Virology 516 (2018) 21–29

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Regulatory CD4 T cells inhibit HIV-1 expression of other CD4 T cell subsets via interactions with cell surface regulatory proteins

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ARTICLE INFO

Keywords:
Regulatory CD4 T cells
HIV-1
HIV-1 integration
HIV-1 mRNA
CTLA-4
PD-1
GARP

ABSTRACT

During chronic HIV-1 infection, regulatory CD4 T cells (Tregs) frequently represent the largest subpopulation of CD4 T cell subsets, implying relative resistant to HIV-1. When HIV-1 infection of CD4 T cells was explored *in vitro* and *ex vivo* from patient samples, Tregs possessed lower levels of HIV-1 DNA and RNA in comparison with conventional effector and memory CD4 T cells. Moreover, Tregs suppressed HIV-1 expression in other CD4 T cells in an *in vitro* co-culture system. This suppression was mediated in part via multiple inhibitory surface proteins expressed on Tregs. Antibody blockade of CTLA-4, PD-1, and GARP on Tregs resulted in increased HIV-1 DNA integration and mRNA expression in neighboring CD4 T cells. Moreover, antibody blockade of Tregs inhibitory proteins resulted in increased HIV-1 LTR transcription in co-cultured CD4 T cells. Thus, Tregs inhibit HIV-1 infection of other CD4 T cell subsets via interactions with inhibitory cell surface proteins.

1. Introduction

Regulatory CD4 T cells (Tregs) are essential for maintaining immune homeostasis, preventing autoimmunity, and regulating chronic inflammatory diseases (Campbell and Koch, 2011), and they are characterized by expression of the FoxP3 transcription factor. In the context of HIV-1 infection and AIDS, a significant volume of data has accumulated over the last decade about the role of Tregs, but there remains debate as to their help or hindrance during infection with HIV-1 (Whiteside, 2015). Some studies have concluded that Tregs facilitate control of AIDS development via inhibition of pathology associated with persistent immune activation which occurs during HIV-1 infection (Card et al., 2009; Chase et al., 2008; Lopez-Abente et al., 2016). By contrast, other data support the notion that Tregs restrict HIV-1-specific immune responses making viral eradication more difficult and resulting in persistently chronic infection (Legrand et al., 2006; Li et al., 2008). Therefore, there is not a simple answer to whether or not Tregs are beneficial or disadvantageous during individual cases of HIV-1 infection (Chevalier and Weiss, 2013; Phetsouphanh et al., 2014). In addition, CD4 Tregs function can be altered following direct HIV-1 infection (Tran et al., 2008). Understanding the role that Tregs play during HIV-1 infection, thus, remains a challenge (Imamichi and Lane, 2012).

Others have reported that Tregs were more susceptible to CXCR4 HIV-1 infections following polyclonal activation *in vitro*, but their definitions of Tregs by flow cytometry are not identical to our work and others (Moreno-Fernandez et al., 2009). In contrast, we previously reported that FoxP3-transduced primary human CD4 T cells function as Tregs *in vitro* and are partially resistant to HIV-1 infection by down-regulating HIV-1 LTR transcription via an NFAT-dependent pathway (Selliah et al., 2008). Moreover, FoxP3-expressing regulatory CD4 T cells also limited HIV-1 expression in neighboring non-Tregs CD4 T cells in co-culture. In the current study, we now demonstrate that Tregs themselves are relatively resistant to HIV-1 infection, and also suppress viral expression in adjacent CD4 T cells in a cell contact-dependent manner. Specifically, GARP (Glycoprotein A Repetitions Predominant), an important Tregs cell surface inhibitory protein, participates in suppression of HIV-1 expression in neighboring CD4 T cells. These results provide novel insights regarding alterable mechanisms involving the role of Tregs during HIV-1 infection.

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https://doi.org/10.1016/j.virol.2017.12.036
Received 23 August 2017; Received in revised form 28 December 2017; Accepted 29 December 2017

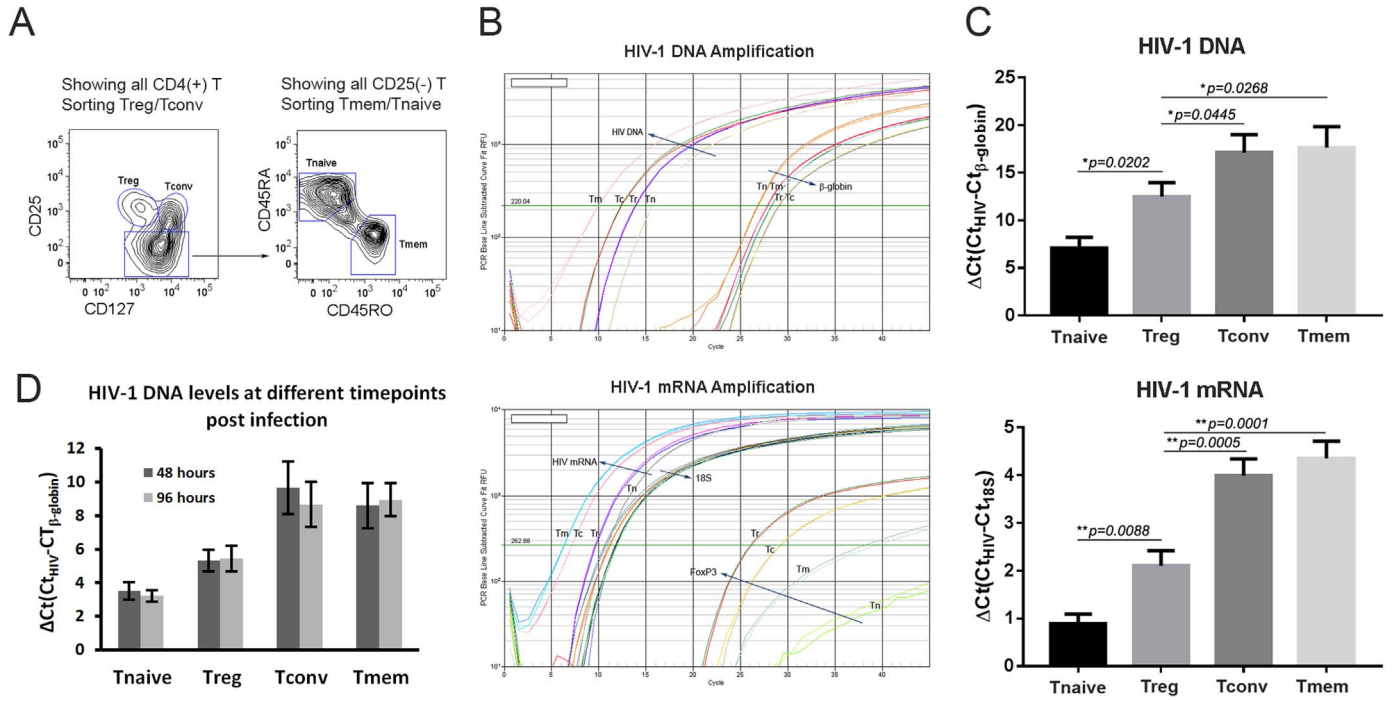

Fig. 1. HIV-1 infection of CD4 T cells *in vitro* demonstrates lower levels of HIV-1 DNA and gag mRNA in Tregs compared to Tconvs and Tmems. (A) FLOW contour plot shows the cell sorting strategy. The *in vitro* bulk infected ((B) and (C)) or freshly isolated ((D)) CD4 T cells were stained and sorted into Tregs ($CD25^{high}$ $CD127^{low}$), Tconvs ($CD25^{low}$ $CD127^{high}$), Tmems (CD25(-) CD45RO(+)), and Tnaives (CD25(-)CD45RA(+)) populations. (B) Semi-quantitative real-time PCR plots of HIV-1 viral DNA (top) and mRNA (bottom) levels, relative to control β-globin DNA and 18 S RNA, for Tmems (Tm), Tconvs (Tc), Tregs (Tr), and Tnaives (Tn) populations. (C) Bar graphs depict the mean results of viral DNA (top) and mRNA (bottom) levels from three independent experiments. Standard error bars are shown along with statistically significant comparisons between CD4 T cell populations. (D) Bar graph shows the viral DNA levels over two different time points as indicated post infection (n = 3).

2. Results/Discussion

2.1. Tregs express lower levels of HIV-1 than other effector and memory CD4 T cell subsets

It is well known that HIV-1 readily infects and is expressed in activated CD4 T cells, but resting CD4 T cells are largely resistant to HIV-1 infection. However, within a heterogeneous T cell population it is not entirely clear which CD4 T cell subsets are infected and how efficiently these subsets express HIV-1. To address this question and mimic natural infection, peripheral blood CD4 T cells were isolated from healthy blood donors and infected promptly in bulk with HIV-1 without any prior cell activation. The cells were maintained in culture for 5–7 days in the presence of moderate amounts of recombinant human IL-2 (30–50 U/ml) to maintain cell survival. The infected CD4 T cells were then phenotypically sorted by flow cytometry into Tregs – $CD25^{high}CD127^{low}$, conventional effector CD4 T cells (Tconvs) – $CD25^{low}CD127^{high}$, memory CD4 T cells (Tmems) – CD25(-)CD45RO(+), and naïve CD4 T cells (Tnaives) – CD25(-)CD45RA(+) (Fig. 1A). Real-time RT-PCR confirmed that Tregs expressed the highest mRNA levels of the Tregs master transcription factor, FoxP3, with lower levels in CD4 Tconvs, Tmems, and Tnaives, in that decreasing order (Fig. 1B). Having sorted the CD4 T cell subsets, HIV-1 gag mRNA and total HIV-1 DNA levels were measured. As shown in Fig. 1B and C, both CD4 Tconvs and Tmems expressed the highest levels of HIV-1 mRNA, and contained the highest levels of HIV-1 DNA, but there were no statistically significant differences between these subsets. In contrast, Tregs had markedly lower HIV-1 mRNA and DNA levels. As expected, CD4 Tnaives contained viral mRNA and DNA, near the lower limits of detection (Fig. 1B and C). Furthermore, to address the kinetics of infection in the different CD4 T cell subsets, cells were immediately sorted from freshly isolated peripheral blood mononuclear cells by flow cytometry. The four CD4 T cells subsets were then separately infected with HIV-1 to observe potential changes of HIV-1 DNA levels in each subset. As shown in Fig. 1D, both Tconvs and Tmems showed the highest levels of HIV-1 DNA at 48 h and 96 h post infection, with similar levels detected at both time points. By comparison, Tregs and Tnaives had notably lower viral DNA levels but with similar levels detected at each time point for each respective subset. The relative levels of HIV-1 DNA in the different CD4 T cells infected in isolation (Fig. 1D) mirrored the results seen in the bulk infections (Fig. 1C). Moreover, there were no significant changes in HIV-1 DNA levels in the individual subsets noted at the 2 time points analyzed.

In order to confirm the results obtained *in vitro*, HIV-1 levels in CD4 T cell subsets from HIV-1 infected patient samples were examined immediately *ex vivo*. Patient A was acutely infected [not receiving antiretroviral therapy (ART)] with an HIV-1 viral load of 360,000 copies/ml and a CD4 T cell count of 254 cells/μl. Patient B was chronically HIV-1 infected and receiving ART with a viral load of 69,000 copies/ml and a CD4 T cell count of 652 cells/μl. The patients' peripheral blood samples were independently sorted to obtain the various CD4 T cell subsets, and each subset was assessed for HIV-1 mRNA and DNA by Real-time RT-PCR and PCR, respectively. FoxP3 mRNA levels were used to help confirm the CD4 T cell subsets based on sorting by cell surface phenotypes. Both HIV-1 infected patient samples demonstrated that CD4 Tmems and Tconvs possessed the highest levels of HIV-1 DNA and mRNA (Fig. 2). Consistent with the results from the *in vitro* infection experiments, CD4 Tregs had notably lower levels of HIV-1 DNA and mRNA (Fig. 2). As expected, very low levels of HIV-1 DNA, and undetectable levels of HIV-1 mRNA, were present in the CD4 Tnaives subset (Fig. 2). Therefore, both the *in vitro* infection data and the *ex vivo* HIV-1 patient results support the notion that Tregs, despite expressing the requisite receptors for HIV-1, are less likely to be infected by HIV-1.

2.2. Tregs inhibit HIV-1 expression in other CD4 T cell subsets

Having confirmed our previous *in vitro* data that FoxP3-expressing CD4 T cells are relatively resistant to HIV-1 infection (Selliah et al.,