In-depth characterization of human regulatory T cells using single-cell CITE-Seq and high-parameter flow cytometry

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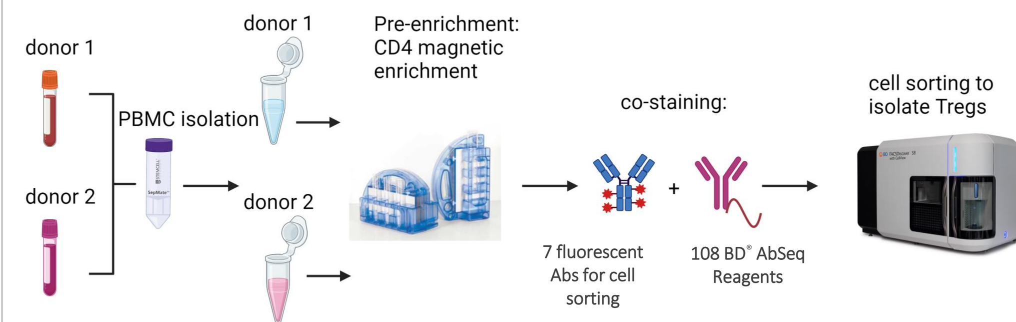
Abstract

Regulatory T cells (Tregs) are an essential population of T cells that are critical for maintenance of peripheral tolerance. Imbalanced Treg frequency or dysregulation of Treg function can lead to the development of autoimmune diseases. Thus, the modulation of Treg frequency or function has become an important therapeutical approach to treat autoimmune disorders, such as colitis and diabetes. Despite increased study, whether distinct populations of Tregs with unique functions exist is still unclear. Here, we used a combination of single-cell CITE-Seq (scCITE-Seq) and high parameter flow cytometry to resolve the heterogeneity of Tregs in humans. Using magnetic cell enrichment in conjunction with fluorescence-activated cell sorting (FACS), we isolated Tregs and performed downstream scCITE-Seq. By using more than a hundred antibody-oligo conjugates, we identified distinct populations of Tregs, uncovered the proteomics signature associated with these different populations, and revealed donor-to-donor variability in the human peripheral Treg compartment. The protein expression pattern and antigen density on Tregs revealed by scCITE-Seq correlated to that of high dimensional flow cytometry. Our analysis segregated Treg subsets into distinct populations with unique proteomic signatures ranging from naïve, activated to memory Tregs. These findings improve our understanding of the heterogeneity and differentiation of Treg cells and provide a single-cell proteomic atlas for dissecting the complex roles of Tregs in health and disease.

Methods



- PBMCs were isolated from whole blood obtained from healthy volunteer donors using Ficoll™ gradient centrifugation
- CD4 T cells were selectively enriched from PBMCs using the BD IMag™ Human CD4 T Lymphocyte Enrichment Set-DM
- Enriched CD4 T cells were co-stained with fluorescent antibody panel for cell sorting and 108 BD® AbSeq Reagents for assessment of protein expression
- Co-stained cells were processed for cell sorting using the BD FACSDiscover™ S8 Cell Sorter
- CD4 FITC and CD4 BUV395 were used to label two donors to allow simultaneous isolation of donor specific CD4+ cells
- BD® AbSeq Reagents:

CD10	CD49f	CD69	CD14	CD43	CX3CR1	CD119	CD196
CD36	CD122	CD80	CD146	CD45	CD11a	CD11b	CD110
CD58	CD18	CD85g	CD15	CD45RA	CD49b	CD126	CD329
CD59	CD29	CD85j	CD16	CD45RO	CD49d	CD13	CD197
CD94	CD132	CD95	CD16b	CD46	CD49E	CD134	CD115
TCRgd	Integrin B7	CD96	CD19	CD47	CD33	CD38	CD31
CD181	CD162	CD98	CD2	CD5	CD35	CD39	CD27
CD182	CD268	CD99	CD21	CD62L	CD360	CD40	CD103
CD183	CD270	CD56	CD210a	CD62p	CD366	CD41a	CD324
CD184	CD272	Invariant NK	CD212	CD7	CD371	CD42b	CD195
CD185	CD278	CD335 (NKp46)	CD226	CD71	CD9	TIGIT	
CD192	CD279	CD337 (NKp30)	CD235ab	CD73	HLA-A,B,C	KLRG1	
CD193	CD294	CD101	CD24	CD8beta	HLA-DR	R-PE	
CD194	CD314	CD102	CD26	CD28	TCRab	CD152	

Results (1)

Figure 1

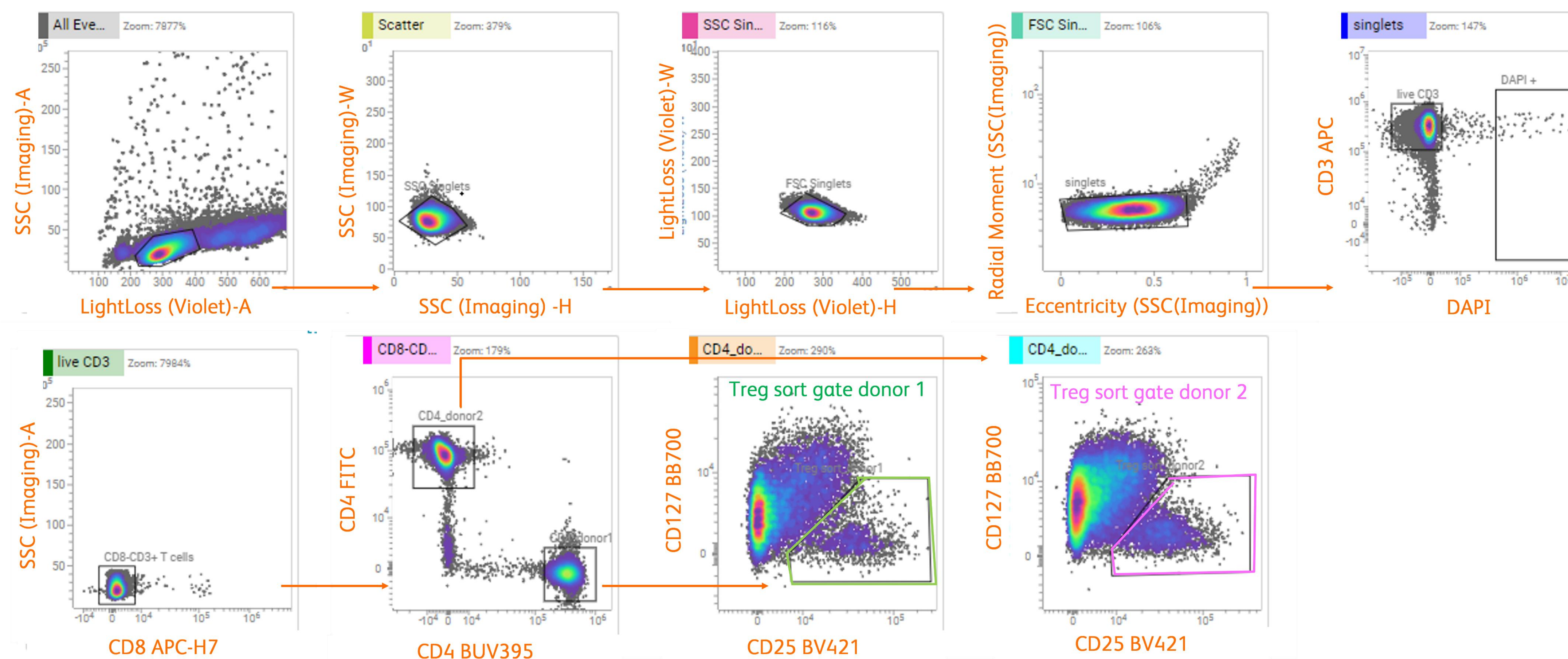


Figure 1. Flow cytometric sorting strategy for Treg isolation. Representative flow cytometry plots demonstrating the gating strategy for the isolation of regulatory T cells (Tregs) from two donors. Flow cytometry panel included: CD3 APC, CD8 APC-H7, CD4 FITC, CD4 BUV395, CD127 BB700, CD25 BV421, DAPI. BD CellView™ Image Technology on the BD FACSDiscover™ S8 Cell Sorter allowed investigation of cell image features (first four plots) including radial moment and eccentricity for doublet discrimination.

Figure 2

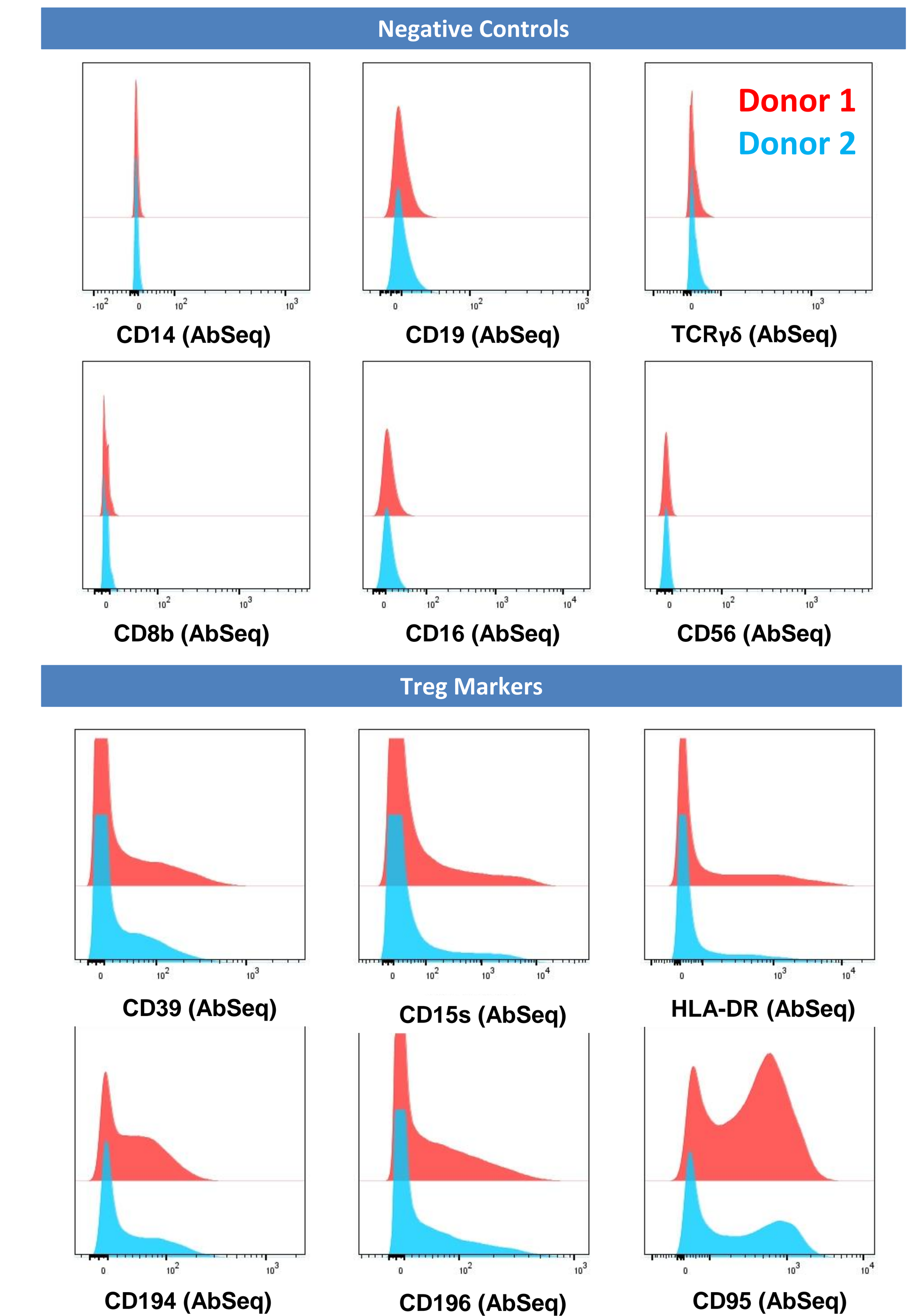


Figure 2 . Assessment of AbSeq protein expression in isolated Tregs. Representative data showcasing assessment of donor variability in human peripheral Treg compartment using AbSeq protein expression evaluated for several key internal negative control markers and Treg cell markers.

Results (2)

Figure 3

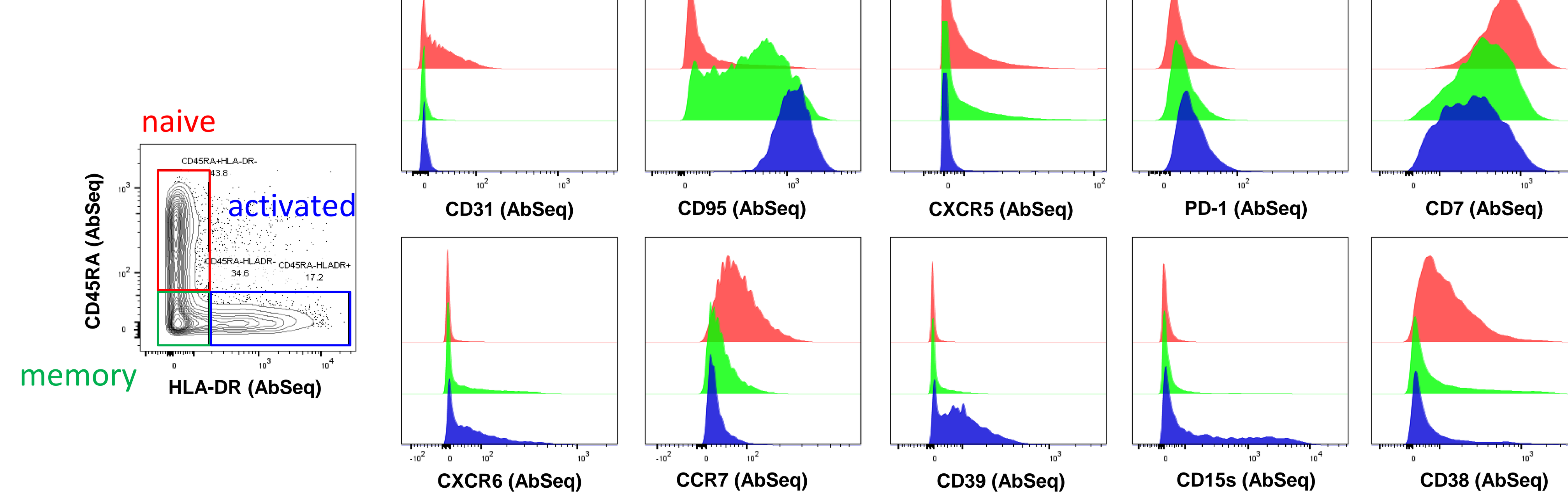


Figure 3. Cellular heterogeneity of regulatory T cells characterized by expression of CD45RA and HLA-DR. Representative histogram overlays of naïve, activated and memory Treg subpopulations highlight CD31expression exclusively in a small subset of CD45RA+ HLADR- naïve cells representing recent thymic emigrants (RTEs). CD95, PD-1 and CD39 are expressed on activated Tregs at higher levels as compared to memory and naïve subsets. Increased expression of CCR7 is observed in naïve Tregs. Representative data from one donor.

Figure 4

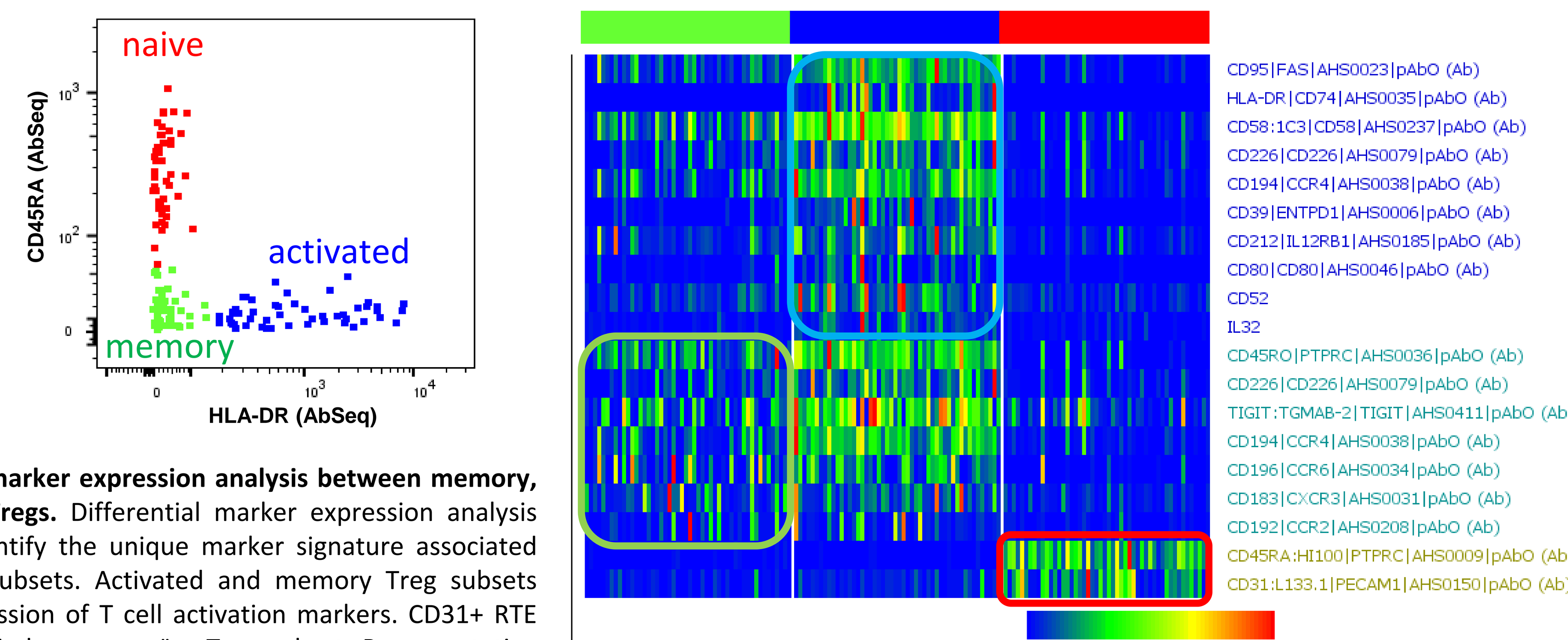


Figure 4. Differential marker expression analysis between memory, naïve and activated Tregs. Differential marker expression analysis was performed to identify the unique marker signature associated with the three Treg subsets. Activated and memory Treg subsets show a gradient expression of T cell activation markers. CD31+ RTE population was identified among naïve Treg subset. Representative data from one donor.

Results(3)

Figure 5

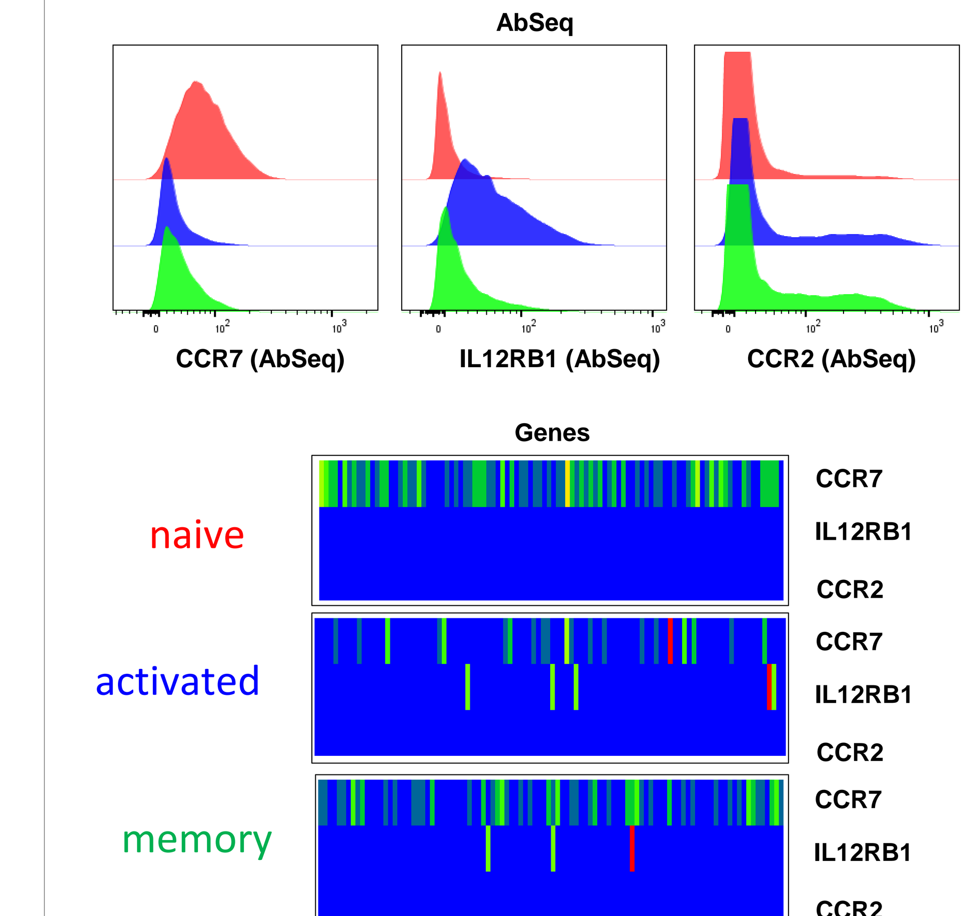


Figure 5. AbSeq data provide additional proteomic information not captured by mRNA analysis. Representative data of protein expression of markers (top) and mRNA expression (bottom) of markers differentially expressed on Treg subsets on the same cells. Representative data from one donor.

Conclusions

- Using 108 BD® AbSeq Antibody-Oligo Conjugates, we identified and characterized three different subsets of Tregs: naïve, memory and activated subpopulations.
- We uncovered the proteomics signature associated with different subpopulations and revealed donor-to-donor variability in the human peripheral Treg compartment.

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