Poster#

Evaluation of Activation and Homing Markers on Regulatory T cells using the 12-Color BD FACSLyric™ Flow Cytometer

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Introduction

Deeper understanding of regulatory T cell (T_{reg}) biology is critical for the successful development of therapeutic and diagnostic applications. T_{reg} heterogeneity in terms of phenotype, function, and distribution is widely documented, thereby making a more detailed characterization of these cells critical for downstream applications. Several reports have identified distinct T_{reg} immunophenotypic signatures correlating with different biological functions including activation and tissue homing[1-9]. Furthermore, others have combined high parameter mass cytometry approaches with high dimensional data analysis tools to reveal additional heterogeneity within the human T_{reg} compartment[10,11]. Here, we report the development and utility of a more conventional flow cytometry based assay to explore T_{reg} heterogeneity. We developed an 8-color backbone plus 4-color drop-in modular flow cytometry assay to identify and characterize T_{reg} subsets in greater depth. Specifically, we developed two different T_{reg} characterization drop-in panels to explore two critical facets of T_{reg} biology:

- Homing (CD31, CD183, CD194 and CD196)
- Activation (PI16, CD39, CD147 and HLA-DR)

The 8-color backbone panel enabled clear identification of $CD3^+CD4^+CD127^{low/neg}CD25^+FoxP3^+$ T_{reg} cells, and further categorized as $CD45RA^+$ (naïve) and $CD45RA^-$(effector) subsets. The inclusion of CD15s and CD161 in the backbone panel enabled identification of functionally suppressive effector[1] and/or pro-inflammatory cytokine-secreting [2,3] T_{reg} cells within the heterogeneous $CD45RA^-$ population, respectively.

The markers in the homing drop-in panel helped us identify recent thymic emigrants within the naïve T_{reg} cell population, and $T_{effector}$-like T_{reg} subsets based on a gating strategy that has been shown previously[4]. Simultaneous assessment of the markers in the activation panel enabled us to study the correlation between them. Our 12-color activation panel recapitulates some of the earlier observations and more importantly expands the knowledge of the interplay of activation markers with the inclusion of CD15s and PI-16.

Overall, our results highlight an interesting interplay between different T_{reg} markers, their putative biological function, and the underlying heterogeneity within the T_{reg} population. Altogether, this modular 12-color flow cytometry assay presents a new approach to enable deeper and more comprehensive analysis of different aspects of T_{reg} biology in a simplified workflow.

Methods

Fresh whole blood from healthy donors was used for peripheral blood mononuclear cell (PBMC) preparation. The PBMCs were surface stained using BD Horizon™ Brilliant Stain Buffer (Cat. No. 659611) with the antibodies listed below. For intracellular (IC) staining of FoxP3, BD Pharmingen™ Transcription Factor (TF) Buffer Set (Cat. No. 562574) was used. Samples were analyzed on a 12-color BD FACSLyric™ flow cytometer, and data analysis was performed using BD FACSuite™ software.

8-Color Backbone Panel

	Fluorochrome	Target Antigens
1	BV421	CD25
2	BV510	CD15s
3	FITC	CD4
4	PE	CD161
5	BB700	CD127
6	PE-Cy7	CD45RA
7	AF647	FoxP3
8	APC-H7	CD3

4-Color Homing Drop-In Panel

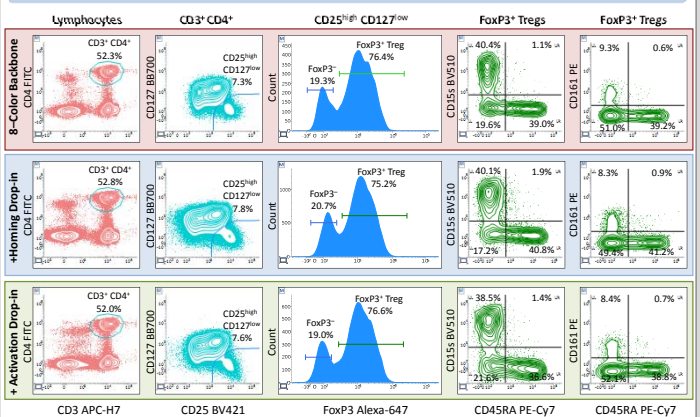
	Fluorochrome	Target Antigens
1	BV605	CD31
2	BV711	CD183
3	BV786	CD194
4	APC-R700	CD196

4-Color Activation Drop-In Panel

	Fluorochrome	Target Antigens
1	BV605	PI16
2	BV711	CD39
3	BV786	CD147
4	APC-R700	HLA-DR

12-color modular flow cytometry panel for T_{reg} identification (8-color backbone panel) and characterization (4-color activation/homing drop-in panels)

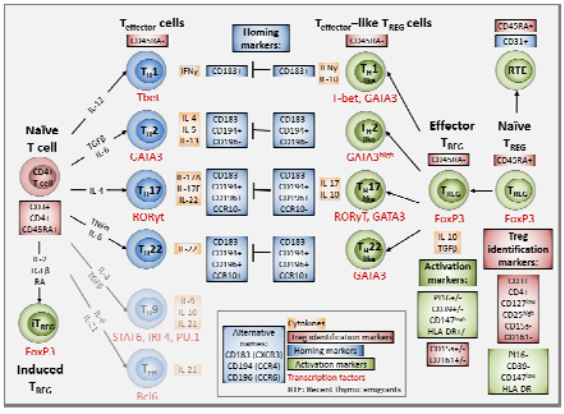
Results 1. Resolution of the 8-color plus 4-color modular panel

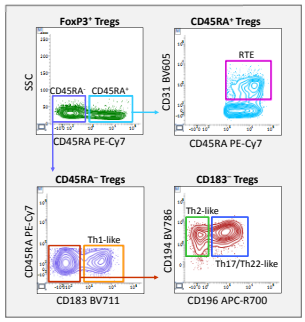


Resolution of the 8-color backbone panel for T_{reg} identification was not impacted by the addition of 4-color T_{reg} homing or activation drop-in panels significantly:

The 8-color backbone panel helps identify T_{reg} cells as $CD3^+$ $CD4^+$ $CD127^{low}$ $CD25^{high}$ $FoxP3^+$ cells, and further characterize them as naïve ($CD45RA^+$), effector suppressive ($CD45RA^-$ $CD15s^+$)[1] or pro-inflammatory cytokine secreting ($CD45RA^-CD161^+$) T_{reg} cell subsets [2,3]. There is minimal impact in resolution and population percentage of major T_{reg} subsets upon addition of 4-color homing or activation drop ins.

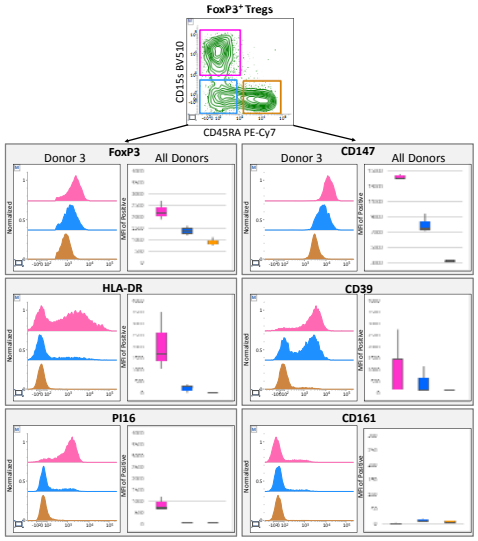
Results 2. Evaluation of the modular 12-color T_{reg} homing panel

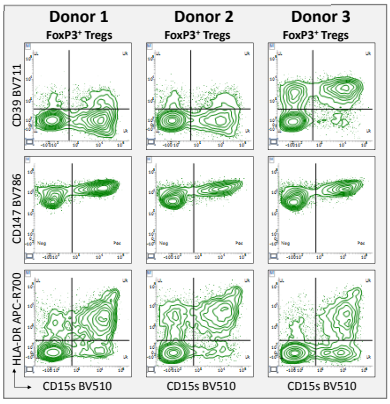




The 4-color homing drop-in panel helps identify recent thymic emigrants (RTE) and $T_{effector}$-like subsets within the T_{reg} population

Results 3. Evaluation of the modular 12-color T_{reg} activation panel





The 12-color T_{reg} activation panel reveals donor specific differences in the interplay between activation markers

Population		% Parent Donor 1	% Parent Donor 2	% Parent Donor 3
CD39+	CD15s-	5.1	5.5	19.6
	CD15s+	12.7	8.5	35.2[12]
CD39-	CD15s-	37.0	55.9	39.6
	CD15s+	45.1	30.1	5.7
CD147+	CD15s-	43.2	62.3	59.9
	CD15s+	56.8	37.7	40.1
HLA-DR+	CD15s-	12.1	15.5	6.2
	CD15s+	47.0	32.1	26.6
HLA-DR-	CD15s-	31.0	46.6	53.6
	CD15s+	9.9	5.8	13.7

Min / Max

Correlation between CD15s expression and other markers:

The results indicate a correlation between high levels of CD15s and FoxP3, and activation markers HLA-DR, PI16, CD147 and CD39. No correlation with CD15s was seen for CD161.

Conclusions

- The 8-color backbone panel reported here enables high resolution identification of the T_{reg} compartment and subpopulations therein.
- Supplementation of the backbone with 4-color drop-in panel(s) for activation or homing markers enables deeper characterization of T_{reg} subsets without any significant impact on the resolution of parent populations.
- Addition of the 4-color homing drop-in panel enabled identification of recent thymic emigrant T_{reg} cells within the naïve T_{reg} population as well as T-effector like subsets within the effector T_{reg} population as reported previously[4].
- Addition of the 4-color activation drop-in panel enabled identification of a previously reported highly immunosuppressive subpopulation expressing high levels of CD15s, HLA-DR, PI-16, CD147 and CD39 within $CD45RA^-$ effector T_{reg} population[1-3, 5-9]. Furthermore, our 12-color assay was able to pick up variations in donor-specific counts of T_{reg} subsets.
- Overall, by combining multiple markers in a single tube this 12-color modular flow cytometry assay on BD FACS Lyric system enables researchers to study different facets of T_{reg} biology, such as homing, activation, proliferation, maturation, stability in the context of a workflow optimized for ease of use (time and cost).

References

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