Epigenetic control of human regulatory T cells (Tregs) during HIV-1 infection

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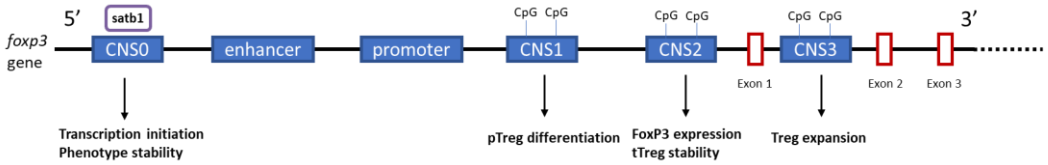
Background

Tregs are immunosuppressive cells that are essential for the maintenance of immune homeostasis and prevention of autoimmune diseases, and the transcription factor FoxP3 is known as the master regulator of these cells. Tregs can originate either from the thymus, or in the peripheral blood under inflammatory conditions. Epigenetic modulation, more specifically demethylation of *foxp3* locus is required for stable FoxP3 expression and suppressive functions of Tregs. Conserved non-coding sequences (CNS) regulate *foxp3,* controlling either thymic (CNS2) or peripheral (CNS1) origin of Tregs, overall phenotype stability (CNS0, CNS3), as well as transcription initiation (CNS0, proximal promoter). HIV infection is characterized by increased Treg frequencies and functions which contribute in HIV-related immune dysfunction. However, the epigenetic control of Tregs during HIV infection is understudied.



Adapted from Mohr et al., 2018

Figure 1. Non-coding regions involved in FoxP3 induction and stable expression in Tregs.

Methods

PBMCs from different cohorts of individuals

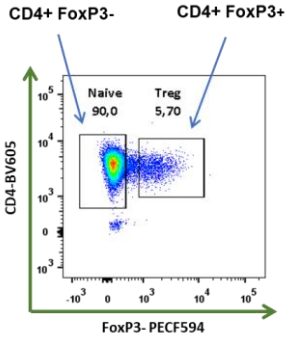
CD4 T cell purification by negative selection with microbeads (STEMCELL)

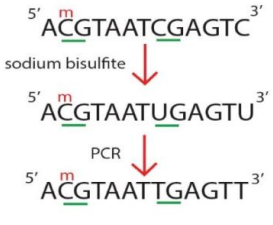
Sort Treg population based on CD4 and FoxP3 expression

Genomic DNA extraction

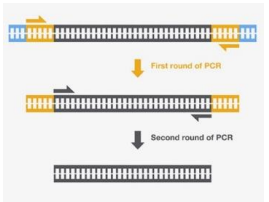
Ex vivo phenotypic characterization of human Tregs by multi-parameter flow cytometry

Healthy subject	HIV- CD4 >400 c/mm³ N = 7	**ART-treated**	VL=U, ART+ ≥3 yrs CD4 > 400 c/mm³ N= 7
Acute Non-Treated	HIV+ <2 yrs, no ART CD4 >300 c/mm³ N= 14	**Elite Controller**	VL= UD, no ART CD4 > 400 c/mm³ N= 11
Chronic Non-Treated	HIV+>2 yrs, no ART CD4 >300 c/mm³ N= 8	**Immunological Controller**	VL<5000/ml, no ART CD4 >400 c/mm³ N= 9





Bisulfite conversion (QIAGEN bisulfite kit)



Nested PCRs using specific primers for bisulfited DNA

Methylation sequencing (MiSeq)

Bioinformatics analysis of *foxp3* methylation status on around 25000 sequences per region from each individual

Figure 2. Flowchart for the phenotypic characterization and methylation analysis of the study samples

Increase in Treg frequencies in both acute and chronic HIV infection. ART-treated individuals and HIV controllers were able to maintain Treg frequency similar to healthy levels.

In acute phase, the increase in FoxP3+ cell frequency was due mainly to their peripheral generation (Helios-), while in the chronic phase, the higher FoxP3+ cell frequency derived from both the periphery and the thymus. ART resulted in a decrease in recent thymic output of FoxP3+ CD4 T-cells.

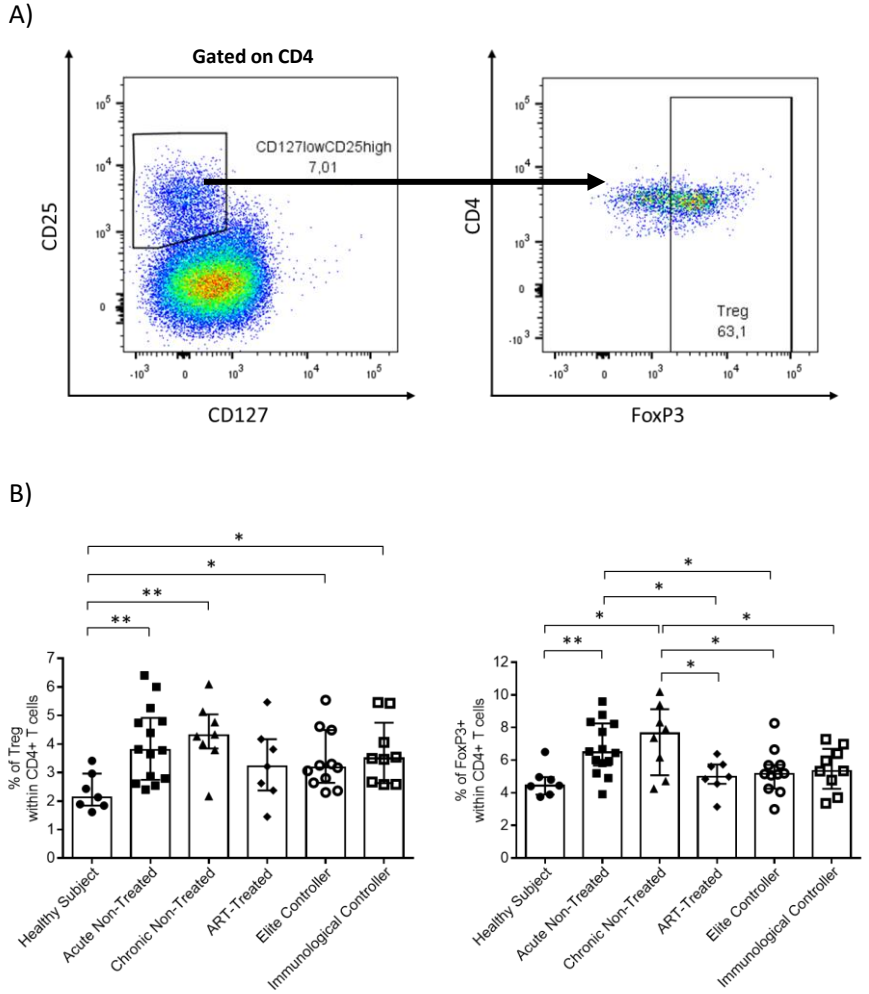


Figure 3. Gating strategy (A) and frequency of Tregs (CD4+CD25+CD127-FoxP3+) and CD4+FoxP3+ cells (B) in groups with different clinical outcomes.

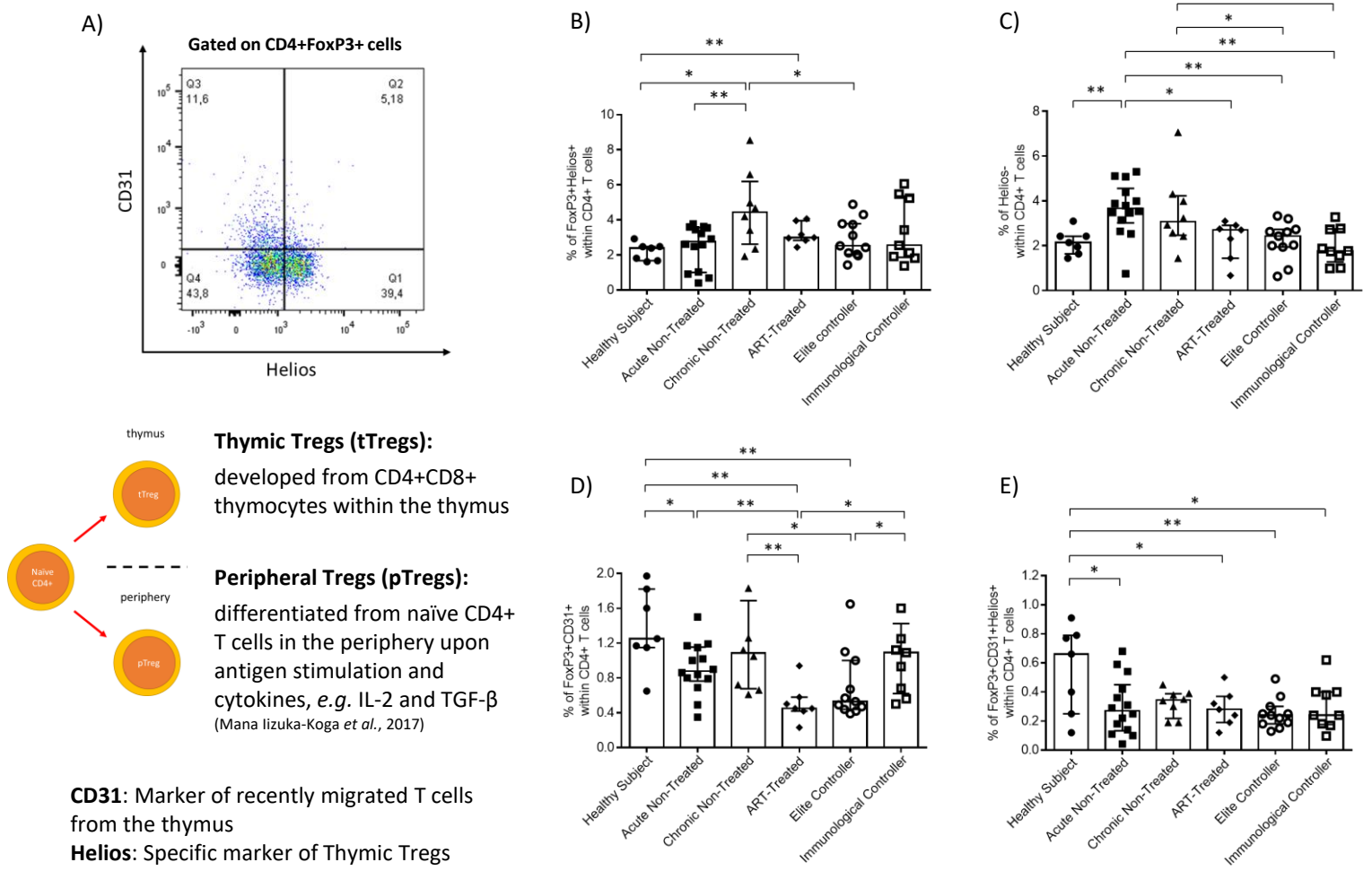
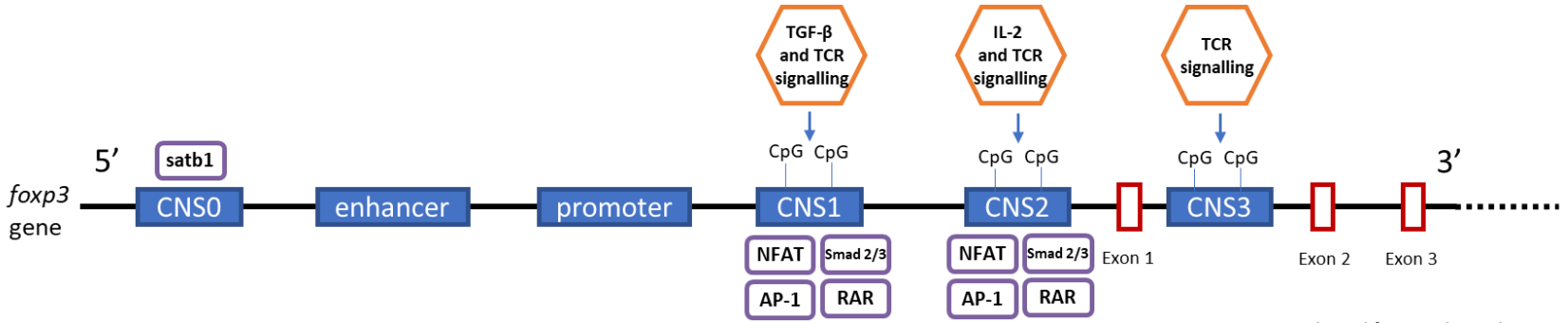


Figure 4. Gating strategy (A) and frequency of FoxP3+ cells of thymic (Helios+) (B) and peripheral (Helios-) (C) origin, as well as recent thymic emigrants (D), and recently migrated thymic FoxP3+ cells (E).

No difference in methylation status of CNS0, proximal promoter, and enhancer region among study groups



Adapted from Mohr et al., 2018

CNS0

- Interacts with Satb1, a global genome organizer that induces both transcriptional regulation and epigenetic regulation (Kitagawa et *al.,* 2016)
- Satb1 binds to CNS0 and initiates activities of the other CNS elements that leads to FoxP3 expression (Kitagawa et *al.,* 2016)

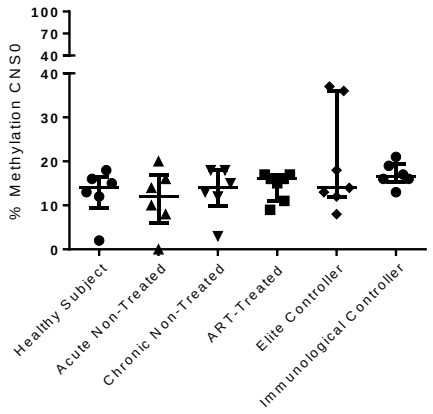
Enhancer

- Contains CpG-rich region, highly demethylated in nTregs but methylated in TGFβ-induced Tregs (Lal *et al.,* 2009)

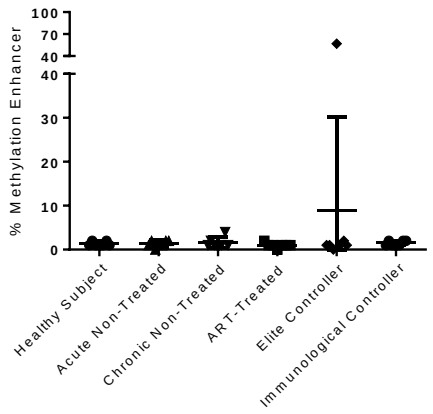
Proximal promoter

- Has weak transcriptional activity, enhanced by the binding of several transcription factors to the cis regulatory elements located in the introns (Sekiya *et al.,* 2016)
- Demethylation is a prerequisite for stable FoxP3 expression and subsequent suppressive phenotype of Tregs (Janson *et al.,* 2008)

A)



C)



B)

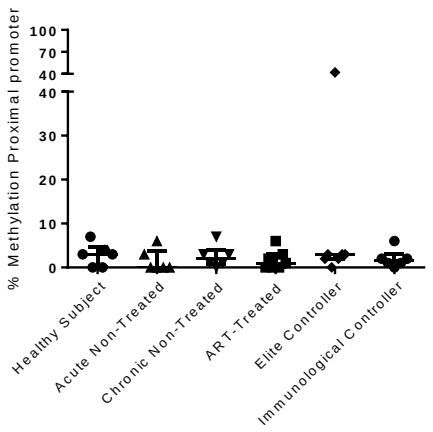


Figure 5a. Foxp3 methylation status in sorted CD4+FoxP3+ cells in CNS0 (A), and enhancer (B) proximal promoter (C) region .

Methylation levels in CNS1 decreased during both acute and chronic infection, while HIV elite controllers had similar CNS1 methylation levels to healthy individuals

CNS1

- Controls **peripheral (not thymic)** induction of FoxP3 expression (Sekiya *et al.,* 2016)
- Deletion of CNS1 abrogates pTreg differentiation (Mohr et al., 2018)

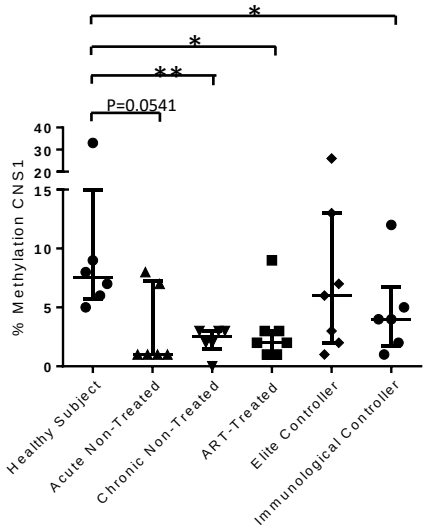
CNS2

- Required for FoxP3 expression and maintenance of **thymic Tregs** (Floess et al., 2007)
- Fully demethylated in tTregs and methylated in non-Tregs, which contributes to a sustained expression of FoxP3 (Takashi Sekiya et al., 2016)

CNS3

- Contain binding sites for c-Rel and plays important roles in the differentiation of both tTregs and pTreg cells (Takashi Sekiya *et al.,* 2016)
- Deletion of CNS3 results in significant tTreg number reduction (Mark Angel *et al.,* 2013)

D)



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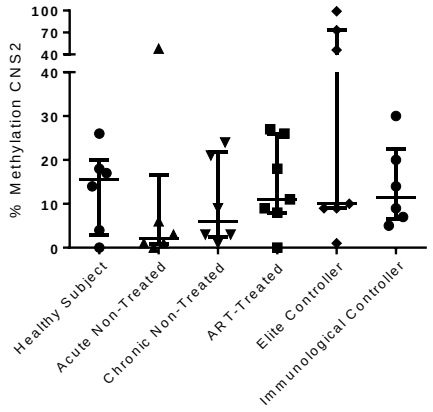

F)

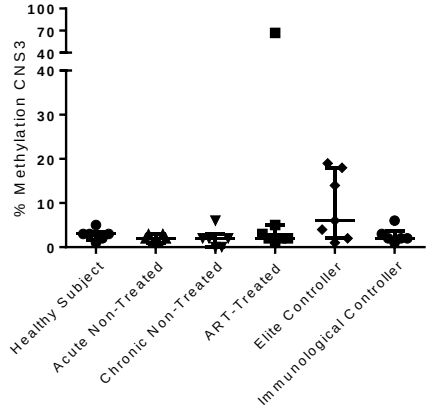

Figure 5b. Foxp3 methylation status in sorted Tregs (CD4+FoxP3+) in CNS1 (D), CNS2 (E), and CNS3(F).

Conclusion

- HIV infection resulted in an increase in Tregs and FoxP3+ T-cell frequencies. This increase was mainly due to peripheral generation in the acute phase, while in the chronic phase, the generation occurred both in the periphery and in the thymus.
- Accordingly, decreases in CNS1 methylation were observed in both acute and chronic HIV infection.
- ART normalized Treg frequencies by decreasing both thymic Tregs output and pTreg generation.
- HIV controllers were able to maintain a Treg frequency similar to that of healthy individuals and had similar epigenetic status of CNS1 to healthy individuals.

Acknowledgements

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