Regulatory T cells: the ultimate HIV reservoir?

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Abstract

Despite significant advances in the understanding of HIV-1 infection, a cure remains out of reach. This is, in part, due to a long-lived HIV-1 reservoir in resting CD4+ T cells, which do not express viral antigens, and thus are invisible to the immune system. These latently infected cells carry replication-competent proviruses and can cause rebound viraemia if antiretrovirals are interrupted. Characterising this HIV-1 reservoir is a challenging task, requiring identification of CD4+ T cell subsets carrying intact proviruses, as well as defining their distribution within the body. Regulatory T cells (Tregs) comprise a subset of CD4+ T cells that are essential for maintaining immune tolerance. HIV-1 is known to infect Tregs *in vivo* but there is limited understanding of their role in HIV-1 persistence. Recent studies of well-controlled HIV-1 infection on antiretroviral therapy (ART) have shown higher frequencies of inducible, intact proviruses in Tregs compared to other CD4+ T cells, and provirus-containing Tregs have been found in lymphoid tissues at substantial frequencies. This evidence is supportive of a latent HIV-1 reservoir in Tregs, but greater detail is needed, including tissue distribution. An HIV-1 reservoir in Tregs could pose a significant barrier to HIV-1 eradication because Tregs are known to be long lived and resistant to apoptosis. Tregs are also immunosuppressive, and can inhibit cell-mediated immunity through multiple mechanisms. Non-specific depletion of Tregs would be likely to result in severe autoimmunity. Additional research is needed to further characterise regulatory T cells as a reservoir of HIV-1 and as an obstacle to eradication, or immune control, of HIV-1 infection.

Keywords: Treg, HIV, persistence, reservoir

Introduction: HIV persistence and the viral reservoir

Suppression of HIV-1 replication and prevention of immunodeficiency can be achieved with antiretroviral therapy (ART) regimens as simple as one pill a day, but a cure for HIV-1 infection remains elusive. Upon cessation of ART, infected CD4+ T cells carrying replication-competent (intact) proviruses cause rebound viraemia and recurrence of progressive immunodeficiency if ART is not restarted [1]. It is believed that part of the HIV-1 reservoir persists in resting CD4+ T cells that do not express viral antigens and thus are not recognisable by the immune system [2,3]. These latently infected cells are thought to be intermittently activated to produce infectious virus that can then infect additional cells if antiretrovirals are not present to block new cycles of productive infection. An important challenge in the search for a cure of HIV-1 infection is better characterisation of cells that constitute the reservoirs of HIV-1 beyond the general phenotype of 'resting' CD4+ T cells. Elucidating the immunophenotype of reservoir cells is critical for their effective targeting.

Characterising the HIV-1 reservoir is a difficult task, however, because most (>95%) infected cells contain defective proviruses, incapable of producing infectious virus [4–6]. Of all the CD4+ T cells carrying HIV-1 DNA, it is estimated that only about 1% carry intact proviruses [5,6]. In addition, identifying which CD4+ T cell subsets can be activated to produce infectious virus is not sufficient, and information is needed on where these cells reside within the body. Most CD4+ T cells are found in lymphoid tissues, and large populations of infected cells are known to reside in the spleen, lymph nodes (LN) and gut-associated lymphoid tissue (GALT) [1,7,8].

The initial studies describing a latent HIV-1 reservoir in the 1990s recognised it primarily in resting memory CD4+ T cells after removing activated T cells expressing CD25, the interleukin-2 receptor alpha chain [7]. These activated T cells were removed because they were transcribing and expressing HIV-1 antigens and thus were not part of a latent reservoir [7]. Since that time, research has progressed to identify latent HIV-1 provirus in a variety of resting memory T cell populations, including those with central, transitional and stem-cell memory phenotypes [9–11]. More recently, another potential reservoir in CD4+ T-follicular cells has been described, a unique cell population expressing the CXCR5 chemokine receptor, which serves as a homing signal to B-cell follicles [12]. Similar studies have also identified potential reservoir populations within polarised memory CD4+ T cell subsets, including those with Th1 and Th17 phenotypes [13,14]. There is still ongoing investigation of additional T cell subsets that may contribute to the latent HIV-1 reservoir. One of the least studied and potentially most durable reservoir cells are regulatory T cells (Tregs). This potentially important cellular component of the HIV-1 reservoir is the topic of the current review. Evidence suggests that the Treg reservoir of HIV-1 may be one of the most difficult to control or eliminate, raising the possibility that it is the 'ultimate' reservoir cell.

CD4+ regulatory T cells (Tregs)

Regulatory T cells were initially described in the 1970s as a subset of CD4+ T cells with suppressive capabilities for other immune cells [15,16]. Originally termed 'suppressor T cells', it was not until the late 1990s that they were recognised as playing a major role in maintaining immune tolerance to self and foreign antigens [17,18]. Tregs can suppress host-adaptive immune responses with salutary effects on autoimmunity but a negative impact on cancer immunity. On average, Tregs account for approximately 1–10% of circulating CD4+ T cells [19]. They were initially defined by the surface expression of CD25, but this is not considered a specific marker as it is also expressed on activated effector T cells [17]. Further studies have revealed that Tregs lack other T cell activation markers such as HLA-DR and CD69, and usually express low levels of the IL-7 receptor alpha chain (CD127) [6,20]. The combination of CD25+ and CD127lo is often used to isolate Tregs; however, other studies have separated cells by positive

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selection for CD25+ and negative selection to remove HLA–DR+ and CD69+ activated effector T cells (Table 1) [6]. The suppressive function of Tregs is mediated by the expression of additional surface proteins, primarily CTLA-4 and CD39. CTLA-4 is a checkpoint inhibitor that blocks the B7-CD28 costimulatory signal and therefore inhibits maturation of antigen-presenting cells [21]. CD39 is the ectonucleoside triphosphate diphosphohydrolase-1, which acts in association with another ectoenzyme, CD73, to convert ATP to adenosine, which has multiple immunosuppressive functions [21]. However, CTLA-4, CD39, and CD73 are not commonly used to isolate Tregs as their expression is variable and based on cellular activation. It was not until 2003 that the most specific marker of Tregs was described: the transcription factor forkhead box-P3 (FOXP3). This intracellular protein plays a critical role in Treg differentiation and suppressive functions [19]. Because it is located intracellularly, it cannot be used to separate or sort live cells, but is commonly used to confirm the purity of Treg populations. Because there is currently no universal surface marker of Treg suppressive function, studies of Treg populations purified by surface marker expression must be complemented by further assessment of their suppressive function.

Initial studies of Treg biology focused on their capacity to suppress cell-mediated immunity in tumour microenvironments, and on the breakdown of their suppressive function in autoimmune diseases. More recently, work has focused on their role in HIV-1 disease pathogenesis during different stages of the infection [22]. The debate continues as to whether Tregs are beneficial or harmful across the spectrum of HIV-1 disease. Their suppressive abilities may decrease immune activation and the inflammation associated with chronic HIV-1 infection, and thus limit new cellular targets for the virus [23]. In contrast, they may be detrimental to the immune clearance of HIV-1-infected cells by inhibiting cytotoxic T cell responses, especially in the context of therapeutic strategies to enhance HIV-1-specific immunity [19,24]. There are several recent reviews detailing the current perspectives on the role of Tregs and immune dysfunction during HIV-1 infection, but few studies have examined Tregs as a potential reservoir of HIV–1 (Table 2) [22,25]. The current evidence supporting Tregs as an important HIV-1 reservoir is reviewed below along with the characteristics of Tregs that could make them particularly difficult to eliminate or control as a reservoir.

Table 1. The most common cell surface markers used for isolating regulatory T cells (Tregs)

	Cell surface marker	Description of marker
Positive selection	CD4+	Co-receptor of the T cell receptor
	CD45RO+	Memory T cell marker
	CD25+	IL-2 receptor α
Negative selection	CD127– or lo	IL-7 receptor α
	HLA-DR-	T cell activation marker
	CD69–	T cell activation marker

Tregs: a potential site of HIV-1 persistence

Initial studies of Tregs were performed in HIV-1-infected individuals not on suppressive ART, and so they could not assess the possibility of latently infected Tregs. *In vitro* infection studies have suggested that Tregs are preferentially infected compared to other CD4+ T cells, but these findings have not been reproduced *in vivo*. There is no evidence for a preferential infection of Tregs as compared to other CD4+ T cell populations, although it is well-established that HIV-1 infects Tregs *in vivo* [28,29]. Studies in chronic and untreated HIV-1 infection have noted that there is an absolute decrease in Tregs with progressive immunodeficiency. This decline occurs at a slower rate than for other CD4+ T cells, leading to a relatively higher frequency of Tregs in the chronic and untreated phase of HIV-1 infection when compared to other CD4+ T cell subsets [19,30,31]. These findings suggest that infected Tregs may be selectively spared from immune clearance during chronic HIV-1 infection, making them candidates for a persistent reservoir of HIV-1.

To assess the HIV-1 reservoir *in vivo*, the study population should consist of individuals who have been virally suppressed on ART for several years. Tran *et al.* published the first study in 2008, which directly examined Tregs as a site of HIV-1 persistence on ART [6]. Participants had been virally suppressed on ART for a median of 5 years. PBMCs were collected, and Tregs, defined as CD4+/CD25hi/HLADR-/CD69–, were isolated via cell sorting from fresh PBMCs [6]. This cell population was confirmed to be >99% FOXP3+. HIV-1 DNA was detected in the Treg compartment

Table 2. Summary of studies directly evaluating the contribution of regulatory T cells (Treg) to an HIV/SIV reservoir

Study	Comparison	Treg definition	Findings
Tran *et al.* 2008 [6]	Treg *vs* CD4+ non-Treg	CD4+/CD25hi/HLADR-/CD69– (**FOXP3+ >99%)**	HIV DNA in Tregs was 1.5–8-fold greater than non-Tregs Supernatants from activated, infected Tregs were capable of infecting new cell lines
Jiao *et al.* 2015 [18]	Treg *vs* CD4+ non-Treg	CD4+/CD25+/CD127lo **(FOXP3+ >90%)**	HIV DNA in Tregs at 10-fold higher levels than non-Tregs Cultured Tregs produced p24 antigen but transmissible virus not confirmed
Dunay *et al.* 2017 [26]	Treg *vs* Tcm *vs*. Tem	CD4+/CD25+/CD127lo (**FOXP3+ >95%**)	No significant difference in HIV DNA levels between Tregs, Tcm and Tem Only study to demonstrate infectious virus production in all three subsets using VOAs
McGary *et al.* 2017 [27]	Tregs were not directly measured – cells were separated based on inhibitory receptors	CTLA-4+PD-1– (**CD25+/CD127lo/FOXP3+ about 60%**)	CTLA-4+PD-1– T cells contained significantly more SIV DNA in the blood, LN and spleen compared to CTLA-4-PD-1+ and CTLA-4+PD-1+ populations Supernatants from activated, infected Tregs capable of infecting uninfected cell lines

Tcm: central memory T cells; Tem: effector memory T cells.

with a frequency 1.5 to 8-fold higher than in the non-Treg CD4+ subset. Using a limiting cell dilution PCR, they estimated that a median of 1 in every 10,000 Tregs was infected compared to 1 per 25,000 non-Tregs. Although they were unable to isolate enough cells to perform quantitative viral outgrowth assays (qVOA), when using a smaller number of cells, they demonstrated that these latently infected Tregs produced viral RNA upon activation [6]. Supernatants from these activated Tregs could infect new cells, as measured by p24 antigen production, confirming that the inducible virus was infectious [6].

A follow-up study by Jiao *et al.* in 2015 was performed on a similar study population of HIV-1-infected individuals with suppressed viraemia on ART for 1–5 years [18]. The investigators defined Tregs somewhat differently as CD4+/CD25+/CD127lo, with purification by magnetic bead separation rather than cell sorting. The purity was somewhat lower than reported by Tran *et al.* but still >90% based on FOXP3+ expression. Results showed more frequent HIV-1 DNA detection in Tregs than reported by Tran *et al.* with a 10-fold higher HIV-1 frequency in Tregs compared to non-Tregs [18]. Although this study did not measure inducible viral RNA production, it did show that cultured Tregs produced more p24 antigen at 72 hours than Treg-depleted PBMCs. However, the production of infectious HIV-1 from Tregs was not assessed [18].

Most recently, a study by Dunay *et al.* went a step further and compared the amount of HIV-1 DNA in Tregs to that in central memory and effector memory CD4+ T cells [26]. This contrasts with prior studies, which had compared Tregs to non-Tregs. Cells were sorted and Tregs defined as CD4+/CD25+/CD127lo with FOXP3+ in >95% of cells. Central memory CD4+ T cells (Tcm) were defined as CD45RO+/CCR7+ and effector memory T cells (Tem) by CD45RO+/CCR7– expression. Droplet digital PCR was used to measure HIV-1 DNA levels, with no significant difference found between any of these subsets (Tregs, Tcm, Tem). Quantifying the reservoir size by qVOA was performed, but was limited due to low cell yields. Infectious virus was isolated from all three T cell subsets in only two of ten participants [26]. The difference in inducible infectious virus recovered among the different T cell subsets, reported as infectious units per million, was not significant. This study was the first to quantify the amount of replication-competent HIV-1 in Tregs compared with other CD4+ T cell subsets, and no difference was found, although there was limited power for comparison because of the fact that most qVOA results were negative [26].

Although the above studies support the existence of latently infected Tregs in peripheral blood, they do not address Tregs in tissue compartments. It has been previously established that a significant population of Tregs reside in gut-associated lymphoid tissue (GALT) [32]. It is therefore plausible that a proportion of these Tregs carry latent HIV-1 as well. Although no human study has been performed to further evaluate this hypothesis, there is supportive evidence from the simian immunodeficiency virus (SIV) model in rhesus macaques. In 2010, Allers *et al.* examined colonic biopsies of chronically SIV-infected macaques and found that gut mucosal Tregs contained 11.7 times more SIV DNA than non-Tregs [32]. SIV RNA was detected in these mucosal Tregs as well. Of note, this study was carried out in the setting of chronic and untreated SIV infection. It demonstrates that a significant number of infected Tregs are found in the gut mucosa, but it is not possible to comment on their persistence as a reservoir on effective ART.

An extensive study by McGary *et al.* provided more evidence for a widespread SIV Treg reservoir in different tissue locations [27]. Authors have investigated CD4+ T cells from SIV-infected rhesus macaques, with well-controlled disease, on ART for at least 50 days. Unfortunately, Tregs were not purified directly, as T cell populations were sorted based on inhibitory receptor expression. CD4+ T cells were isolated from peripheral blood, lymph nodes (LNs), spleen, and gut mucosa and were separated based on PD-1 and CTLA-4 receptor expression (CTLA-4+PD-1–, CTLA-4+PD-1+, CTLA-4-PD-1+). Interestingly, CTLA-4+PD-1– T cells in blood, LNs, and spleen harboured significantly more SIV DNA compared to other inhibitory receptor combinations [27]. More SIV DNA was also recognised in gut mucosa in CTLA-4+PD-1– T cells when compared to CTLA-4+PD-1+ T cells. Further characterisation of this CTLA-4+PD-1– T cell population revealed that about 60% of these CD4+ T cells were Tregs, defined as CD25+CD127loFOXP3+ by flow cytometry [27]. The CTLA4+PD-1– T cells also carried replication-competent virus as evidenced by SIV RNA and p27 antigen production after co-culture with uninfected cell lines. Not enough T cells were isolated to perform qVOA to compare the amount of latent, but inducible, infectious virus in each of the cell subgroups [27].

These studies in SIV-infected macaques describe increased numbers of infected Tregs in GALT during chronic and untreated infection, which may persist in the setting of well-controlled infection on ART. Similar studies in uncontrolled HIV-1 infection have also shown an increase in gut mucosal Tregs in combination with a decrease in Th17 helper T cells and disruption of the Th17/Treg ratio [8]. This abnormal ratio can persist even in well-controlled HIV-1 on ART, and may have played a role in the chronic systemic immune activation observed in the individuals studied [8]. However, the impact of the altered Th17/Treg ratio on the HIV-1 reservoir size and persistence are undefined. Although the SIV tissue study described above did not sort specifically for Tregs, these results are supportive, at least in macaques, of a latent Treg reservoir in lymphoid tissue. When combined with the aforementioned human studies, which clearly demonstrate replication-competent HIV-1 in peripheral blood Tregs, there is strong evidence that Tregs can contribute to the HIV-1 reservoir. It is thus important to consider the Treg characteristics that could pose a significant barrier to their elimination.

Treg function could support HIV-1 persistence

Tregs have multiple unique properties that could make them particularly difficult to eliminate as an HIV-1 reservoir. They are hyporesponsive to antigens compared to other CD4+ T cells, and have several biochemical features that resemble an anergic cell population [33]. As these features limit cellular and proviral activation and expression of viral proteins, it makes them less likely to be targeted by cytotoxic T cells. *In vitro* studies provide evidence that FOXP3 expression can inhibit HIV-1 long-terminal repeat transcription, which could favour the establishment of latently infected cells and a barrier to latency reversal [34,35]. Tregs have also been shown to be resistant to apoptosis [27,36,37]. This is mediated at least partially by CTLA-4 cross-linking and activation, which can prevent apoptosis by reducing Fas and FasL expression. This cross-linking is also associated with an upregulation of Bcl-2, a common regulatory protein with anti-apoptotic properties [36]. Additional studies have shown that FOXP3 alone can downregulate FasL expression, thereby providing another mechanism for Treg resistance to apoptosis [37].

HIV-1 has been found in one study to preferentially integrate in genes encoding transcription factors, such as BACH2 and STAT5B, both of which are specifically enriched in CD4+ central memory and Treg subsets [38]. Furthermore, *in vitro* studies of BACH2

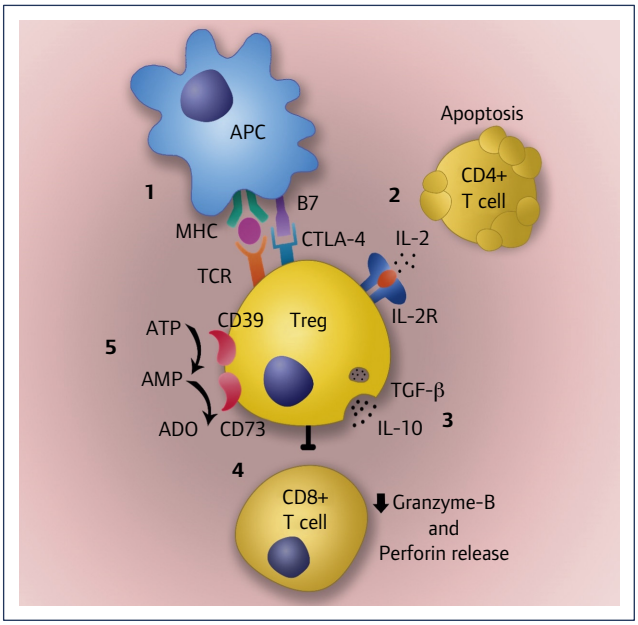

Figure 1. Primary immunosuppressive functions of Tregs. **1:** CTLA-4 binds to B7, blocking CD28 co-stimulation, thereby inhibiting dendritic cell maturation and antigen presentation [18]. **2:** IL-2R on Tregs consumes IL-2, preventing CD4+ T cell activation, inducing apoptosis and inhibiting CD8+ T cell differentiation [15,38]. **3:** Production of inhibitory cytokines, such as TGF-β and IL-10 [36,37]. **4:** Direct cellular interactions with CD8+ T cells, suppressing granzyme-B and perforin release [6]. **5:** CD39 and CD73 ectonucleotidases convert ATP to AMP and then to adenosine, activating a variety of inhibitory immune pathways [18]. ADO, adenosine; AMP, adenosine monophosphate; APC, antigen-presenting cell; ATP, adenosine triphosphate; MHC, major histocompatibility complex; TCR, T cell receptor.

Table 3. Regulatory T cell (Tregs) properties that promote their persistence as a cellular reservoir of HIV-1

Characteristics promoting survival	• Biochemical characteristics similar to an anergic cell population	[26]
	• FOXP3 expression inhibits HIV long-terminal repeat transcription favouring latency	[32]
	• CTLA-4 activation upregulates Bcl-2, promoting resistance to apoptosis	[33]
	• FOXP3 expression and CTLA-4 activation downregulate Fas-FasL production making Tregs resistant to apoptosis	[33,34]
Immunosuppressive properties preventing elimination	• Direct cellular interactions with CD8+ T cells suppresses granzyme-B and perforin release	[6]
	• IL-2R consumption of IL-2 prevents CD4+ T cell activation and induces apoptosis; this process also inhibits CD8+ T cell differentiation	[15,38]
	• Production of inhibitory cytokines such as TGF-β and IL-10	[36,37]
	• Blockade of B7-CD28 co-stimulation inhibits dendritic cell maturation and antigen presentation	[18]
	• CD39 and CD73 ectonucleotidases convert ATP to adenosine, activating a variety of inhibitory immune pathways	[18]

and STAT5B show that they promote Treg survival and proliferation, and do not affect their suppressive function [38]. This suggests that proviral integration may promote a self-renewing, persistent HIV-1 reservoir in specific T cell populations, although further studies are needed to assess the frequency and mechanisms involved and whether integrated proviruses are intact and replication-competent.

Tregs have also been shown to retain their immunosuppressive function while infected with HIV-1 [19]. Therefore, a population of these latently infected cells could provide another barrier to eradication through their multiple immunosuppressive properties (Figure 1) [19]. Activated Tregs can dampen cell-mediated immunity through direct cell-to-cell interactions and production of inhibitory cytokines [39–41]. They can disrupt cytotoxic T cell functions, such as granzyme-B and perforin secretion. In the study by Tran *et al.*, Treg depletion (CD25+CD4+ cells) led to a significantly increased release of granzyme-B from cytotoxic T cells during non-specific activation with PMA/ionomycin and upon stimulation with HIV-1-specific peptides [6]. Similar results were found by Jiao *et al.* who noted that Treg (CD25+CD4+ cells) depletion led to an increase in CD4+ activation markers and frequency of HIV-1-specific cytotoxic T cells [18]. These findings are consistent with studies of well-controlled HIV-1 patients receiving therapeutic vaccination with dendritic cells loaded with HIV-1 peptides [42]. Vaccination led to a modest increase in Treg frequency but a marked enhancement in HIV-1-specific Treg-mediated suppression. Treg depletion revealed a heightened polyfunctional cytotoxic T cell response in vaccinated patients, as well as an increased percentage of cytokine-producing cytotoxic T cells [42]. These findings show that Tregs have immunosuppressive capabilities in the setting of long-term, well-controlled HIV-1. It is therefore possible that HIV-1-infected Tregs could survive in the long term because of antigenic hypo-responsiveness and suppression of cell-mediated immune responses that would otherwise target them for elimination. These properties suggest that Tregs could be one of the most difficult HIV-1 reservoirs to eliminate (Table 3).

Current strategies to reduce the CD4+ T cell reservoir size include latency reversal agents, such as histone deacetylase inhibitors, TLR-agonists, and other approaches that activate resting T cells leading to antigen expression and cytotoxic T cell- or natural killer (NK)-cell-mediated elimination. Since Tregs normally lack immunosuppressive functions at rest, it is possible that activating them would not improve HIV-1 clearance and may actually suppress immune responses, similarly to that demonstrated in the therapeutic vaccination study described above [42]. Treg-depleting agents are being developed, such as denileukin diftitox, which is a genetically engineered protein composed of IL-2 and the diphtheria toxin. It has been shown to target and remove up to 75% of cells expressing the IL-2 receptor alpha chain, including Tregs [43]. A study in SIV-infected macaques that had received this compound showed an increase in CD4+ T cell activation and a modest viraemia rebound after Treg depletion, which was accompanied by a dramatic boost in SIV-specific cytotoxic T cells and rapid viral clearance [43]. Treg depletion could serve as a powerful 'kick and kill' strategy, not only through viral reactivation, but also due to enhanced cytotoxic T cell function targeting HIV-1 reservoirs. However, peripheral Tregs are essential in maintaining host immune tolerance. Strategies aimed at removing this cell subset would likely be complicated by significant autoimmunity and need to be tested with extreme caution.

In summary, a latent viral reservoir in a functioning Treg population could provide a significant barrier to HIV-1 eradication and

potential cure. Further studies are needed to fully characterise the size of the Treg reservoir and the function of Tregs in well-controlled HIV-1 infection. New treatment approaches are needed to specifically target infected cells in this unique subset, while also minimising disruption of immune tolerance.

Difficulties in studying Tregs

Despite the current evidence supporting Tregs as a potentially problematic HIV-1 reservoir, there have been few studies classifying their quantitative contribution to the reservoir. The lack of research in this area is due to by the inherent difficulties in studying Tregs. They constitute only about 1–10% of total human T cells, and of the cells carrying HIV-1 DNA, only 1% will carry intact provirus [4,5,44]. It is extraordinarily difficult to isolate large numbers of Tregs to perform more detailed investigations. Of the studies cited in this review, none could consistently isolate >400,000 Tregs at a time, and many did not reach that number. This makes it very difficult to run quantitative analysis, such as the qVOA to compare the size of this reservoir to other T cell populations.

Additionally, there is no accepted definition or definitive marker of Tregs. The most specific marker is FOXP3 expression, which is an intracellular marker and cannot be used to isolate this population. Tregs are frequently defined by different combinations of cell surface receptors, causing variable purities when FOXP3+ expression is subsequently measured by flow cytometry. This leads to heterogeneous studies and limits the generalisability of the results. It is essential to also perform studies of suppressive function on the isolated Treg population, but given the aforementioned issues with Treg yield, isolating adequate numbers to perform both qVOAs and suppressive studies is problematic. Additionally, freezing cells prior to bead separation or sorting can dramatically decrease Treg yields as compared to using fresh samples.

It is essential to improve the Treg isolation process and to simplify their functional analysis. Promising new technologies, such as single-cell analyses, may help and can improve the classification of rare cells based on gene expression profiles. Single-cell transcriptomic analysis has already led to enhanced identification and purification of other T helper cell subsets [45]. Additional research is needed to enhance the Treg recovery, and such advances would likely have far-reaching applications beyond HIV-1 infection, because of the pivotal role of Tregs in immune regulation and tolerance.

Conclusions

Human studies have shown that Tregs can harbour latent and intact HIV-1 proviruses, which are replicative competent. However, the size, anatomic distribution and importance of the Treg reservoir in terms of rebound viraemia after ART cessation are unknown. The SIV studies in macaques suggest that the viral reservoir in Tregs is distributed throughout the lymphoid tissues, with a large component in gut-associated lymphoid tissue. Inherent characteristics of Tregs could provide a challenge for their elimination, including their hyporesponsiveness to stimulation, resistance to killing, and immunosuppressive functions, but many questions remain unanswered. Additional research is needed to better define the importance of Tregs as an obstacle to HIV-1 eradication or immune control.

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