

SDC – TABLE S1

	%CD4+CD25+CD127lowFoxp3+ of lymphocytes	% Foxp3+ of lymphocytes
Healthy controls, n=10 Median (IQR)	1.5 (0.9-1.8)	2.1 (1.6-2.5)
HIV-infected patients, n=10 Median (IQR)	1.5 (1.0-1.9)	2.3 (1.8-3.2)
P-value	0.967	0.426

Activated T cells may express Foxp3. This may impose a bias especially in the setting of untreated HIV-infection with high level of immune activation. Thus, we cannot rule out that the some Foxp3+ cells in the tonsils are activated T cells and not Tregs. However, at present there seems to be consensus that Foxp3 is the best way to identify Tregs if a functional assay is not available.

To determine if using Foxp3 alone without CD4, CD25 and CD127 overestimated the number of Tregs, we analyzed Tregs as % of lymphocytes expressing Foxp3+ and as % of lymphocytes expressing CD4+CD25+CD127low in peripheral blood samples from 10 HIV-infected patients and 10 healthy controls. Using the lymphocytes Foxp3+ phenotype opposed to lymphocytes CD4+CD25+CD127lowFoxp3+ overestimates the frequency of Tregs by approximately one third in healthy controls and in HIV-infected patients, the relative overestimation possibly being slightly higher in HIV-infected patients. Thus, we cannot rule out that increased immune activation in HIV-infection may result higher percentage of lymphocytes expressing Foxp3 in lymphoid biopsies. Ideally, all markers of Tregs including graduation of the Foxp3 expression and CD45RA to define Treg subsets including activated, resting and non-suppressing Tregs as suggested by Miyara and used in this study should be used in peripheral blood and biopsies. However, this technique is unfortunately not available in our lab.