Identification of Circulating Myeloid Cell Populations in NLRP3 Null Mice

TECHNICAL INFORMATION BULLETIN

CytoFLEX Research Cytometer

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Introduction

Surface markers are commonly used to discriminate populations in circulating blood. Using the surface markers Ly6C (hematopoietic cells), Ly6G (granulocyte/neutrophil) and CD115 (MCSF receptor), we are able to describe the circulating cell populations from fresh blood in C57BL/6J wild type control mice and NLRP3 null samples. Due to NLRP3's implication in forming the inflammasome, we hypothesize that there will be a difference in the monocyte to granulocyte ratio. Moreover, we are interested in observing the expression of the CD115 (MCSF receptor) between the control and null groups.

The CytoFLEX flow cytometer is excellent for interrogating different populations circulating in blood. The proprietary Wavelength Division Multiplexing (WDM) detection module uses solid-state, high efficiency, low-noise Fiber Array Photodiode Detectors (FAPD), giving exceptional resolution for more precise data and better detection of rare events. This protocol describes the use of the CytoFLEX flow cytometer with a red and blue laser configuration for analysis.

Materials & Methods

Reagents

Laser	405nm					488nm					638nm		
Fluor	KrO	PB	V610	V660	V780	FITC	PE	ECD	PC5	PC7	APC	APC A700	APC A750
Marker						Ly6G	Ly6C				CD115		

Sample Preparation

1. Aspirate 100-600 µL of mouse blood via cardiac puncture.
2. Place fresh blood into 1.5mL tubes with 40 µL 0.5M EDTA.
3. Lyse red blood cells with 1X hypotonic lysis solution for 5 minutes.
4. Centrifuge tubes at 400 x G for 10 minutes at room temperature.
5. Remove supernatant and resuspend in 500 µL MACS buffer (Miltenyi) or equivalent.
6. Centrifuge cells at 400 x G for 10 minutes at 4 °C.
7. Resuspend in 100 µL MACS buffer with 5% Normal Mouse Serum and 5% Normal Rat Serum.
8. Add antibodies and stains. Incubate for 30 minutes on ice.

Antibody	Quantity
Ly6C – PE	0.5 µL
Ly6G – FITC	1.0 µL
CD115 – PE	2.5 µL

9. Centrifuge at 400 x G for 10 minutes at 4 °C
10. Aspirate supernatant
11. Resuspend in 600 µL MACS buffer
12. Acquire on CytoFLEX flow cytometer

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Data Acquisition on CytoFLEX

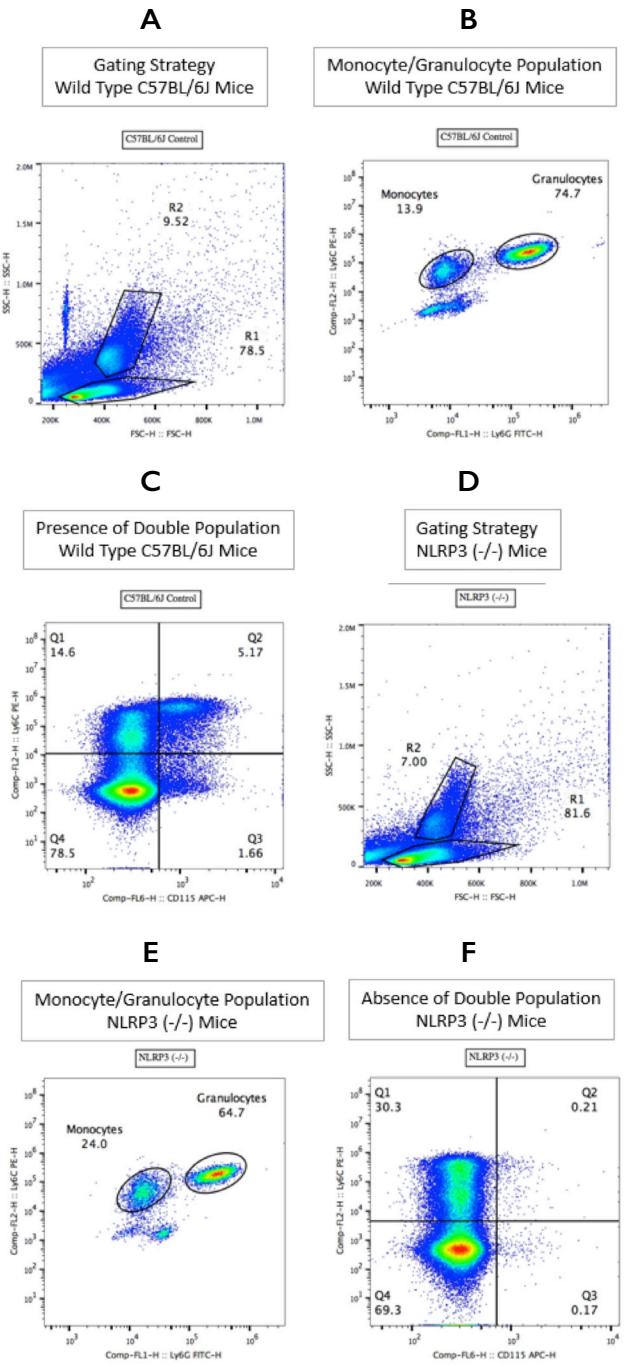
1. Create new experiment
2. Create FSC/SSC, PE/FITC, and PE/APC plot.
3. Import previously established compensation settings for FITC, PE and APC.
4. Create gates for monocytes and granulocytes
5. Run the sample on Fast rate setting.
6. Auto-adjust for scaling.
7. Acquire a minimum of 50,000 events.
8. Save data.

Conclusions

Through the use of surface markers Ly6C and Ly6G, we are able to observe a change in the dynamics of the circulating monocyte and granulocyte population between our wild type controls and the NLRP3 null samples. Furthermore, a double positive population for CD115 (MCSF receptor) and Ly6C observed in the wild type controls is absent in the NLRP3 null samples. This observation was previously not detected on other instrumentation. The CytoFLEX flow cytometer is simple to operate and is a sensitive and reliable method to interrogate the differences in populations circulating in blood.

Data

Plots A and B show the gating strategy. Plots C and D depict the percentage of monocytes and granulocytes in wild type vs. NLRP3 null mice. Plots E and F demonstrate the presence/absence of a double positive population.



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