A comprehensive characterization of regulatory T cells using BD Rhapsody™ Single-Cell Analysis System

Simultaneous measurement of surface protein and mRNA expression enables the identification of distinct subsets of regulatory T cells

Features

- BD® AbSeq and BD Rhapsody™ Human Immune Response Panel enable sensitive and specific resolution of markers that define major regulatory T cell subsets
- Integration of high-parameter protein and gene expression analysis allows refined resolution of putative differentiation states of regulatory T cells

Regulatory T cells (Tregs) play an essential role in maintaining immune homeostasis and their alterations can have many implications including tumor progression or onset of autoimmune diseases. Tregs are not a functionally homogenous population and can be subgrouped based on the expression of CD45RA and HLA-DR as naïve or activated Tregs, respectively. The use of either high-parameter protein analysis or single cell RNA sequencing has further revealed additional subgroups representative of intermediate states of Treg differentiation. The advent of single cell multiomic technologies offers the unprecedented ability to perform an even deeper cell characterization by integrating analysis of mRNA and proteins from the same individual cells. In this study, we utilized BD Rhapsody™ Human Immune Response Panel and BD® AbSeq to further dissect Treg subsets and identify putative cell differentiation trajectories. Twenty-two proteins including stimulatory/inhibitory receptors and cytokine/chemokine receptors and 399 mRNA transcripts were analyzed at a single cell level.

BD

Figure 1

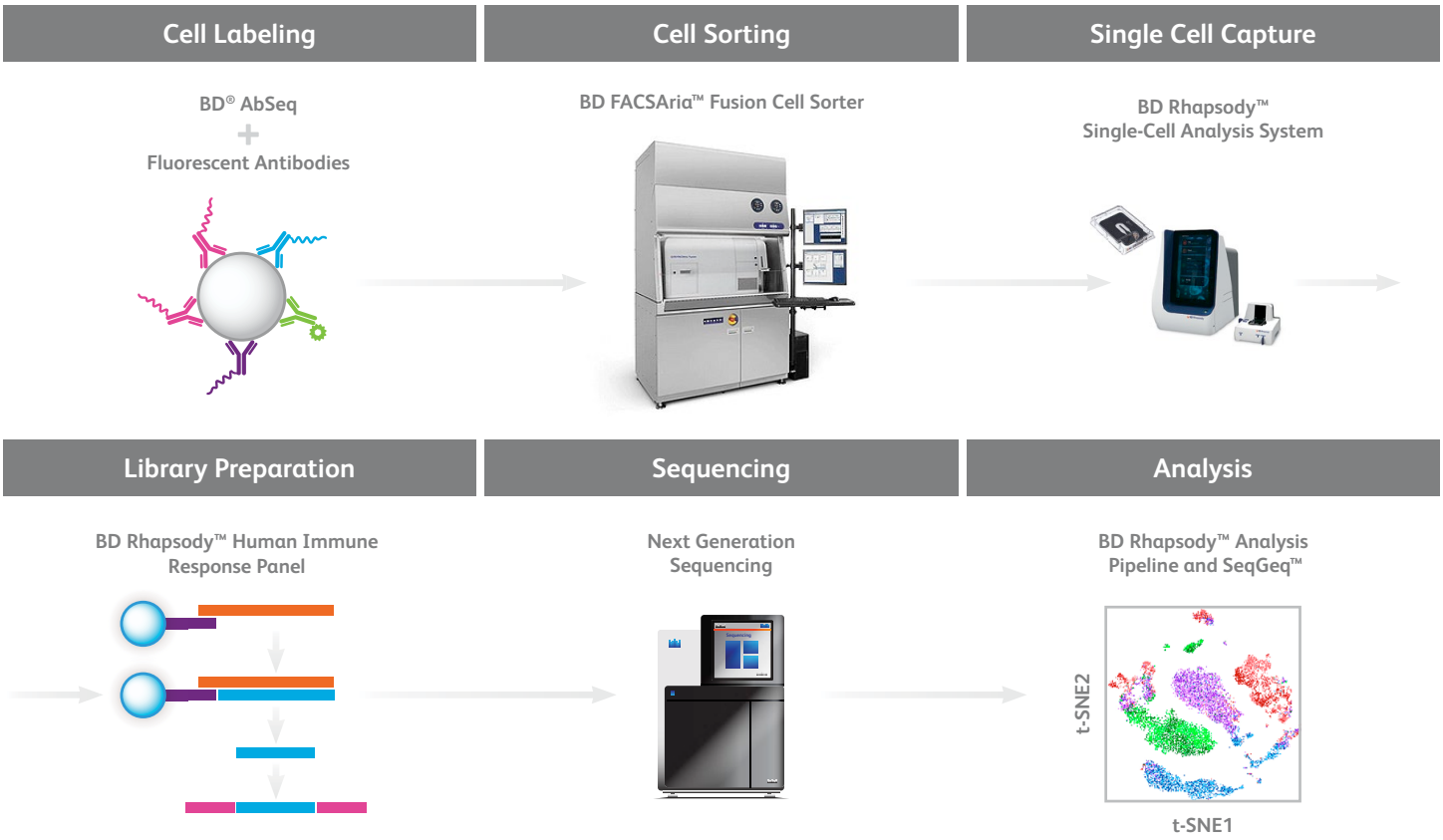

Figure 1. Experimental workflow using the BD Rhapsody™ Single-Cell Analysis System

Peripheral blood mononuclear cells (PBMCs) from a healthy donor were co-stained with fluorochrome conjugated antibodies for cell sorting and BD® AbSeq antibodies for downstream sequencing-based protein detection. The sample was sorted for $CD4^+CD25^+CD127^{low/-}$ Tregs and 5,000 sorted Tregs were loaded onto the BD Rhapsody™ Single-Cell Analysis System for single cell capture. After sample retrieval, BD® AbSeq and mRNA (BD Rhapsody™ Human Immune Response Panel) libraries were prepared for sequencing. The sequencing results were analyzed with the BD Rhapsody™ Analysis Pipeline and SeqGeq™.

We first assessed the ability of BD® AbSeq to clearly identify major Treg subsets previously defined using flow or mass cytometry. The result shows that BD® AbSeq enabled a clear resolution of antigens expressed over a continuum such as CD45RA and HLA-DR, which define naïve and activated Tregs respectively (Figure 2A). We then measured the expression of selected markers within the $CD45RA^+HLADR^-$ naïve cells (Figure 2A, red), $CD45RA^-HLA\text{-}DR^+$ activated cells (Figure 2A, blue) and $CD45RA^-HLA\text{-}DR^-$ cells (Figure 2A, green). In agreement with previous reports, CD31 was exclusively detected in a small subset of $CD45RA^+HLADR^-$ naïve Tregs (Figure 2B), representing recent thymic emigrants (RTEs). On the other hand, CD95 was expressed in both $CD45RA^-HLA\text{-}DR^-$ and $CD45RA^-HLA\text{-}DR^+$ activated populations (Figure 2B), consistent with previous report that identified $CD95^+$ apoptosis-sensitive cells within activated Tregs. Distinct expression patterns were also observed for the chemokine receptor CD185 (CXCR5) and the inhibitory receptor CD279 (PD-1), as shown in Figure 2B.

Figure 2A

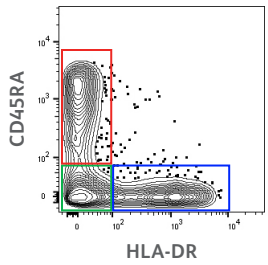

Figure 2B

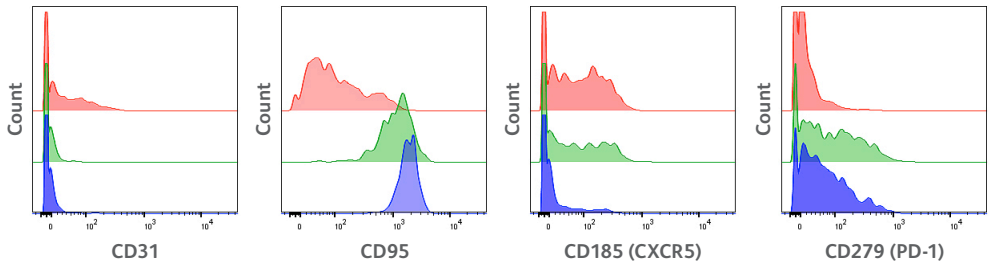

Figure 2. Phenotypic characterization of Tregs cells with BD® AbSeq

A. Contour plot of CD45RA and HLA-DR surface protein expression on Tregs. **B.** Expression of selected markers on different Treg subsets, $CD45RA^+HLADR^-$ naïve cells (red), $CD45RA^-HLA\text{-}DR^-$ cells (green), and $CD45RA^-HLA\text{-}DR^+$ activated cells (blue).

While bi-variate plots and histograms are useful to assess expression patterns of limited number of markers, unsupervised high-dimensional data analysis makes it possible to assess expression patterns of high number of proteins and genes. Thus, we used unsupervised high-dimensional data analysis to refine the identification of Treg subsets. The algorithm Monocle was used to perform single cell trajectory analysis and define transitional states of differentiation processes. Based on the expression of 22 protein and 399 mRNA targets, eight distinct states were identified, likely representing distinct differentiation states of Tregs (Figure 3A). We then performed differential protein and gene expression analysis to reveal the identity of Tregs that belong to each state. The single cell heatmap in Figure 3B displays selected proteins and genes differentially expressed across the eight states. Cells belonging to each state were arranged, from left to right, based on progressive loss of CD45RA and upregulation of HLA-DR, to represent a progression from naïve to activated cells (Figure 3B). This analysis facilitated the identification of unique signatures defining putative transitional states of Treg differentiation. For example, three distinct subsets of Tregs expressing high, medium and low levels of CD45RA (Clusters 7, 6, 5) were identified. Such dissection of CD45RA^{+} Tregs could not be accurately achieved by subjective manual gating (Figure 2A), demonstrating the utility of unsupervised data analysis. In addition to the differential levels of CD45RA expression, these three states could be further discriminated because CD31 is exclusively expressed in state 7 cells, consistent with the signatures of RTEs. On the other hand, the exclusive expression of CD95 and concurrent downregulation of CD45RA in state 5, may suggest a transition from naïve to apoptosis-sensitive and activated cells. Overall, unique signatures for each state could be defined by the virtue of the differential gene and protein expression analysis performed at the single cell level.

Figure 3A

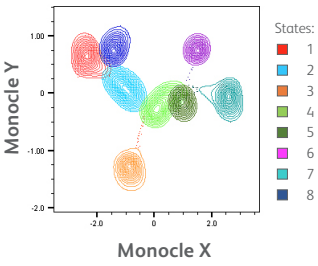

Figure 3B

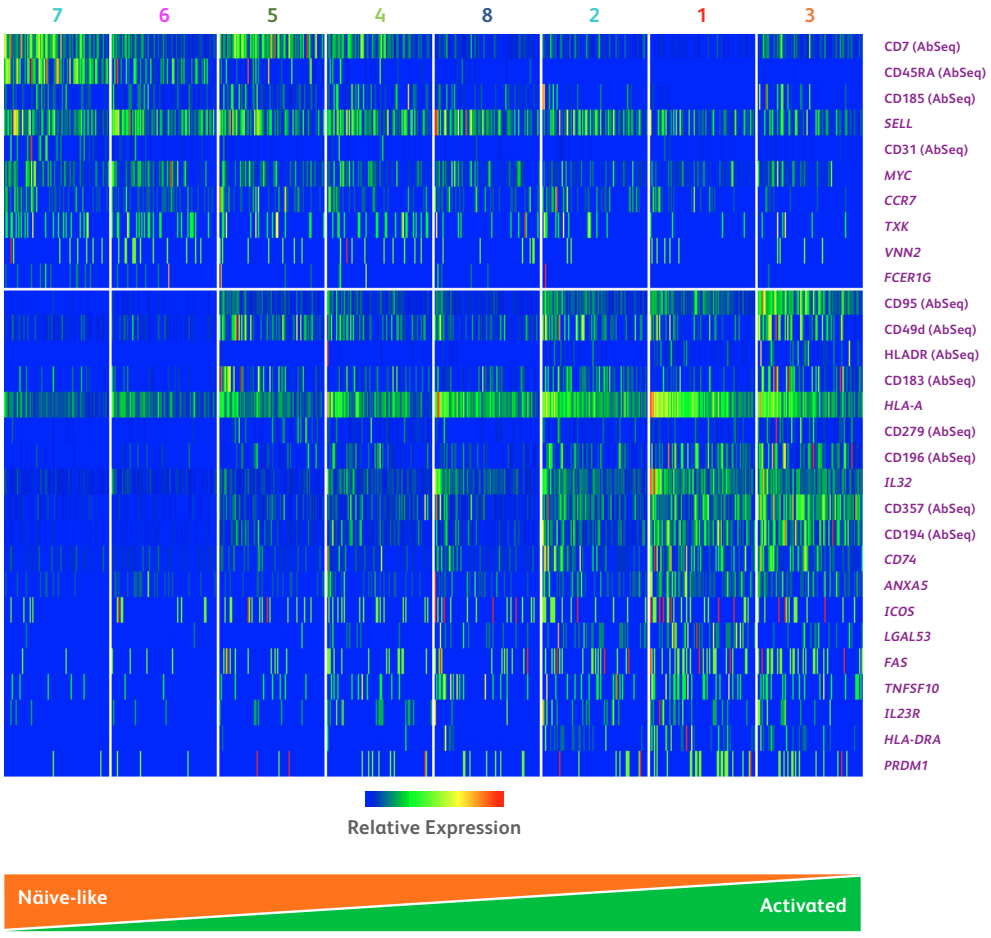

Figure 3. Characterization of Treg differentiation by Monocle analysis

A. Monocle algorithm identified eight distinct states within the total Treg cell population. **B.** Heatmap showing expression of different proteins (AbSeq) and genes in distinct Treg states.

Altogether, we have demonstrated the sensitivity and specificity of BD® AbSeq for the identification of Treg subsets based on known surface marker signatures. The combination of single cell multiomics and high-dimensional data analysis further enabled a clear resolution of distinct subsets of Tregs defined by unique protein and gene expression signatures, likely representing differentiation states of Tregs.

Ordering information

Systems and software
Description
BD Rhapsody™ Single-Cell Analysis System
BD FACSAria™ Fusion Cell Sorter
SeqGeq™

Reagents
Description
BD Rhapsody™ Targeted mRNA and AbSeq Reagent Kit
BD® AbSeq
BD Rhapsody™ Human Immune Response Panel

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

23-22159-00

BD Life Sciences, San Jose, CA, 95131, USA

bdbiosciences.com