Impact of *in vitro* HIV infection and TGF-β stimulation on human thymic regulatory T-cell development

Sharada Swaminathan [1], Tatiana Scorza [1], Omar Farnos [1], Stephanie C. Burke Schinkel [2], Jonathan B. Angel [2,3], Mohammad-Ali Jenabian [1]

1. Department of Biological Sciences, University of Quebec at Montreal (UQAM), Montreal, Quebec, Canada.

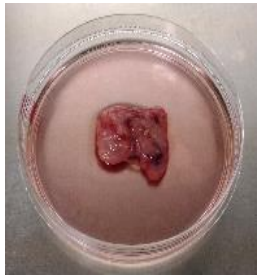
2. Division of Infectious Diseases, Ottawa Hospital-General Campus, Ottawa, ON, Canada

3. Ottawa Hospital Research Institute, Ottawa, Ontario, Canada.

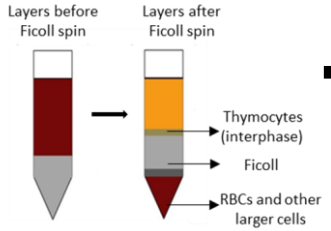
Contact: jenabian.mohammad-ali@uqam.ca

No conflict of interest to declare

Background: Regulatory T-cells (Tregs) are immunosuppressive T-cells expressing the master transcription factor FoxP3 that controls their differentiation and suppressive functions. HIV infection is associated with increased Treg frequencies and immunosuppressive functions in the peripheral blood and lymphoid tissues, which contribute to disease progression and immune dysfunction. Furthermore, thymic dysfunction during HIV infection is associated with rapid disease progression. FoxP3 expression by thymic Tregs (tTregs) is partly regulated by TGF-β, which also contributes to Treg development in the peripheral blood and lymphoid tissues. TGF-β-mediated fibrosis of lymphoid tissues in HIV-infected individuals is associated with disease progression. However, the role of TGF-β in the induction and maintenance of Tregs within the thymus during HIV infection remains unclear.



Recovery of thymus from pediatric patients undergoing emergency corrective cardiac surgery



Mechanical disruption of the tissue and Ficoll density gradient separation

Experimental approaches:

HIV-1 infection, TGF-β treatment of human thymocytes co-cultured with OP9-DL1

+ Polybrene (3µg/mL)

1 hour

Infection of thymocytes with 110NB R5 and NL4.3 X4 HIV-1 strains

Centrifuge for 3 hours at 1200xg, 24°C

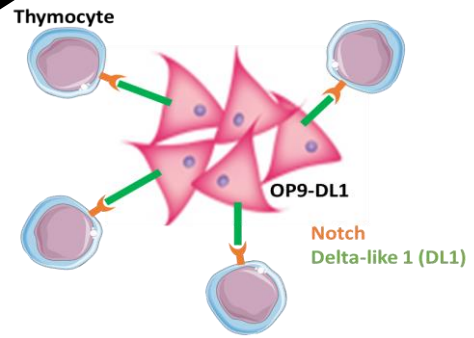
Wash twice with media to remove virus

+/- Pirfenidone (PFD) 2.69mM

1 hour

Remove media with pirfenidone

+/- TGF-β1 (10 ng/mL)
co-culture with OP9-DL1
(Thymocyte : OP9-DL1 = 25:1)

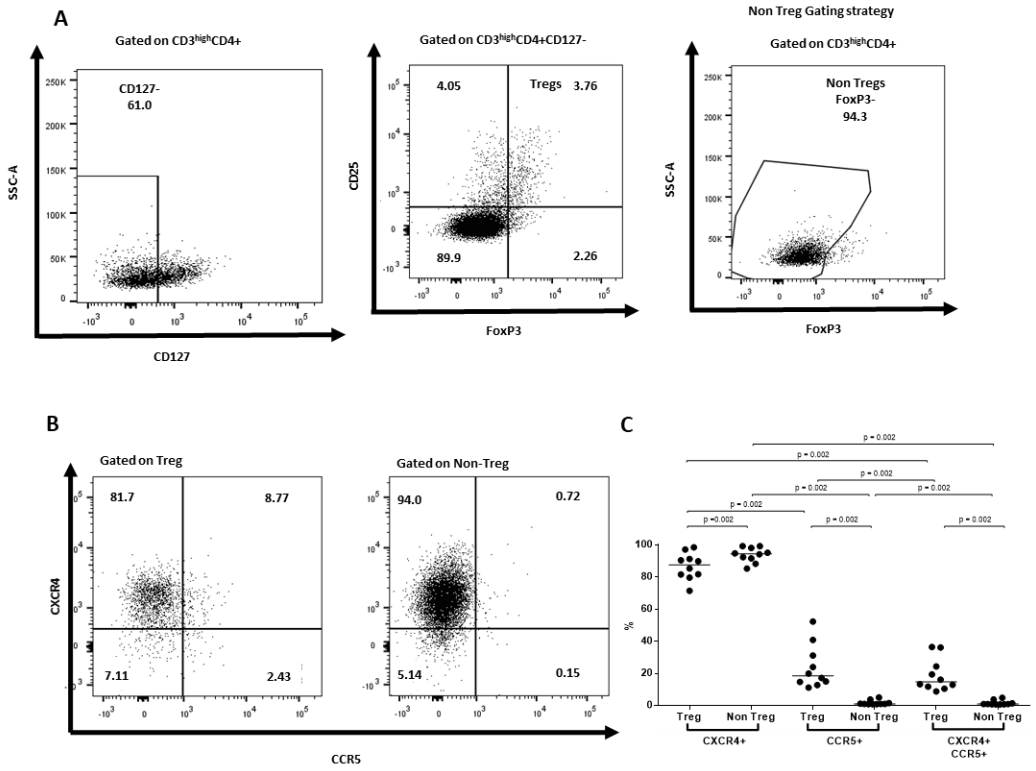


2 days

a) Flow cytometry
and/or
b) mRNA extraction, cDNA preparation and qPCR

Under review, JAIDS

Figure 1: tTregs have higher expression of HIV co-receptor CCR5 compared to non-Tregs. A) Gating strategy used to characterize Tregs (CD3high CD4+CD8-CD127-CD25+FoxP3+) and non-Tregs within CD3high thymocytes. (B) The frequencies of thymocytes expressing CXCR4 and/or CCR5.



Under review, JAIDS

Figure 2: FoxP3+ thymocytes are not infected by HIV. Human thymocytes were infected with R5-tropic 110NB and X4-tropic NL4.3 HIV-1 viral strains for 3 h.

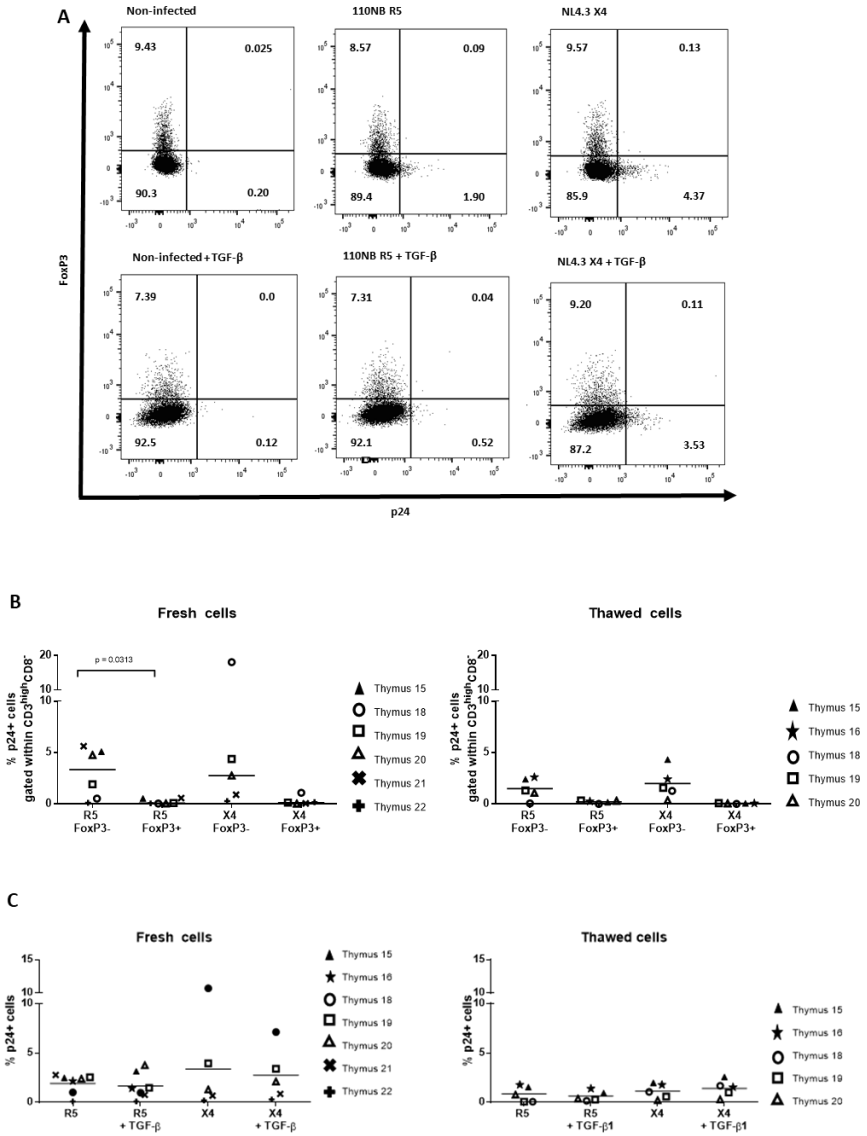
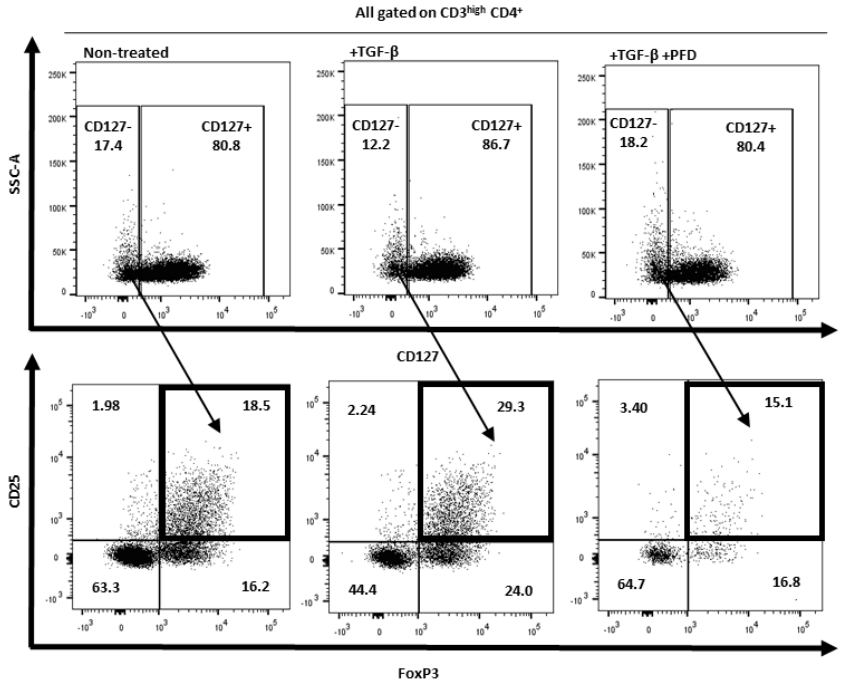


Figure 3: Impact of TGF-β treatment on thymic Treg frequencies. Thymic Tregs were characterized as CD3highCD4+CD127-CD25+FoxP3+ cells. The gating strategy for CD4+ cells excluded CD4+ CD8+ thymocytes



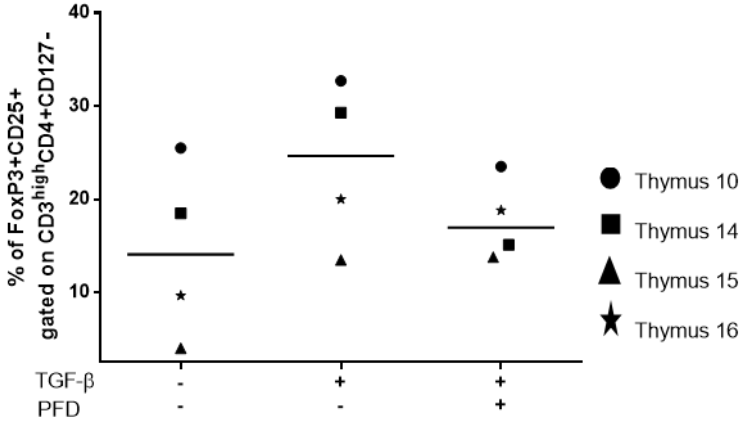
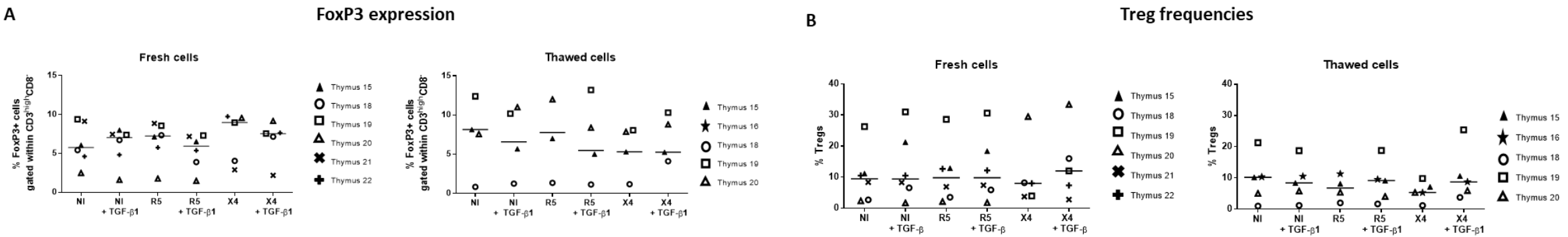
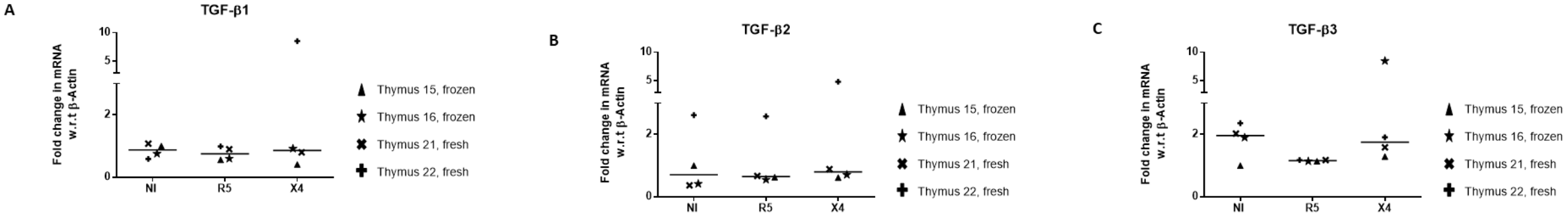


Figure 4: FoxP3 expression and thymic Treg frequencies are not altered by HIV infection and TGF-β treatment.



Under review, JAIDS

Figure 5: HIV infection has no influence on TGF-β production by thymocytes. Fold change in mRNA expression of (A) TGF-β1, (B) TGF-β2 and (C) TGF-β3 in thymocytes infected with R5- and X4-HIV-1 strains, compared to the non-infected control (NI) determined by qPCR



Under review, JAIDS

Conclusion

In vitro HIV infection of human thymocytes alone does not increase FoxP3 expression and tTreg differentiation, nor does the combination of HIV infection and TGF-β. Our results suggest that differentiation of tTregs within thymus is not the same as in blood and secondary lymphoid tissues. Additional inflammatory mechanisms might be involved in differentiation and thymic outputs of tTregs during HIV infection.

Acknowledgements

IRSC CIHR
Instituts de recherche en santé du Canada | Canadian Institutes of Health Research

The Ottawa Hospital RESEARCH INSTITUTE | L'Hôpital d'Ottawa INSTITUT DE RECHERCHE

Réseau SIDA/MI du FRSQ

Canada Research Chairs
www.chairs-chaires.gc.ca

INNOVATION.CA
CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION