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Foxp3 and Treg cells in HIV-1 infection and immuno-pathogenesis

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Abstract

FoxP3$^+$CD4$^+$CD25$^+$ regulatory T (Treg) cells are implicated in a number of pathologic processes including elevated levels in cancers and infectious diseases, and reduced levels in autoimmune diseases. Treg cells are activated to modulate immune responses to avoid over-reactive immunity. However, conflicting findings are reported regarding relative levels of Treg cells during HIV-1 infection and disease progression. The role of Treg cells in HIV-1 diseases (aberrant immune activation) is poorly understood due to lack of a robust model. We summarize here the regulation and function of Foxp3 in Treg cells and in modulating HIV-1 replication. Based on recent findings from SIV/monkey and HIV/humanized mouse models, a model of the dual role of Treg cells in HIV-1 infection and immuno-pathogenesis is discussed.

Keywords

Regulatory T cells; AIDS; Chromatin; Epigenetic; Humanized mouse; DKO-hu; ONTAK

Regulatory T cells play an important role in self immune tolerance and in balanced immune responses

The regulation of immune tolerance is a critical aspect of immunology. The balance between recognition of self versus non-self is essential for maintenance of immune homeostasis. Regulatory T cells (CD4$^+$CD25$^+$) are a crucial component for the control of deleterious effects from excessive immune responses as seen in autoimmune disease or allergic insult [1, 2]. Treg cells have been implicated in a number of pathologic processes including elevated levels in cancers [3–5] and infectious diseases [6–9], and reduced levels in autoimmune diseases [1, 10–13]. It is apparent that Treg cells are induced (or recruited and expanded) by most infections to modulate host immune responses to avoid overreactive immunity (Fig. 1). As a result, Treg play a critical role in immune responses, vaccinations as well as in immunopathogenesis of pathogens. For example, Leishmania infection leads to induction of Treg that help to maintain the balance of immune response and pathogen persistence [18, 19]. For the host, persistence of the pathogen is beneficial to maintain effective immunity against these pathogens [18]. In a number of chronic viral infections, Treg are induced to subdue the anti-viral immune responses and allow persistent infection. For example, Treg cells are implicated in establishing persistent infections of viruses

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including HCV in human and chimps [14–16] and friend leukemia virus in mice [7]. Recent experiments with Herpes Simplex virus (HSV) infection in mice showed that depletion of $CD4^+CD25^+$ T cells 3 days prior to infection resulted in elevated HSV-specific $CD8^+$ T-cell response in vivo in the acute and memory phases, and elevated HSV-specific $CD4^+$ T-cell responses [20, 21].

The majority of HIV infection efficiently leads to viral persistence even though a seemingly robust immune response is induced. In addition, it has become increasingly clear that HIV disease is associated with chronic immune hyper-activation. In fact, systemic immune hyperactivation is the most reliable predictor of AIDS progression. Therefore, the role of $CD4^+CD25^+$ Treg in HIV-1 diseases is likely critical. However, the findings from HIV-infected patients and animal models are confusing, and the exact role of Treg cells in HIV infection is poorly understood. Here we will summarize recent progresses in the role of FoxP3 and Treg cells in HIV infection and immuno-pathogenesis.

Treg cells and FoxP3 protein

$CD4^+CD25^+$ T cells with regulatory activity that suppressed autoimmune diseases in mice were first identified in the thymus [1, 2]. The molecular mechanism of Treg lineage development is not clearly understood, but recent genetic studies in both mouse and human have identified Scurfin or FoxP3, a fork-head transcription factor, as a critical determinant of Treg development and function [22–26]. First, scurfy (sf) mice carrying mutations in the FoxP3 gene exhibit lymphoproliferative diseases and autoimmune phenotypes, and the sf mutant mice lack functional Treg cells. Indeed, targeted inactivation of FoxP3 in mice leads to loss of Treg function [25, 26]. Second, human IPEX patients, due to mutations in the human FoxP3 gene, also develop multiple organ autoimmune symptoms consistent with lack of functional Treg. Finally, ectopic expression of FoxP3 in naïve $CD4^+CD25^-$ T cells converts them to look and act like Treg cells [8, 27].

Removal of the thymus from neonatal mice leads to multiple organ autoimmune diseases that can be prevented by Treg [1, 2]. Thus, it has been proposed that functional Treg cells are either not made in the neonatal thymus or fail to emigrate from the thymus in neonatal mice. However, it is not clear when and where functional $FoxP3^+CD4^+CD8^-CD25^+$ Treg cells are generated in postnatal thymus.

We and others independently report that neither FoxP3 mRNA nor protein is expressed in $CD4^+CD8^-CD25^+$, or $CD4^+CD8^-CD25^-$ thymocytes until 3–4 days post birth, even though mature $CD4^+CD8^-CD25^{+/-}$ thymocytes are present in the thymus of days 1–2 neonatal mice [28, 29]. As expected, $FoxP3^-CD4^+CD8^-CD25^+$ thymocytes from day 2 newborn mice proliferate in vitro and show no suppressive Treg activity [29]. $FoxP3^+$ thymocytes are detected dispersedly in the medullary region of the thymus even from 3- to 4-day-old mice. Therefore, expression of FoxP3 or Treg maturation is ontogenically distinct and kinetically delayed from generation of $CD4^+CD8^-CD25^+$ or $CD4^+CD8^-CD25^-$ thymocytes in the postnatal thymus. It is reported that CD28-mediated signaling is required for the induction of FoxP3 and natural Treg generation [30]. It is recently reported that the Hassel's corpasel in the human thymic medulla preferentially expressed the cytokine TSLP, which promotes maturation of DC and induce natural Treg generation [31]. As APC enriched at the thymus cortical-medullary junction can provide the B7 ligands to signal CD28, additional paracrine factors must be provided in the medulla to lead to functional maturation of $FoxP3^+$ Treg thymocytes. These paracrine signals in the medulla distinct from positive or negative selection are probably involved in late stages of thymocyte maturation. TSLP produced from medullary thymic epithelia cells (mTEC) may contribute to FoxP3 expression and maturation of natural regulatory T cells in both mouse thymus [29] and human thymus [31].

Mechanisms of CD4 T-cell differentiation

T-cell lineage commitment and activation of T cells are usually accompanied by changes in patterns of gene expression. During T-cell responses, T helper (Th) progenitor cells will undergo Th1 or Th2 lineage commitments involving well-defined initiation cytokines, transcription factors, and effector cytokines. Expression of T-bet and GATA3 in Th1 and Th2 cells, respectively, is epigenetically regulated by histone modification and DNA methylation. As T lineage determinants, T-bet or GATA3 are also involved in epigenetically reprogramming the T-cell genome to silence the Th2 or Th1 effector cytokines, respectively, and to activate Th1 or Th2 cytokine genes for transcription (Fig. 2). Similarly, Th17 cell differentiation requires IL-6/TGF-β (mouse) or IL-1β, IL-2, and IL-23 (human) and concomitant expression of the transcription factor RORγt. Treg cells in the thymus or generation of peripheral Tregs through persistent activation of T cells with their cognate antigens in vivo or activation of naïve $CD4^+CD25^-$ T cells in the presence of TGFβ requires Foxp3 for Treg maintenance and function [36, 37]. Similar to the Th1/Th2 paradigm, epigenetic regulation is required to balance suppressive (Treg) or pathogenic (Th17) T cells development programs. Treg cells can be driven to differentiate into Th17 cell [38, 39] given the proper cytokine mileiu, and blocking HDAC activity by chemical inhibitors in vitro blocked Th17 cell differentiation, thus favoring Treg emergence [40].

Foxp3 is also able to override other developmental T-cell pathways, such as Th17, Th1, and Th2 programs, as demonstrated by the increased expression of related cytokines in the absence of Foxp3 [41–44]. Thus, Foxp3 was labeled a “master regulator” of Treg development. However, the true nature of Foxp3 in Treg development and function has emerged, and recent studies based on microarray and ChIP-Chip analysis of FoxP3 promoter occupancy suggests that Foxp3 as a master regulator of Treg lineage is perhaps an oversimplification. Investigation into the gene signature of Treg cells (natural or induced Treg) suggests that Foxp3 stabilizes or amplifies features of chronic TCR stimulation, although characteristic features of Treg function, such as suppression and cell cycle progression, are highly dependent on Foxp3 [45, 46]. This supports previous findings wherein a non-functional Foxp3 allele expressing eGFP results in a gene expression pattern in GFP^+ T cells typical of Treg cells but lacking suppressive function [47]. Therefore, Foxp3 has been defined as a determinant of functional differentiation and/or maintenance of Treg cells, but not critical for Treg lineage development.

Mechanisms of Foxp3 in programming gene expression in T cells

One clear function of Foxp3 in T cells is to suppress expression of IL-2 during T-cell activation, consistent with its role as a gene repressor [48]. However, genomic analysis of gene expression profiles with $Foxp3^+$ and $Foxp3^-$ CD4 T cells has revealed multiple genes that are repressed or induced by Foxp3 [26, 49]. Extensive studies on the gene profile of Treg cells along with ChIP-Chip (microarray of genomic DNA sequences with immuno-precipitated chromatin) experiments determining the direct targets of Foxp3 protein have produced insight into the direct and indirect effect of Foxp3 protein for the Treg signature. It is now evident that multiple signals, including TGF-β signaling, IL-2 receptor signaling, converge with Foxp3 to impart this Treg signature. Nevertheless, there are multiple promoters that are directly regulated by Foxp3, including *IL-2, Ctla4, Tnfrsf18 (GITR)* among others [43, 50]. The mechanisms by which Foxp3 regulates these promoters will be discussed in the following paragraphs.

Induction of IL-2 expression during TCR/CD28 activation is highly dependent on activation of three transcriptional factors (NFAT, AP1, and NF-κB) that bind the IL-2 promoter and activate IL-2 gene expression. Foxp3 was initially described by Bettelli et al. to functionally

interact with and inhibit the transactivation activity of the transcription factors NFAT and NF-κB [32]. More biochemical and structural analysis of the interaction of Foxp3 and NFAT was described by Wu et al., wherein mutations in the forkhead domain, abrogating NFAT and Foxp3 interaction, inhibited Foxp3 promoter occupancy and regulation of several characteristic Treg genes. Thus, a model was proposed wherein Foxp3 inhibits an activating NFAT:AP-1 complex by promoting a repressive NFAT:Foxp3 complex at the *IL-2* promoter [51]. More recently, Foxp3 was also demonstrated to alter AP-1 DNA binding activity to further inhibit AP-1-dependent genes such as *IL-2* [52]. Factors involved in T-cell development are also required for Foxp3-mediated promoter regulation. The Sakaguchi lab described an interaction of Foxp3 and AML1/Runx1 transcription factor. In this study, AML1/Runx1 bound to a region upstream of the core enhancer region of the *IL-2* promoter and is required for optimal *IL-2* expression. A Foxp3/AML1 complex binds this region to form a repressive complex that is required for Foxp3 repression of *IL-2* and Treg function [53]. Interestingly, two studies showed an increase or 'stabilization' of transcription factors at Foxp3-bound promoters, correlating with the notion that Foxp3 functions to enhance or stabilize a TCR-mediated signal [51, 54].

In conjunction with Foxp3 regulation of gene expression by transcription factor modulation, epigenetic regulation of Foxp3-targeted genes has also been described. In eukaryotic cells, histone modifying enzymes and also ATP-dependent chromatin modifying complexes play a crucial role in unraveling or relaxing tightly bound chromatin to allow access to transcription factors [55, 56]. Enzymes such as histone acetyltransferases (HAT) and histone deacetylases (HDAC) modify specific residues on histone tails, and other molecules regulating the phosphorylation, methylation, and ubiquitination of histone tails are all involved in tightly regulating histone dynamics [56–58]. While covalent modifications actively repress or activate transcription of various promoters, other mechanisms of chromatin remodeling are in place, including DNA methylation, the recruitment of linker histone variants, and histone displacement. Several groups have demonstrated an increase or decrease in histone acetylation of promoters regulated by Foxp3, associated with activation and repression of transcription, respectively [51, 54, 59]. In accordance with this finding, factors modulating histone acetylation, such as HATs and HDACs, have been shown to interact with and be recruited by Foxp3 to various promoters. Li et al. describes an ensemble of Tip60 (HAT), HDAC7, and Foxp3 in the regulation of *IL-2*. This Foxp3/HAT/HDAC complex is required for optimal suppression of IL-2 gene expression, and knockdown of any one of these factors inhibited Foxp3 function [60]. It is unclear the requirement for this complex at other promoters regulated by Foxp3, or in the suppressive function of Tregs. Recently, it was demonstrated that the inhibition of HDACs in vivo resulted in increased Treg activity and response. Administration of TSA over time in mice resulted in increased Treg numbers and suppressive function. The latter was associated with increased Foxp3 expression in Treg cells [61]. The role for multiple HDACs in Treg function is becoming apparent. Mice lacking HDAC7 and HDAC9 have Treg cells that are more suppressive, pointing toward a role of class II HDACs in Treg function [61].

More recently, work from our lab and others has shown an interaction of Foxp3 with linker histone H1b (Mackey–Cushman manuscript in preparation) and histone H1/H5 [62], respectively, which are required for Foxp3 regulation and suppressive function. Foxp3 appears to recruit linker histone H1b but not other linker histones to *IL*-2, and is required for both complete *IL-2* repression and, importantly, Treg suppressive function. Thus, it is becoming abundantly clear that Foxp3 regulates gene expression through multiple layers of gene regulation, and we are just now beginning to unravel the complex nature of Foxp3 gene regulation in Treg cells. It will be important to elucidate the factors required for FoxP3 function with respect to phenotype and suppressive activity.

Relative levels of Foxp3 and Treg during HIV-1 infection and disease progression

HIV disease progression can be separated into three distinct phases: (i) acute infection occurring in the first 3–6 weeks in humans and 1–4 weeks in macaques, which is associated with a spike in viral load and a subsequent decrease in viral load to the viral set point; (ii) the chronic phase of infection lasting 6–10 years. This asymptomatic phase coincides with a gradual increase in viral load and decrease in $CD4^+$ T-cell counts over time; (iii) and the final phase lasting roughly 12–18 months and is associated with AIDS and immune system failure [63]. The focus of past research has been to determine the mechanism of immune activation during the chronic phase and the resultant AIDS progression. More recently, we have shifted our focus on what is happening during the acute phase of infection as a predictor of disease progression. Now, HIV pathogenesis can be divided into two major phases; the acute infection phase associated with a dramatic loss of $CD4^+$ T cells residing in mucosal tissue, and a chronic phase characterized by immune activation and gradual loss of peripheral $CD4^+$ T cells over time [64].

Looking more closely at the acute phase of infection, plasma viral load increase coincides with $CD8^+$ T cell increases and a drop in $CD4^+$ T-cell counts [65]. This leads to an inversion of the $CD4^+/CD8^+$ ratio. Until recently, the magnitude of $CD4^+$ T-cell depletion and its consequence were not fully appreciated. Initial studies in 1998 using an SIV model described a profound depletion of $CD4^+$ T cells in both the gut and gut-associated lymphoid tissue (GALT) [66, 67], and more recent studies have described a similar depletion in the gut of HIV-1 infected individuals [68–71]. The importance of these findings is underscored by the fact that between 60–80% of the total $CD4^+$ T cell population resides in the gut associated lymphoid tissue [72]. The $CD4^+$ T cell population that is most affected and depleted by SIV and HIV has a resting memory $CD4^+$ phenotype, Ki-67- and CD69-, and CD45RA- [73]. Of greater importance is that the majority of mucosal $CD4^+$ T cells are CD45RA-, and up to 75% express the HIV-1 coreceptor required for T-cell infection, CCR5 [68, 74, 75]. Thus, during the acute stage of infection, the resting memory T cell is a major target of SIV and HIV infection and depletion.

The chronic phase of HIV infection is associated with a steady decline in peripheral $CD4^+$ T cell numbers, systemic hyper-immune activation, and a slow and steady rise in viral load in patients not on ART (Table 1). Catastrophic depletion of MALT-associated CD4 T cells in the acute phase of infection occurs in both pathogenic HIV/SIV and non-pathogenic HIV/SIV infection, and is therefore not sufficient or predictive of AIDS progression (reviewed by Paiardini et al. [76]). To date, the best correlate of AIDS progression is hyper-immune activation, and expression of specific activation markers on T cells has been shown to be a predictor for AIDS progression [77, 78]. Several factors play a key role in immune-dysregulation. During acute infection, levels of proinflammatory cytokines are upregulated systemically, largely consisting of IL-6, IL-1β, and TNF-α, consequently induced by HIV-1 surface glycoproteins in in vitro cultured PBMC [79–81] and in vivo [82], or associated with viral replication [83]. Similarly, HIV infection and several HIV-specific gene products are responsible for T-cell activation. Both HIV-1 envelope gp120 and accessory factor nef are capable of modulating T-cell activity [84–86]. In the gut, the depletion of $CD4^+$ memory T cells continues into the chronic stage of infection, and the restoration of this population is never achieved in both pathogenic SIV and HIV infection [67, 68]. As a consequence, chronic immune activation occurs due in part to homeostatic proliferation of the T-cell pool to replenish the HIV-depleted pool. Recently, a mechanism was described for immune activation stemming from a breakdown of the immunological barrier at mucosal sites, resulting in LPS translocation and systemic activation [87, 88]. Similarly, SIV infection

leads to the breakdown of mucosal barrier function and subsequent *Salmonella typhimurium* dissemination as a result of Th17 cell destruction [89]. In support of immune activation as an important factor for AIDS progression, Cecchinato et al. utilized antibody to block CTLA-4 function in SIVmac251 infection both in acute and chronic stages of disease. This treatment led to increased viral load in plasma and tissue, correlating with increased T-cell activation, and exacerbation of MALT $CD4^+$ T cell loss, but it surprisingly had no effect on HIV-specific T-cell responses [90]. A clear understanding of immune balance and the alteration of immune-homeostasis in HIV infection is required to elucidate mechanisms contributing to HIV pathogenenis. Therefore, the remainder of this review will focus on the role of Regulatory T cells in HIV disease progression, both as a target of HIV and regulator of immune activation, along with emerging models for the study of HIV replication and pathogenesis.

Characterization of the role of Treg cells in HIV-1 infection has been controversial, most notably for the lack of consistent determination of a Treg phenotype along with technical methods for determining Treg numbers in HIV patients, and lack of understanding of the dynamics of Tregs in peripheral blood over the time course of disease (Table 2). Thus, several groups have shown that Tregs numbers are either decreased [8, 96, 97, 104, 105] or increased [91, 95, 100, 101] in HIV-1 infection. A clearer understanding of Tregs in disease progression has come to light with the use of monkey models of HIV infection. It is well established that SIV infection in African green monkeys and sooty mangabeys does not result in AIDS-like disease, while several groups have shown that rhesus macaque infection is an accelerated and consistent model for HIV disease progression [106–109]. Using this model, Periera et al. demonstrated that Treg numbers in peripheral blood of SIV-infected sooty mangabeys did not change over the course of infection, while there was severe depletion in rhesus macaques, nicely correlating with disease state and progression. Dynamics of Tregs in SIV-infected macaque's was dependent on the stage of infection, where acute infection resulted in transient increase in Treg numbers followed by a decrease in Treg numbers inversely correlated with immune activation and viral load. This might somewhat explain the discrepancies of Treg numbers found in HIV-infected patients. While Treg numbers declined during the chronic stage of infection in macaques, the Treg percentage remained constant, suggesting a lack of preferential infection of Treg cells over T-effector cells. Interestingly, the function of Tregs ex vivo was decreased in SIV-infected macaque compared to SIV-infected sooty mangabey, and the apparent numerical or function loss of Tregs correlated with viral load [99]. Similarly, Chase et al. described severe depletion of Treg cells in the gut of SIV-infected rhesus pigtail macaques during the acute and chronic phase of the infection, consistent with the finding that the majority of the $CD4^+CCR5^+$ T-cell population is depleted in acute SIV infection [102]. Unlike the findings of Periera et al., the percent of $FoxP3^+$ Tregs decreased compared to the $CD4^+$ T-cell population. Differences in the virus used in these two studies might account for this discrepancy, although the use of an accelerated pigtail macaque model in the second study suggests the rate of disease progression might correlate with a preferential loss of Treg cells resulting in immune activation. Surprisingly, none of these studies determined the relative level of infection of Tregs compared to memory T cells, although the Treg population was described in brief to harbor genomic SIV and is therefore a target of infection [102]. Inconsistencies in the dynamics of Treg population in both HIV and SIV are summarized (Table 2), clearly demonstrating the need for a unified and consistent method for Treg quantification, along with a more robust model to further dissect Tregs in HIV pathogenesis.

Emerging humanized mouse models for the study of HIV infection and immuno-pathogenesis

Until recently, the best model for the study of HIV pathogenesis in vivo is pathogenic and non-pathogenic SIV infection of non-human primates. As previously stated, insight into the mechanisms of T-cell dynamics and immune activation during SIV infection has been invaluable. The use of primate models does have drawbacks, including cost of primate maintenance and housing and natural differences between HIV and SIV in genomic organization. The need for a robust model that mimics HIV infection in both replication and disease progression is of great importance. The development of this model began with the discovery in 1988 that mice carrying a mutation in *prkdcscid* (protein kinase, DNA activated, catalytic polypeptide; severe combined immunodeficiency) allowed engraftment of human cells from PBMCs, fetal haematopoietic tissue, and later hematopoietic stem cells [110–112]. However, the efficiency of engraftment was low due in part to spontaneous mouse T- and B-cell generation and increased radiosensitivity and high levels of natural killer (NK) cell [113, 114], which was all but eliminated (sans the high NK cell level) in mice with a targeted mutation in recombination-activating gene 1 (*Rag1*) and *Rag2* [115, 116]. A breakthrough occurred with the generation of mice with mutations in the interleukin-2 receptor γ-chain (*IL2R*γ), or the common cytokine receptor γ-chain, a component of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 signaling cascade. This opened the door for decades of research and development of mouse models with the capacity of reconstituting a human immune system. The combination of *IL2R*$\gamma^{-/-}$ and *Rag2*$^{-/-}$ (or NOD-scid mice) are currently utilized for the high engraftment efficiency and lack of development of T cell, B cell, and NK cell [117, 118].

The goal for humanized mouse model is to generate a functional human immune system capable of primary and secondary responses. Using the BALB/c-*Rag2*$^{-/-}$ $\gamma_c^{-/-}$ model (referred to as BALB/c-DKO-hu), Traggia et al. was able to demonstrate a functional immune reconstitution in central and peripheral lymphoid organs following engraftment of HSC, including development of human dendritic cell, myeloid cells, and importantly human Treg cells [119]. More importantly, an antibody response to T cell-dependent antigen was demonstrated, along with tolerance to both mouse and human-self antigen, indicating both positive and negative selection occurs in this model [120]. Taken together, the BALB/c-DKO-hu is an important model for the study of infectious diseases (such as HIV) and human immune responses.

Several groups including our own have demonstrated the efficient use of the BALB/c-DKO-hu HSC model for HIV infection utilizing either cord-blood or fetal liver CD34$^+$ cells [121–123]. Although all groups were able to show infection of HIV (both CCR5- and CXCR4-tropic virus) in peripheral blood, long term viremia, and depletion of CD4$^+$ T-cell populations similar to HIV-infected patients, they were unable to demonstrate appreciable levels of anti-HIV antibody responses. Interestingly, Watanable et al. was able to demonstrate in the NOD/Shiscid *IL2R*$\gamma^{-/-}$ mouse sustained viremia and humoral immune response specific to HIV envelope and gag proteins [124]. In an attempt to evaluate the humanized mouse model as an appropriate model for HIV transmission studies, two independent groups were able to demonstrate transmission of HIV via mucosal routes of infection. Utilizing the NOD/scid or BALB/c-DKO-hu models it was shown that sites of mucosal transmission were populated by HIV target cells and were capable of transmitting virus [125, 126]. Thus, it appears that the humanized mouse model is a robust model for the study of HIV infection.

HIV-1 pathogenesis in the humanized mouse model

As discussed earlier, the humanized mouse model has the potential of being a robust model for the in vivo study of HIV infection and pathogenesis and the effect on the immune system. Currently, SIV has provided the greatest insight into the pathogenic mechanisms of HIV infection, namely the mechanisms of immune-pathology that differ between natural and unnatural hosts of SIV. At the forefront of this research is the evaluation of the role of Treg cells in both viral clearance mechanisms and immune-pathology described in chronic HIV infection. An earlier study from our lab demonstrated HIV infection in the BALB/c-DKO-hu mouse model mimicked infection in HIV^+ patients, namely an increase in viral load followed by a subsequent depletion of $CD4^+$ T cells and an inversion of the $CD4^+/CD8^+$ T-cell ratio in the peripheral blood [123]. Both naïve and memory subsets ($CD45RO^+$ and $CD45RO^-$) of $CD4^+$ T cells were infected in lymphoid organs similar to HIV-infected patients [127]. Importantly, the depletion of naïve T cells is dramatically seen in both HIV-infected patients and highly pathogenic SHIV infection in macaques, and there is direct evidence correlating CXCR4 emergence with a dramatic depletion of naïve T cells and rapid clinical progression [128].

HIV-1 infection and replication in Treg cells

$CD4^+CD25^+$ Treg cells express both CXCR4 and CCR5 coreceptors for HIV-1 infection. Given that $Foxp3^+$ Tregs cells are thought to be functionally anergic in vitro, characterized by the repression of the T-cell activation-dependent IL-2 gene, it was surprising to find Treg cells support higher levels of infection by HIV-1 or FIV compared to Foxp3-$CD4^+$ T cells in vitro [8, 129]. Recently, a precursor of Treg cells, naïve Tregs ($CD4^+CD25^+CD45RA^+$) capable of in vivo expansion and suppression, also support high levels of HIV infection and replication [130]. Two lines of evidence have also indicated that HIV-1 infection and replication in Treg cells may be important in vivo. First, although <5% of total $CD4^+$ T cells from peripheral blood are $CD25^+Foxp3^+$ Treg cells, up to 50% of $CD4^+$ T cells express FoxP3 in mucosal lymphoid organs from HIV-1 [100] or SIV [101] infected human or monkeys, respectively. Therefore, the $Foxp3^+$ Treg cells can provide a significant number of target cells for HIV-1 infection in lymphoid organs. Second, 13% of the $Foxp3^+$ T cells are shown to be productively infected by SIV in the lymphoid organs of acutely infected animals [101]. Therefore, $Foxp3^+$ Treg cells are important target cells for HIV-1 infection and replication, at least in mucosal lymphoid tissues during acute infection. Treg induction in HIV-1 infected lymphoid organs may contribute to suppressed anti-HIV immunity and establishment of persistent HIV infection. It is, therefore, critical to investigate how HIV-1 infects and replicates in these T cells for both virological and immuno-pathogenic reasons.

It appears that more efficient infection of Tregs stems from several critical features of Treg biology. First, HIV-1, as well as FIV, preferential infection of Treg cells, to a certain extent, correlates with increased viral coreceptor expression on human and feline Treg cells, respectively [8, 129]. Second, transcriptional regulation of the viral promoter is altered in Treg cells, namely the effect of Foxp3 on lentivirus gene expression. FoxP3 both activates and inhibits multiple genes through modulation of transcription factors NFAT, AP-1, and NF-κB, factors also critical for HIV gene expression. Reports from Grant et al. and our published data point to a role of Foxp3 in transcriptional regulation of HIV-1 LTR promoter. In both cases, the HIV-1 promoter was regulated by Foxp3-mediated modulation of NF-κB, although with disparate results [54, 131]. A study by Grant et al. demonstrated an inhibitory effect of FoxP3 on both HIV and HTLV gene expression through NF-κB- and CREB-dependent mechanisms, respectively. Conversely, findings from our lab determined that Foxp3 differentially regulates HIV LTR gene expression. In expanded primary Treg cells and $CD4^+CD25^-$ T cells ectopically expressing FoxP3 by retroviral transduction, HIV gene

expression was enhanced [54]. This enhancement required NF-κB, and FoxP3 expression was associated with increased NF-κB binding and histone 3 acetylation at the integrated LTR promoter. Support for FoxP3 enhancement of LTR comes from the recent study by Dunham et al., wherein they demonstrated in HIV-infected patients a twofold increase in viral DNA on a per cell basis in $CD4^+$ CD25hi CD127lo Tregs compared to T memory or T effector cells [132]. Furthermore, $FoxP3^+$ Treg cells in humanized mice infected with a CCR5-tropic JRCSF strain of HIV were not only preferentially target for infection, but intracellular HIV gene expression was enhanced compared to $FoxP3^-$ T cells (our unpublished results). Although ongoing studies are investigating the dynamics of Tregs in infection, the targeting of Tregs by HIV and the functional role of HIV-infected Treg cells requires further investigation.

The dual role of Treg in HIV-1 infection and immuno-pathogenesis

The role of Tregs in establishing chronic versus acute diseases has been established for several pathogens, and the importance of this cell population in HIV-1 infection is of great importance. Since the hallmark of progression to AIDS is persistence of HIV-1 infection, hyper-immune activation and the decrease in $CD4^+$ T cells, it is not unreasonable to rationalize the importance of Treg depletion in establishing persistent infection and in controlling chronic immune activation. The best approach to define the role of Tregs in HIV infection is through genetic manipulation in relevant HIV-infection models. The question remains if depletion of Treg prior to or during acute HIV infection will lead to elevated anti-HIV immunity and reduced acute viremia, and whether depletion of Treg in chronic HIV-1-infected patients contributes to uncontrolled immune activation and accelerates AIDS progression. Thus determining the kinetics and functional response of Tregs is of great importance.

Given the importance for Tregs in immune-homeostasis, depletion would likely have a dramatic effect on T-cell activation. As stated previously, the strongest predictor of AIDS progression is immune activation, and depletion and loss of Treg function could contribute to disease progression. Interestingly, multiple groups have determined that Treg cells from HIV-infected patients or SIV-infected macaques are capable of suppressing viral-specific $CD4^+$ and $CD8^+$ T-cell responses in vitro [99, 133]. Therefore, in both SIV and HIV infection, Treg are becoming a more integral player in immune activation, viral replication, and pathogenesis.

Our lab recently directly addressed the role of Treg cells in HIV infection in the BALB/c-DKO-hu mouse model [134]. $CD4^+FoxP3^+$ T cells developed in all lymphoid organs and display normal Treg phenotype and function. These $FoxP3^+$ Treg cells in lymphoid organs are preferentially infected and depleted by a pathogenic strain of HIV (NL-4-R3A [135]) and depletion of Treg cells is correlated with induction of their apoptosis in vivo. To assess the role of Treg cells in the control of viral replication during acute infection, Treg cells were depleted with the IL2-toxin fusion protein (ONTAK) prior to infection. The result was a significant impairment of HIV replication and infection, probably due to a robust anti-HIV immune response. This is demonstrated by reduced levels of productively infected cells in lymphoid organs and lower plasma viremia. Interestingly, we see increased inflammatory cytokines (IFN-γ, TNF-α by intracellular staining) in ONTAK-treated HIV-infected lymphoid organs compared to mock, supporting the presence of increased antiviral T-cell response in the absence of Treg cells (Fig. 3). To determine the importance of Tregs in the chronic phase of HIV-1 infection, we depleted Treg cells at 40 weeks post infection with the CCR5-tropic HIV-JRCSF by administration of ONTAK. Two important finding resulted from this experiment. First, a reduction in the numbers of Tregs significantly enhanced human T-cell activation, consistent with the model of Tregs actively suppressing the

immune activation and viral replication during chronic HIV-1 infection. Second, Treg depletion resulted in a significant increase in HIV-1 viral infection, consistent with the fact that immune activation during chronic infection will enhance HIV-1 replication (Fig. 3 and Jiang and Su, unpublished results). Therefore, $FoxP3^+$ Treg cells are productively infected by HIV, and Treg cells play an important role in suppressing antiviral immunity to enhance viral replication in acute HIV-1 infection. However, during chronic infection, Tregs are important in controlling immune activation, HIV infection and potentially slowing down disease progression.

Perspectives

The current model from our data and previous reports is that Treg cells play multiple roles during HIV-1 infection and pathogenesis. Following initial acute infection, HIV-induced immune response works to control the virus and upregulation of Treg cells may contribute to suppress anti-HIV immunity, promote acute viremia, and persistent infection. In addition, Treg cells serve as efficient target cells for HIV infection during acute infection. Over the course of chronic infection, HIV infection leads to gradual depletion of Treg cells and immune activation. The remaining Treg cells during chronic HIV infection are critical to down-modulate the immune activation because HIV has established infection in lymphoid tissues and immune activation will benefit HIV-1 replication. Using the robust models of HIV-1 infection and immuno-pathogenesis will allow the more complete study of the mechanisms of HIV-1 interaction with Treg cells, and the role of Treg cells in HIV-1 immune-pathogenesis. It will be important to determine the mechanism of Treg depletion by HIV. Although ONTAK or anti-CD25 mAb are widely used to deplete $CD4^+$ $CD25^+$ Treg cells in vivo, it is of concern that neither is specific for Treg cells. To genetically prove the role of $FoxP3^+$ Treg cells in HIV infection and immuno-pathogenesis, we can genetically manipulate the human immune system by in vitro transduction of HSC with lentivirus carrying shRNA to knockdown FoxP3 proteins and determine the effect both on HIV replication and pathogenesis. Finally, the dual role of Treg in HIV infection and pathogenesis suggest that it will be medically beneficial to deplete or reduce Treg cells for enhancing anti-HIV immunity during acute infection. On the other hand, enhanced levels or activity of Treg cells may prevent or slow down the progression of HIV-1 diseases during chronic phases of infection. Various animal models, including humanized mouse models and SIV-monkey models, will be critical to elucidate the role of Treg cells and to develop novel therapeutics targeting Treg cells.

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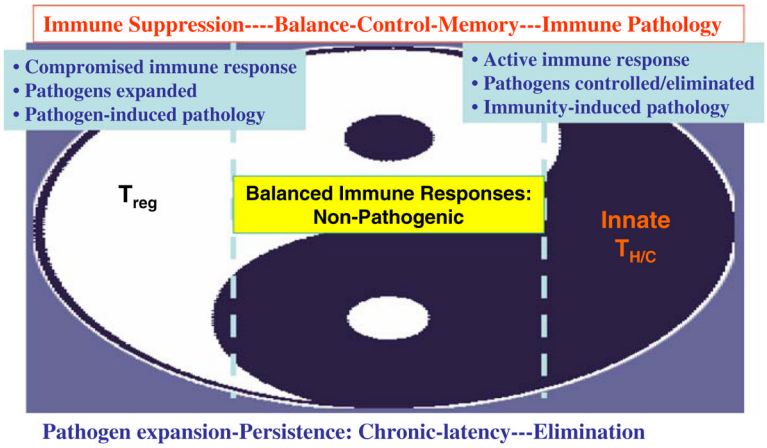

Fig. 1.

Regulatory T cells are induced to balance immune responses: Treg cells are induced (or recruited and expanded) during infections to modulate host immune responses to avoid over-reactive immunity. As a result, Treg play a critical role in immune responses, vaccinations as well as in Immunopathogenesis of pathogens. In a number of chronic viral infections, Treg are induced to subdue the anti-viral immune responses and allow persistent infection, with HCV in human and chimps [14–16] and friend leukemia virus in mice [7, 17]

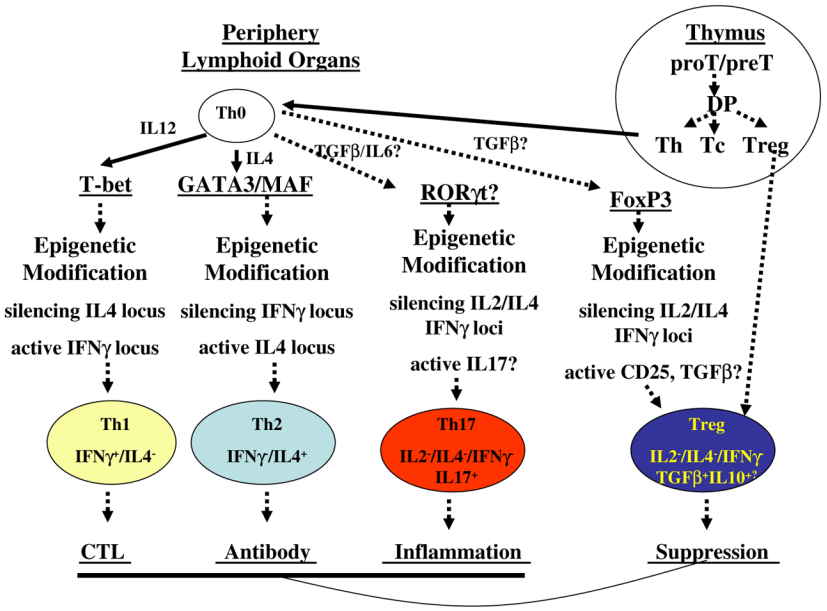

Fig. 2.

T helper and Treg differentiation: cytokines, transcription factors and epigenetic modifications. CD4 T cells can differentiate into Th1, Th2, Th17, or Treg cells when activated in the presence of IL12, IL4, IL6/TGFβ, or TGFβ, respectively. Treg cells are also generated in the thymus during development. Master lineage determinants of Th1 (T-bet) and Th2 (GATA3/MAF) have been well-characterized and are induced by initiating cytokines (IL12 for T-bet and IL4 for GATA3) during T cell activation. T-bet leads to gene silencing of Th2 effector cytokines such as IL4 and "open" chromatin at Th1 cytokine gene loci such as IFNγ. Likewise, GATA3-MAF will lead to gene silencing of Th1 cytokines and activation of Th2 cytokine genes. Induction of FoxP3 and its molecular mechanism in driving Treg differentiation are not as clear, although TGFβ has been implicated to contribute as an "initiation" cytokine and possible Treg effector cytokine. Th1, Th2 effector cytokines, and IL2 genes are silenced by FoxP3. Induction of the newly reported Th17 is even less clear, probably involving IL6/TGFβ [32, 33] and RORγt [34]. IL23 seems important for Th17 cell survival or proliferation [33, 35]

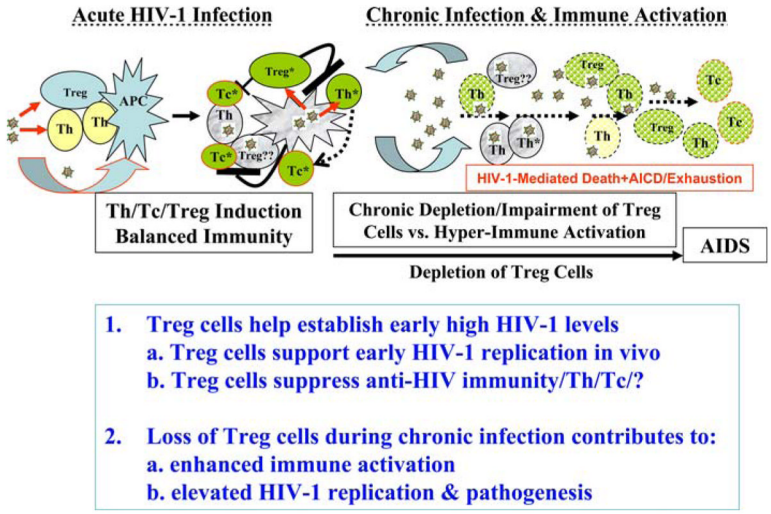

Fig. 3.

The role of Treg in HIV diseases. Based on our hypotheses, Treg cells before or at acute infection will reduce anti-HIV immunity and provide more HIV target cells, thus allow high acute phase viremia and persistent infection [134]. During chronic phase of infection, Treg cells is expected to reduce systemic immune responses (T-cell activation and pro-inflammatory cytokines), reduce viremia (reduced number of activated T cells) and slow down T-cell depletion

Table 1

Correlation of immune activation and AIDS

Host	Virus	Viral load	$CD4^+$ T-cell depletion	Immune activation	AIDS
Human	HIV-1	High	+	High	Yes
Human	HIV-2	Low	−/+	Med/High	Yes/No
Human/HAART	HIV-1	Low	−	Low	No
Human-LTNP	HIV-1	Low	−	Low	No
Rhesus macaque	SIV	High	+	High	Yes
African green monkey	SIV	High	−	Low	No
Sooty mangabey	SIV	High	−	Low	No

Table 2

Modulation of Treg levels during HIV-1 or SIV infection

	Treg levels[a]	Blood	Lymphoid tissue
HIV-1	↑	Epple et al. [91] Montes et al. [92] Mozos et al. [93] Lim et al. [94]	Andersson et al. [95] Epple et al. [91] Nilsson et al. [100]
	↓	Oswald-Richter et al. [8] Andersson et al. [95] Apoil et al. [96] Eggena et al. [97]; Baker et al. [98]	Mozos et al. [93]

	Treg levels[a]	Blood		Lymphoid tissue	
		Acute	Chronic	Acute	Chronic
Macaque	↑	Pereira et al. [99]	–	Estes [101]	–
	↓	–	Pereira et al. [99]	Chase [102] Qin et al. [103]	Chase [102] Pereira et al. [99] Qin et al. [103]
Mangabey	No change	–	–	Pereira et al. [99]	Pereira et al. [99]

[a]Treg levels compared to total $CD4^+$ cell population