Identification of NK subsets in mice

APPLICATION NOTE

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IN THIS PAPER YOU WILL LEARN

The preparation of a 10 color immunophenotyping panel to identify mouse Natural Killer cells (NK)

The gating strategy to quantify NK population subsets

Introduction

Human NKs are commonly divided based on their expression of CD56 and CD16, which generally correlates to their activation status. $CD56^{bright}CD16^{neg}$ NKs are abundant in peripheral tissues; they respond to soluble factors by making copious amounts of immunoregulatory cytokines, but acquire cytotoxicity after prolonged activation. In contrast, most circulating human NKs are $CD56^{dim}CD16^{pos}$ and are considered activated; when target cells are recognized they respond by killing the target or producing cytokines. Mouse NKs lack CD56 expression, which has made it difficult to identify functional counterparts to human NKs. However, mouse NKs are commonly identified based on the expression of CD11b and CD27, but these markers focus more on immature versus mature status of mouse NKs. Several reports have shown that mouse NKs express CD127 (IL-7Rα) and may be similar to $CD56^{Bright}CD16^{neg}$ human NKs in that they produce large amount of cytokines and only acquire cytotoxicity function after prolonged activation. The NKp46 (CD335) activation receptor is a common surface marker found on both human and mouse NKs. Together with CD122 (IL-2Rb chain), these markers are used to identify NKs among $CD45^{pos}CD3^{neg}$ cells. There are conflicting reports about NKs in autoimmune models, which could relate to the poor understanding of functional NK subsets in mice.

Sample Preparation

1. Remove mouse spleen.
2. Make single cell suspension. Use 5 mL Hanks per 2 spleens in 35 mM culture dish. Use syringe head to "smash" and transfer suspension to 14mL tube. No more than 2 spleens per tube!! Allow debris to settle to bottom of tube and transfer supernatant to fresh 14 mL tube.
3. Spin cells 5 minutes at 1500 rpm at 4˚C.
4. Pour off supernatant and "flick" pellet to loosen cells.
5. Remove red cells using 2 mL ACK (90% of 0.83% $NHCl_4$ +10% 2.06% Tris pH 7.6-Sterile) per tube. Incubate 1 minute in 37˚C waterbath. Add 8 mL Hanks. Mix by pipetting up and down with 10 mL pipette and allow suspension to settle in the pipette, so that the debris will adhere to the side of the pipette. Slowly place suspension in fresh 14 mL tube leaving the cell debris in the pipette. Repeat steps 3 and 4.
6. Add 10 mL PBS count cells.
7. Stain for fixable live/dead dye for 20 minutes in fridge. Wash with PBS 2X.
8. Surface stain splenocytes for CD45, CD19, CD3. CD335, CD25, CD122, CD127, CD218, and CD117. Incubate 20 minutes in fridge. Wash 2X.

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9. Fix and permeabilize with Foxp3 stain buffer set (e-Bioscience cat. No. 00-5523). For 30 minutes. Wash 2X with Perm buffer.
10. Add antibody for Ki67. Incubate for 20 minutes in fridge. Wash 1X with Perm buffer and 1X with PBS.
11. Acquire on CytoFLEX.

Laser	405nm					488nm					638nm		
Fluor	Krome Orange	Pacific Blue	V610	V660	V780	FITC	PE	ECD	PC5.5	PC7	APC	APC AF700	APC AF750
Marker	CD45	CD218	CD122		CD127	Ki-67	Ckit		CD335		CD19	CD3	Live/Dead
Clone	30-F11	P3TUNYA	TM-b1		SB/199	B56	ACK2		29A1.4		103	17A2	

Data Acquisition on CytoFLEX*

1. Create new experiment
2. Create plots.
3. Run unstained and single color controls for compensation settings
4. Create gates for NK and T-cells
5. Run the sample on fast.
6. Auto-adjust for scaling.
7. Acquire a minimum of 100,000 events.
8. Save data.
9. Export to FSC
10. Analyze in Kaluza

Conclusions

The current panel was aimed at identifying NK subsets with effector and regulatory properties using C57BL/6 mice. We found $CD335^{pos}CD122^{pos}$ cells among $CD45^{pos}CD3^{neg}CD19^{neg}$ cells in spleen. Among bulk NKs, c-Kit^{pos} cells were observed. Unlike T cells where the majority of cells express CD127 (IL-7Rα), only a subset of NK cells express CD127 and among the c-Kit^{pos} NK cells, we found $CD127^{pos}$ and $CD127^{neg}$ cells. The majority of NKs are $CD218^{pos}$ (IL-18R) and about 25% of splenic NKs are proliferating as determined by Ki67 staining.

Figure legend: here we show the gating strategy used to identify subpopulaions of mouse NK cells, CD3 CD19 double negative cells which are CD335 CD122 double positive can be further characterized using CD127 and the proliferation markers c-kit and Ki-67.

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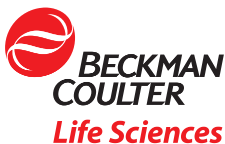

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