

In vitro Pharmacology: Ex Vivo Immunophenotypization

Leukocyte activation and function

Differential cell count and determination of eosinophils and neutrophils within bronchoalveolar lavage fluid (BALF), peritoneal lavage (PL) or whole blood leukocyte population are commonly used methods for measurement of inflammatory response in animal models. Manual cell count is time-consuming and prone to errors due to low number of cells and subjectivity in differentiation.

Fidelta has developed a novel flow cytometric method for the detection of murine granulocytes. The method is applicable in every *in vivo* inflammatory model where identification and count of eosinophils and/or neutrophils in BALF, peritoneal lavage fluid and blood samples is desirable.

Material

- BALF
- Peritoneal lavage fluid
- Whole blood

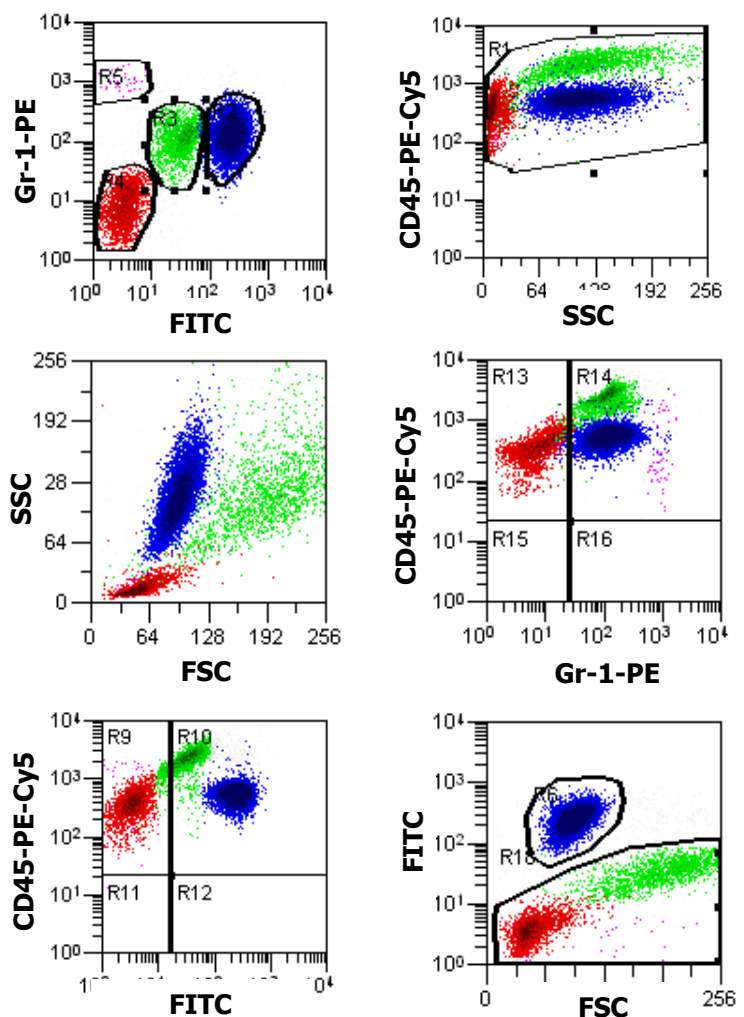
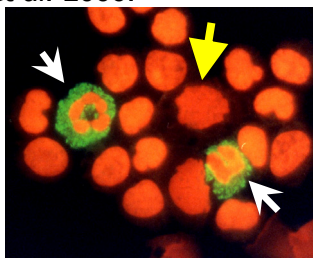
Available read-outs

- Immunophenotypization by flow cytometry
- Absolute cell count
- Differential cell count

BALF cells immunophenotypization - mouse model of chronic lung eosinophilia

A simple 3-color flow cytometry method using FITC, anti-mouse CD45 and anti-mouse Gr-1 antibody provides the possibility for determination of eosinophil (white arrow), neutrophil, monocyte/macrophages (yellow arrow) and lymphocyte subpopulations in BALF, peritoneal lavage fluid and blood. Presented are BALF analysis examples.

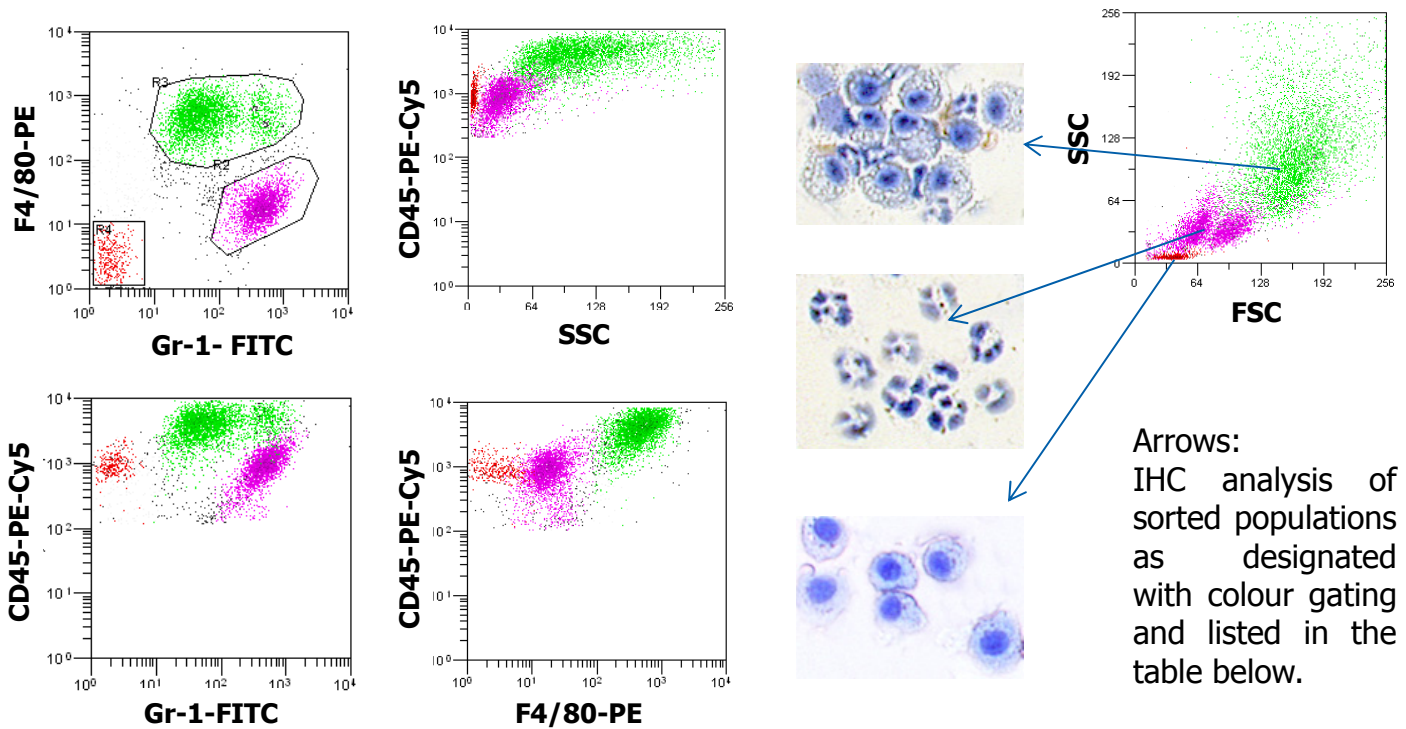
Polanec et al. 2006.



BALF subpopulations				
Region / Gate Colour	Cell type	FITC	CD45	Gr-1
Blue	Eosinophils	+	bright +	From dim+ to bright +
Magenta	Neutrophils	-	bright +	High +
Green	Monocytes/Macrophages	-	bright +	Partially dim+
Red	Lymphocytes	-	bright +	-

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BALF cells immunophenotypization - mouse model of acute lung neutrophilia



Simple 3-color flow cytometry method using anti-mouse F4/80, anti-mouse CD45 and anti-mouse Gr-1 antibody provides a possibility for determination and enumeration of neutrophil, monocyte/macrophage and lymphocyte subpopulations in BALF. Presented are BALF analysis examples.

Polancec et al. 2006.

BALF leukocyte subpopulations				
Region / Gate Colour	Cell type	CD45	Gr-1	F4/80
Magenta	Neutrophils	bright +	High +	-
Green	Monocytes/ Macrophages	bright +	Partially dim+	+
Red	Lymphocytes / Lymphocyte-like leukocytes	bright +	-	-

References

1. Bedner et al. 1999. Cytometry 36, 77.
2. Efthimidiai et al. 1996. Cytometry 26, 75.
3. Leong et al. 2001, J Am Chem Soc. 120,12237.
4. Polancec et al. 2006. ISAC XIII Congress. 160631-p369
5. Polancec et al. 2006. ISAC XIII Congress. 160685-p294