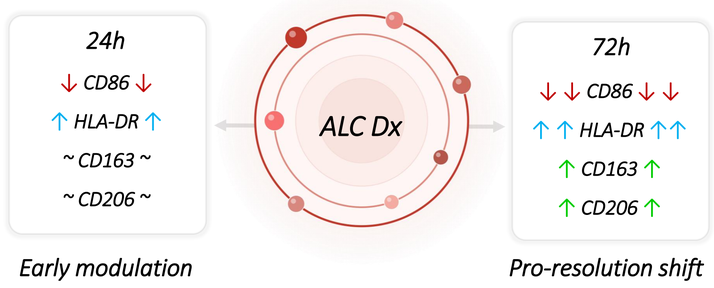


CD206 showed a delayed increase at 72h, most clearly with ALC-Dx

n = 3 independent experiments · two-way ANOVA + Holm-Šídák's test · *$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$, ****$p \le 0.0001$ · median shown · GraphPad Prism 10.3

Conclusion

ALC-Dx showed the most distinct time-dependent macrophage marker profile, with **lower CD86** and **later increases in CD163/CD206**.



Acknowledgements

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