#1056

PD-1-positive population in resting T cells identified using a qualified flow cytometric method

Laïla-Aïcha Hanafi, Philippe Pouliot, Valérie Hébert, Florin Constantin and Jean-François Poulin
Caprion Biosciences Inc, Montreal, Canada.

CAPRION

Abstract

Flow cytometry is an information-rich method for cellular analysis. In the age of immunotherapy the importance of flow cytometry in drug development has increased dramatically. When using flow cytometry to generate data for decision making, it is mandatory that the assays and instruments are well characterized. A critical element in assay optimization is the screening of the monoclonal antibodies. In this poster, we will describe the results obtained with different PD-1 monoclonal antibodies where results and conclusions differed significantly.

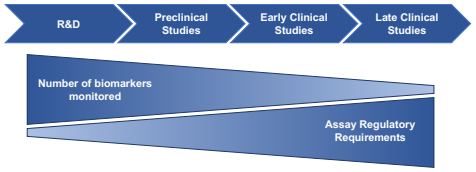
It is generally recognized that the immune checkpoint molecule, programmed cell death protein 1 (PD-1) is expressed at low levels on the surface of resting T and B lymphocytes and is upregulated via signaling through the TCR or B-cell receptors. During the optimization of a PD-1 monitoring flow cytometric method, we observed higher than expected levels of PD-1 expression on resting T cells obtained from healthy donors. The magnitude of this population was both donor and reagent specific.

Five different anti-PD-1 monoclonal antibody clones obtained from different providers were screened (EH12.2H7, EH12.1, eBioJ105, MIH4, D3W4U). They were all titrated to obtain an optimal saturating concentration and then compared. In resting CD4 T cells, the best performance was observed with EH12.2H7 or EH12.1 clones which identified 30-33% of CD4 T cells as PD-1 positive. Clone eBioJ105 staining of the same samples on the same day identified 14 % PD-1 positive CD4 T cells, while clones MIH4 and D3W4U staining indicated no PD-1 expression on CD4 T cells. After *ex vivo* stimulation with SEB, EH12.2H7, EH12.1 and eBioJ105 clones all yielded similar results (76-80% positive CD4 T cells) but fewer positive cells were identified with MIH4 and none with D3W4U clones.

These results illustrate the importance of thorough reagent evaluation in order to achieve a sensitivity coherent with the information-rich promises of flow cytometry.

Introduction

Assay Qualification



Critical Steps of Panel Development

1. **Identify the populations of interest**
   - Choose appropriate markers and gating strategy to identify population of interest.
2. **Identify the markers of interest** (functional readouts)
   - Defining the markers to monitor, their level of expression and co-expression patterns.
3. **Identify the staining procedure**
   - Defined by the markers to reach (extracellular, intra-cytoplasmic, intra-nuclear…)
   - To be optimized according to the matrix (whole blood, PBMC, bone marrow, tissue…).
4. **Select appropriate reagents** (clones, fluorochromes)

In this poster, we will present a case-study demonstrating the importance of selecting the adequate reagents

Clones tested

Clone	Manufacturer	Catalog #
EH12.2H7	Biolegend	329906
EH12.1	BD Biosciences	560795
eBioJ105	eBioscience	12-2799-42
MIH4	BD Biosciences	557946
MIH4	eBioscience	12-9969-42
D3W4U	Cell Signaling	15121

*discontinued

Methods

The marker of interest is **PD-1** investigated on healthy donor cryopreserved PBMC using a surface staining method. To assess anti-PD-1 clones, unstimulated PBMC and SEB-activated PBMC were used to compare 6 different clones from various manufacturers. PD-1 staining was assessed in CD4⁺ and CD8⁺ T cells.

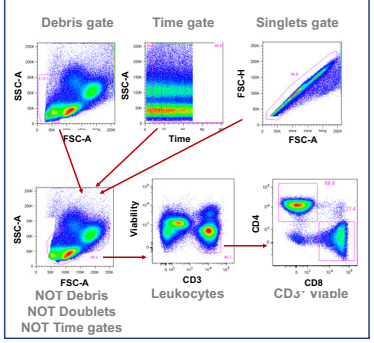
STAINING
FcR block
10 min RT
↓
Antibody
30 min 4ºC
↓
PFA 0.5%
30 min 4ºC

SAMPLES

SEB
HD PBMC stimulated with 25ng/mL of SEB for 4D

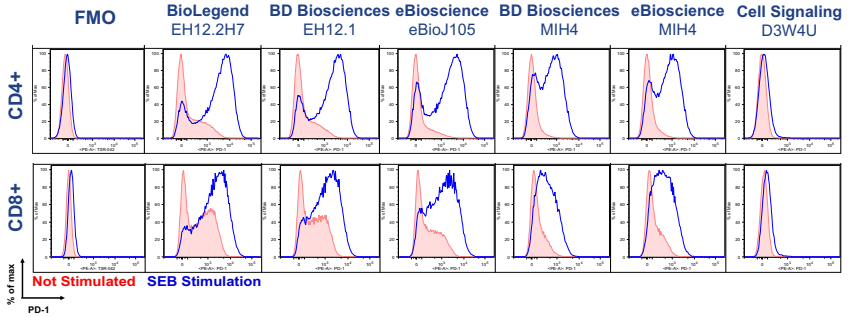
Fresh
HD PBMC stained directly after thawing

GATING STRATEGY



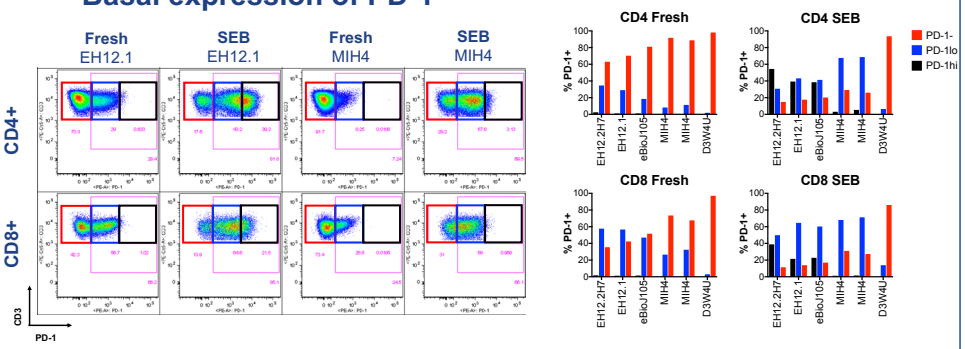
Results

Clone Screening



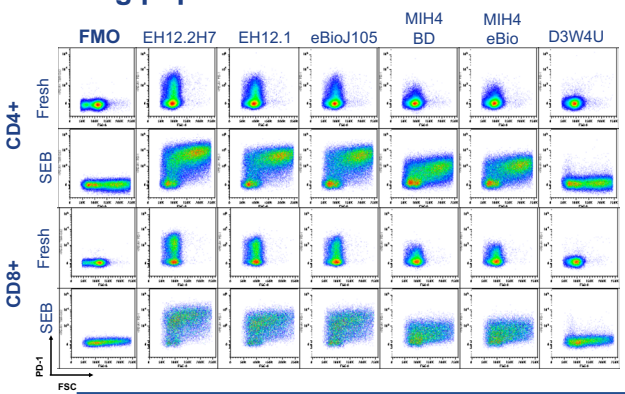
All antibody clones are not created equal. The same cells stained with 6 different antibodies (different clones and different manufacturers) display different staining patterns.

Basal expression of PD-1



EH12.2H7 and EH12.1 have the best resolution and higher signal when compared to the other clones. This leads to the identification of a larger range of PD-1 staining levels in both stimulated and unstimulated PBMC.

Blasting population and PD-1 induction



To confirm the specificity of all anti-PD-1 clones, the staining was observed in non-stimulated and stimulated cells. The staining is consistent with PD-1 induction mainly in stimulated cells (with a larger size as observed by increased FSC). All cells with increased size upregulate PD-1 (except with D3W4U clone).

Conclusions

- **EH12.2H7**, **EH12.1** and **eBioJ105** clones have a better staining resolution and lead to PD-1 staining with a large range of staining.
- **MIH4** (from BD Biosciences and Biolegend) has similar staining profile, with PD-1 positive staining in stimulated cells, but cannot detect PD-1 expression at basal level.
- **D3W4U** clone from Cell Signaling was unable to detect PD-1 expression both in activated and freshly-thawed cells.