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Targeting mast cell surface receptors for enhanced lipid nanoparticle internalization

Abstract

Mast cells (MCs), are immune cells that release inflammatory mediators in response to several stimuli. MCs can provide an effective target for anti-inflammatory therapeutic strategies due to their role in inflammatory responses in the lung and skin, although, the unique phospholipid components of their cell membrane make them resistant to genetic manipulation using canonical drug delivery approach. To facilitate cellular internalization of cationic lipid-based nanoparticles (LNPs) into differentiated bone marrow derived mouse mast cells (BMMCs), we coated them with various apolipoproteins (proteins that bind lipids to promote their transport into cells). Out of all the apolipoproteins tested, ApoC3 most efficiently facilitated cellular internalization of LNPs into BMMCs. ApoC3 did not activate BMMC degranulation and cytokine production, suggesting that they are immunologically inert, a feature that would make them an attractive candidate for promoting LNP transport into MCs. Interestingly, internalization was increased when BMMC were pre-treated with inhibitors of actin polymerization and decreased by inhibitors of clathrin-dependent pathways, suggesting that ApoC3 facilitates LNP internalization via an actin-dependent clathrin-mediated endocytic pathway. We hypothesized that ApoC3 interacts with MC surface receptor apolipoprotein E receptor 2 (ApoER2), that internalizes by an actin dependent clathrin-mediated endocytic pathway, to promote LNP internalization. Our results show that activation of transcription factor peroxisome proliferator activated receptor gamma (PPARγ), that modulates expression of low-density lipoprotein receptors including ApoER2, decreased LNP internalization and reduced the expression of ApoER2 in BMMCs. Furthermore, ApoC3 failed to facilitate internalization of LNPs in BMMCs that do not expressed ApoER2 and significantly enhanced internalization in U-87 MG glioblastoma cells that highly expressed ApoER2 on their cell surface. Based on our results, we suggest that functionalizing cationic LNPs with ApoC3 and co-encapsulating actin cytoskeletal inhibitors along with the payload might help achieve better internalization of LNPs, to genetically manipulate MCs and hence can be utilized towards development of cell directed therapeutics.

Introduction

- LNP delivery to immune cells remains a major unmet challenge, particularly to cell types that are difficult to isolate and culture *in vitro*.
- Cationic lipids have several advantages to polymer-based nanoparticles such as: 1) enhanced interaction with the anionic phospholipids in the plasma membrane to promote its uptake via endocytic pathways; 2) immunologically inert and therefore unlikely to activate nonspecific immune responses; 3) can be easily functionalized to specifically target cell membrane receptors and biomarkers.
- Although cationic lipids are effective in delivering cargo to adherent cells, they are particularly troublesome for cells grown in suspension due to reduced probability of interaction with the delivery vehicle.
- Non-transformed, primary cultured immune cells are extremely difficult to transfect due to their low division rate, intrinsic self-defence mechanisms to prevent introduction of foreign genetic material, and increased susceptibility to apoptosis when exposed to cationic lipids.
- These challenges can be overcome by coating LNPs with apolipoproteins (Apo) which bind both lipids and lipoproteins to form polar structures that act as lipid transport carriers in the lymph, blood and cerebrospinal fluid.
- We hypothesized that Apos may facilitate the internalization of cationic LNPs into terminally differentiated mouse bone marrow-derived mast cells (BMMC).

Methods

- LNPs were formulated by microfluidic mixing of the cationic lipid DODMA and GFP/eGFP reporter plasmid using the NanoAssemblr benchtop instrument and microfluidic cartridges (Precision nanosystems, Vancouver, BC, Canada).
- GFP/eGFP encapsulation was analyzed by picogreen assay. LNP size and morphology were characterized by dynamic light scattering and cryo-transmission electron microscopy respectively.
- LNP internalization efficiency was measured by assessing GFP/eGFP fluorescence either by flow cytometry or fluorescence microscopy.
- β-hexosaminidase and TNF levels were measured by colorimetric assay and ELISA respectively.

Results

1. ApoC3 facilitates internalization of LNPs into BMMC and are immunologically inert

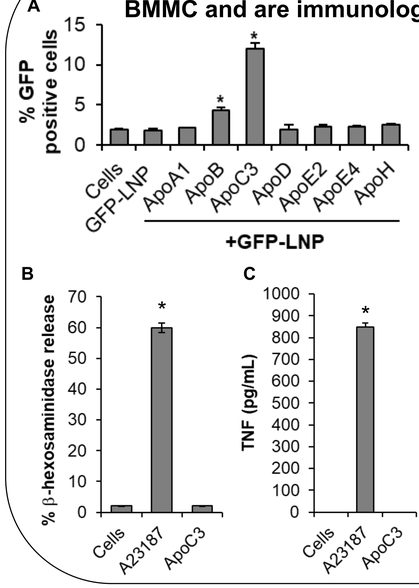

Figure 1. (A) BMMC were treated with GFP-LNP or GFP-LNP complexed with various Apos for 24 hr followed by flow cytometry to determine % GFP positive cells. (B & C) BMMC were treated with the calcium ionophore A23187 or ApoC3 for 45 min followed by measuring the release of β-hexosaminidase enzyme (B) or TNF (C). Untreated cells are represented as "Cells". n=3, a student's t test was performed to indicate statistical significance (p<0.05 indicated by an asterisk).

2. LNPs internalize by actin-dependent clathrin- mediated endocytic pathway

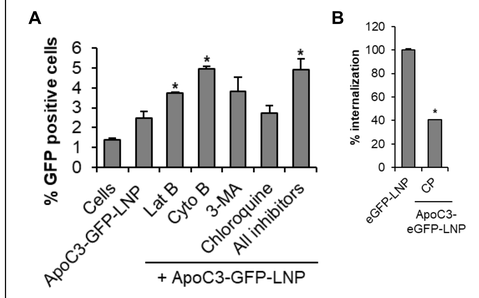

Figure 2. (A) BMMC were treated with endocytosis inhibitors such as Latrunculin B (lat B), Cytochalasin B (Cyto B), 3-methyladenine (3-MA), chloroquine or all inhibitors for 1 hr followed by transfection with ApoC3-GFP-LNP for 24 hr. (B) BMMC were treated with clathrin-mediated endocytosis inhibitor Chloropromazine (CP) as in "A". Flow cytometry was conducted to determine % GFP positive cells. n=3, a student's t test was performed to indicate statistical significance (p<0.05 indicated by an asterisk).

3. Activation of the transcription factor PPARγ reduces LNP internalization, *Lrp8* mRNA synthesis and its encoded protein ApoER2.

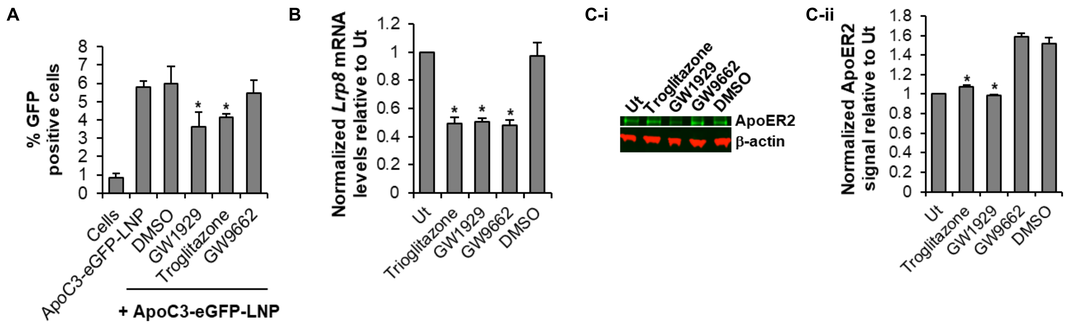

Figure 3. BMMC were incubated with PPARγ agonist (GW1929 and Troglitazone), antagonist (GW9662) or DMSO control for 1 hr prior to transfection using the ApoC3-eGFP-LNP for 24 hr followed by flow cytometry to determine % GFP positive cells (A). Untreated BMMC are represented as "Cells" or "Ut". (B) BMMC were treated as in "A" for 3 hr followed by RNA extraction, cDNA synthesis and qPCR to determine *Lrp8* mRNA levels. Housekeeping gene GapDH was utilized as an endogenous control. BMMC were treated as in "A" for 24 hr followed by Western blot (C-i) or densitometric (C-ii) analysis to determine ApoER2 protein levels. β-actin was utilized as a loading control. n=3, a student's t test was performed to indicate statistical significance (p<0.05 indicated by an asterisk).

4. ApoC3 fails and promotes to facilitate internalization of LNPs into cells that do not or highly express ApoER2

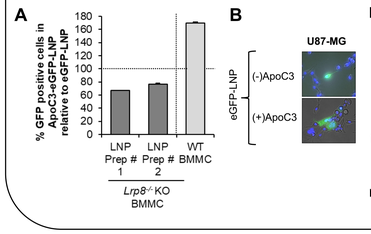

Figure 4. (A) WT or *Lrp8*$^{-/-}$ KO BMMC were transfected with eGFP-LNP or ApoC3-eGFP-LNP complex for 24 hr followed by flow cytometry to determine % GFP positive cells. (B) U87-MG cells were transfected as in "A" and GFP fluorescence (green) was analyzed by fluorescence microscopy. Nuclei are stained using DAPI (blue).

5. Hypothetical Model

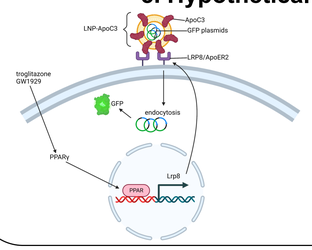

Figure 5. We propose that ApoC3 potentiates internalization of LNPs via interaction with the cell surface receptor ApoER2.

Conclusion

- ApoC3 plays an important role in facilitating the binding and transport of cationic LNPs into BMMC.
- Immunologically inert nature of ApoC3 makes it an attractive candidate for promoting transport into MC.
- Our studies reveal an important role of ApoC3 in facilitating internalization of LNPs into MCs that can have an impact on fabrication of novel nano therapies against inflammatory diseases directed to skin or lungs.

Future directions

Functionalizing DODMA-LNPs with ApoC3 and co-encapsulating actin cytoskeletal inhibitors along with the payload to see if we achieve better transfection efficiency to genetically manipulate MCs and hence can be utilized towards development of cell directed therapeutics.

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References

Alam et al, *Scientific Reports Jan 2023,* https://doi.org/10.1038/s41598-022-25737-7, Apolipoprotein C3 facilitates internalization of cationic lipid nanoparticles into bone marrow-derived mouse mast cells.

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