Detection of lipid nanoparticles phagocytosed by antigen-presenting cells using imaging flow cytometry

Poster#
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Louise M. D'Cruz[1], Anthony T. Phan[2], Xiaoshan Shi[3], Stephanie J. Widmann[1], Mohamad-Gabriel Alameh[4], Christopher A. Hunter[2] Aaron J. Tyznik[1]

[1]BD Biosciences, San Diego, California 92121, USA; [2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA; [3]BD Biosciences, San Jose, California 95131, USA; [4]Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

Abstract

DNA or mRNA vaccines encoding antigens that can elicit host immune responses have shown great efficacy for combating infectious disease and potentially treatment of cancer. Effective delivery of nucleic acid vaccines can be achieved through nucleic acid-encapsulated lipid nanoparticles (LNPs). Despite the proved effectiveness of nucleic acid vaccines in different disease settings, the mechanism by which antigen presenting cells recognize the LNP cargo upon vaccination is not understood. In this study, we utilized the state-of-the-art CellView™ Image Technology on the BD FACSDiscover™ S8 Cell Sorter to visualize LNP phagocytosed by host cells. To determine the mechanisms of antigen presentation, green fluorescent LNPs were formulated *in vitro* and injected into mice. With the imaging capabilities of the BD FACSDiscover™ S8 Cell Sorter, we visualized LNPs in different antigen presenting cells and observed distinct morphology of LNPs in dendritic cell subsets, monocytes, and neutrophils. A time course experiment uncovered the kinetics of antigen recognition upon LNP-mediated cargo delivery. Unsupervised analysis using the imaging parameters derived from the instrument revealed unique cell clusters possibly associated with different morphology of LNP phagocytosis. This work provides an advanced workflow to visualize LNP phagocytosis followed by image-based cell sorting for downstream in-depth characterization enabled by the BD FACSDiscover™ Cell Sorter. With an increased adoption of LNP-mediated nucleic acid therapeutics and vaccines, the image-based cell sorter provides a powerful tool to understand the mechanism of host immunological response upon vaccine injection.

Methods

Experimental workflow

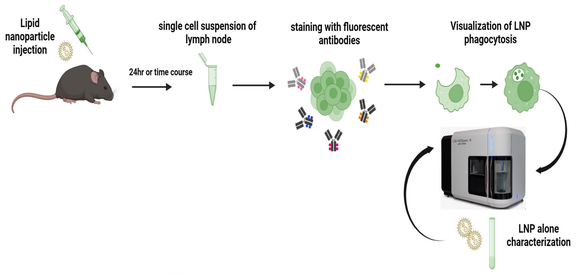


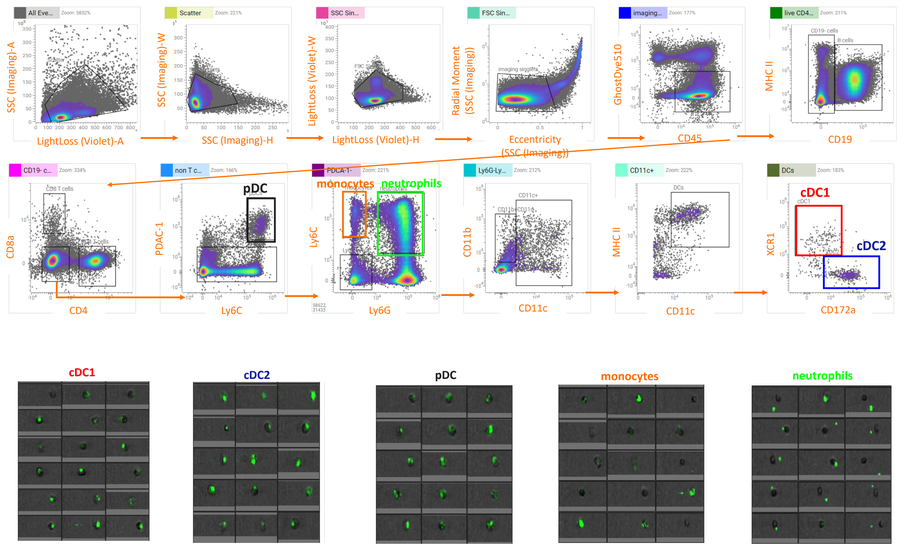
Figure was created using BioRender.com

Table. 18-color fluorescent panel to visualize LNP in antigen-presenting cells

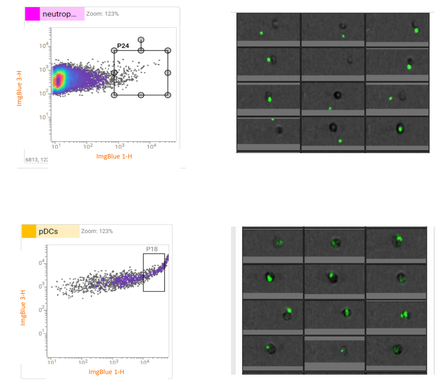
Laser	Fluorophore	Antibody
UV Laser	BUV395	CD19
	BUV496	CD4
	BUV563	Ly6G
	BUV615	CD8
	BUV661	CD11b
	BUV737	CD11c
	BUV805	CD45
Violet Laser	BV421	PDCA-1
	Ghost Dye™ Violet 510	Live dead
	BV605	CX3CR1
	BV650	XCR1
	BV711	CD90.2
	BV786	Ly6C
Blue Laser	FITC (Imaging Channel 1)	DiO
Yellow Green Laser	PE-Cy7	CD64
Red Laser	APC	H2-Kb SIINFEKL
	AF700	MHCII – IA/IE
	APC-Cy7	CD172α

Results (1)

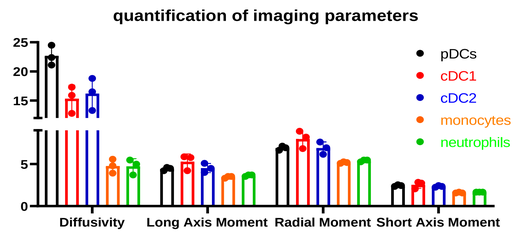
1A Visualization of LNP phagocytosis in different antigen-presenting cells



1B Imaging flow cytometry reveals distinct phagocytosis morphology



1C Key imaging parameters associated with distinct cell populations



1D Unsupervised clustering of distinct antigen presenting cell populations

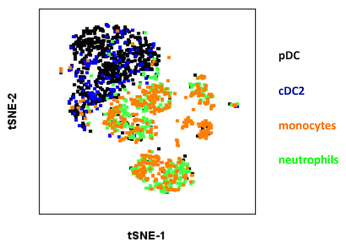


Figure 1. Visualization of LNP phagocytosis in different subsets of antigen presenting cells. (A) Flow cytometry plots identifying different cell populations (cDC1, cDC2, pDC, monocytes and neutrophils) and visualization of LNP phagocytosis in different cell subsets. (B) Images from the BD FACSDiscover™ S8 Cell Sorter revealed the morphology of LNP phagocytosis. Although both neutrophils and pDCs were LNP positive by conventional flow cytometry, by imaging neutrophils did not take up LNPs whereas pDCs phagocytosed LNPs. (C) Measurement of key imaging parameters associated with morphology derived from the BD FACSDiscover™ S8 Cell Sorter indicated differences in LNP phagocytosis in different cell types. (D) Unsupervised tSNE analysis of different antigen presenting cells resulted in separation between dendritic cells (cDC2 and pDC) from monocytes and neutrophils, suggesting distinct morphology of LNP phagocytosis in different cell types.

Results (2)

2A Kinetics of LNP phagocytosis using imaging parameter-driven clustering

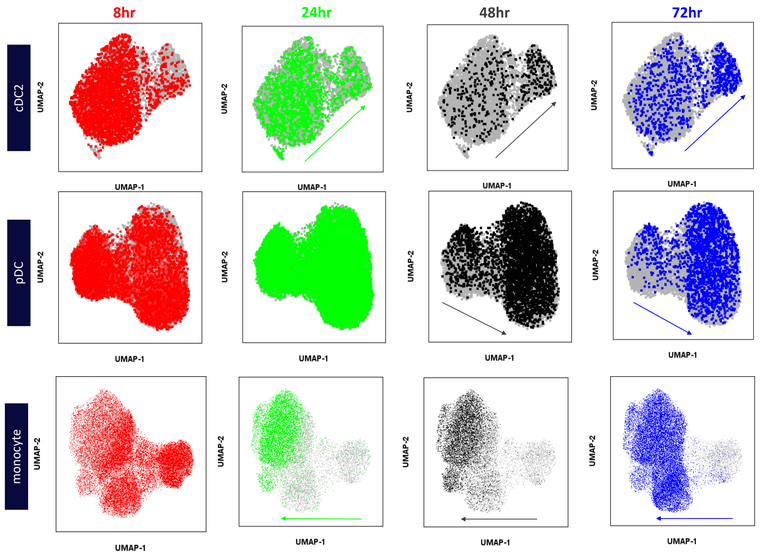
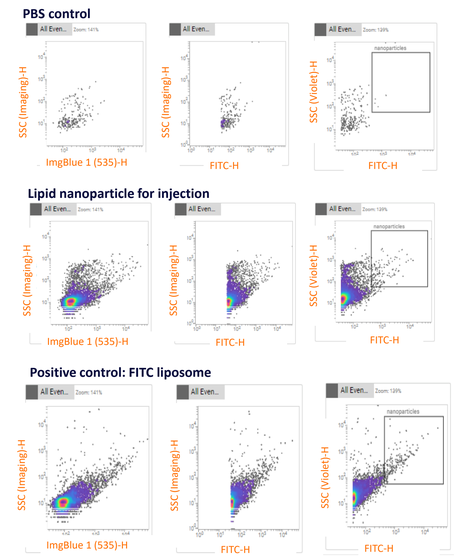


Figure 2A. Kinetics of LNP phagocytosis using imaging parameter-driven clustering. LNPs were injected into mice intramuscularly and mice was sacrificed at 8hr, 24hr, 48hr and 72hr post injection. Single-cell suspensions from lymph node were stained with the 18-color fluorescent panel and acquired using the BD FACSDiscover™ S8 Cell Sorter. Different cell populations (cDC2, pDC, monocyte and cDC1) were concatenated from three mice and UMAP analysis was performed on each cell type using only imaging parameters from the instrument. UMAP plots at different time points indicated possible changes of LNP morphology upon uptake.

2B Acquisition of LNP alone



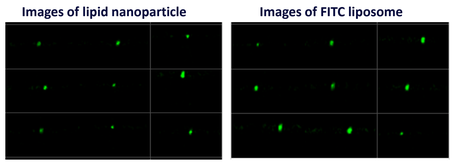


Figure 2B. Visualization of lipid nanoparticles using the BD FACSDiscover™ S8 Cell Sorter. DiO/DiI LNPs used for mice injection were acquired using the BD FACSDiscover™ S8 Cell Sorter. LNPs can be identified using a scatter plot. Green fluorescent signal was detected using both the FITC fluorescent channel and imaging channel 1. Images were acquired for LNPs. Filtered PBS was acquired as a negative control. FITC liposome was run simultaneously to set up the instrument and served as a positive control.

Conclusions

- ❖LNPs were injected into mice intramuscularly to evaluate the biological process of LNP uptake by antigen-presenting cells using imaging flow cytometry.
- ❖Single-cell suspensions from lymph node of mice were stained with an 18-color fluorescent panel to identify different cell subsets of antigen-presenting cells.
- ❖LNP uptake by different antigen-presenting cells was clearly visualized using the BD FACSDiscover™ S8 Cell Sorter.
- ❖Images provided additional information regarding LNP phagocytosis compared to fluorescent staining only.
- ❖Quantification of image parameters in different cell types revealed distinct morphology of LNP uptake by different antigen-presenting cells.
- ❖A time-course experiment uncovered the kinetics of LNP phagocytosis by different antigen-presenting cells.
- ❖Acquisition of LNPs alone on the BD FACSDiscover™ S8 Cell Sorter helped evaluate the size and fluorescence intensity of LNPs, providing additional information on LNP characteristics and serving as an efficient quality control tool of LNP prior to ***in vivo*** injection.