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T Regulatory Cells and HIV Infection

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Abstract

Several issues of HIV pathogenesis remain unsolved. Among them, the reason for uncontrolled viral replication in the majority of infected patients is one of the most investigated but still not completely understood. In the last four years a new player has been incorporated into the HIV field: T regulatory (Treg) cells. They are a subset of CD4+ T-cells whose main function is to maintain peripheral tolerance in order to avoid autoimmunity. However, their role in chronic viral and parasitic infections has also been recognized. Several papers have been published in the last years on the potential role of these cells on HIV disease pathogenesis. From the data available so far, two main, nonexclusive roles have been attributed to Treg cells in HIV: a detrimental effect mediated through the impairment of HIV-specific responses, and a beneficial effect by limiting immune activation. The topic is currently highly controversial for different reasons, one of the most important being the lack of standardized assays to measure levels and function of Treg cells. **(AIDS Reviews 2007;9:54-60)**

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Key words

HIV. Treg cells. T-cell responses. Immune activation.

Biology of T regulatory (Treg) cells

The immune system is constantly recognizing self and nonself structures, being capable of destroying foreign pathogens and at the same time respecting self. The ability of the immune system to recognize self without causing any damage is controlled by different mechanisms of central and peripheral tolerance. Immunologic self-tolerance is critical for the prevention of autoimmunity and maintenance of immune homeostasis. Central tolerance involves deletion of most high-affinity, self-reactive T-lymphocytes in the thymus and that of high-affinity, self-reactive B-lymphocytes in the bone marrow, both in an early stage of development. However, it is well known that under certain circumstances some of these high-affinity, self-reactive clones are able to reach the periphery and stay there without provoking autoimmune diseases in most individuals. This is because of several mechanisms of peripheral tolerance, including T-cell anergy and ignorance. Among all of these mechanisms, studies ongoing for more than a decade in rodents have provided firm evidence for the existence of several professional CD4+ T-lymphocytes with different phenotypes that have the ability of inhibiting the response of other T-cells. They have been grouped under the name "T regulatory (Treg) cells" and seem to have an important role in preventing some autoimmune diseases by keeping autoreactive T-cells that have escaped other mechanisms of tolerance from both the activation and the effector function. Their regulating role seems to be carried out through cell-cell interactions or cytokine release mechanisms and is able to inhibit both Th1 and Th2 responses[1-5].

The best characterized and apparently most important Treg cell population has been identified as CD4+CD25+ T-cells[6]. Many studies have shown that these cells appear naturally in the thymus, thus being called "natural Treg cells"[7-10] and would constitutively express CD25[11]. They represent 5-10% of CD4+CD8– thymocytes in humans, mice, and rats[1-3]. Development of this Treg subset in the thymus would be directed by intermediate avidity interactions between the T-cell receptor and self-peptide ligands

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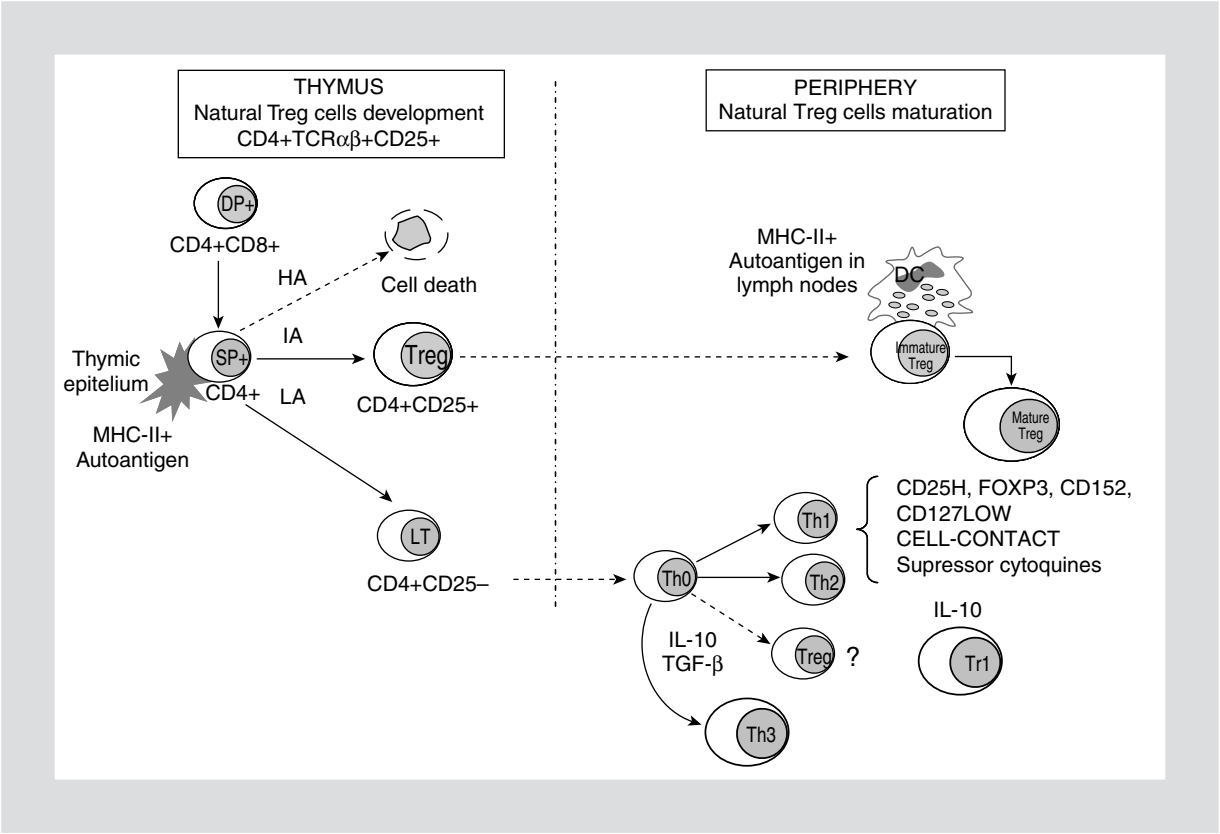

Figure 1. *Origin and maturation of natural and induced T regulatory cells. DP: double positive thymocytes; SP: single positive thymocytes; HA: high affinity; IA: intermediate affinity; LA: low affinity; DC: dendritic cells. Th: T helper lymphocyte.*

expressed on thymic antigen-presenting cells (APC). This affinity is too low for negative selection, yet too high to allow the T-cells to pass through to the periphery. As a result of this process of activation in the thymus, CD4+CD25+ are rendered non-responsive and suppressive. Lower avidity interactions would predominantly promote the development of conventional CD4+CD25– single positive thymocytes and higher avidity interactions would lead to clonal deletions[4] (Fig. 1).

Although the activation of CD4+CD25+ T-cells for the first time requires antigen-specific stimulation via T-cell receptors, once activated they do not require further T-cell receptor ligation, being thus capable of inhibiting both CD4+ and CD8+ T-cell responses in an antigen-nonspecific manner[12,13]. The CD4+CD25+ T-cells do not prevent initial responder T-cell activation, as upregulation of early activation antigens is not affected; however the cells fail to proliferate and undergo cell cycle arrest at the G0/G1 stage. Suppression is overcome by addition of exogenous IL-2 and anti-CD28 to the cultures, which suggest that limiting IL-2 may be responsible for the lack of a sustained T-cell response. This is not attributable to simple consumption of IL-2 by CD4+CD25+ T-cells because Treg cells also prevent IL-2 production by normally responsive T-cells[5]. These data suggest several immunosuppressive properties of naturally occurring CD4+CD25+ T-cells. But how are they carried out? *In vitro* studies show that although when conveniently stimulated these cells are capable of producing small amounts of IL-10 and transforming growth factor-beta (TGFβ), none of these cytokines seem to be necessary for these cells to carry out their regulatory function. This function is mainly mediated through a cell contact-dependent mechanism independent of suppressor cytokines. There is conflicting data on whether the cell contact-dependent inhibitory effects of natural Treg cells are mediated via APC. In fact, some authors show that co-culture with activated CD4+CDC25+ T-cells led to reduced amounts of the co-stimulatory molecules CD80 and CD86 on dendritic cells and B-cells[5,14]. Others suggest the involvement of a direct T-cell/T-cell interaction that is independent of APC, through molecules such as glucocorticoid-induced tumor necrosis factor (TNF) receptor[15]. These natural Treg cells also express high levels of cytotoxic T-lymphocyte-associated antigen 4 (CD152). This molecule has been also involved in cell contact-dependent inhibitory mechanisms[16].

The main problem hindering the study of Treg cells has been the lack of a unique marker that defines all cells with regulatory activity. Although CD25 expression is a useful marker to distinguish Treg cells from resting T-cell populations and seems to be highly specific for their immunoregulatory activity, a complete characterization of natural Treg cells has been difficult to achieve because of the problems to distinguish them from the recently activated CD25+ effector cells. Nevertheless, it has been shown that the expression of CD25 is highly stable on CD4+ regulatory T-cells, in contrast to transient CD25 expression on activated T-cells[17]. Based on this, some authors have classified CD4+CD25+ Treg cells according to the level of CD25 expression. This has been demonstrated to roughly divide activated (low CD25 expression) from regulatory T-cells (intermediate-to-high or only high CD25 expression) in the peripheral blood of healthy subjects[3,18,19].

Other surface markers such as high expression of CD5 (CD5high), CD28, CD38, CD45RO, low expression of CD45RB (CD45RBlow), CD62L, CD134 (OX-40), GITR (glucocorticoid-induced TNF-related receptor), CD127, CD152, CD154 (CD40L) or forkhead box protein 3 (Foxp3) have been correlated to a certain extent with phenotype and/or function of CD4+CD25+ Treg cells[15-17,20-22]. Among them, Foxp3, a member of the "forkhead" or winged helix family transcription factors, seems to be the one which has a stronger association with regulatory T-cell function. In fact, the finding that enforced expression of Foxp3, either via retrovirus or transgene, can convert T-cells to a Treg phenotype suggests its determinant role. These data, coupled with other studies showing that T-cell receptor stimulation of human CD4+CD25– T-cells induces both Foxp3 expression and Treg activity, suggest an active role for Foxp3 in suppressor function. Because of the fact that Foxp3 is expressed in more than 95% of peripheral blood CD4+CD25+ T-cells with high CD25 expression and only in 35% of CD4+CD25+ T-cells with intermediate CD25 expression, some authors conclude that most CD4+CD25+ Treg cells would be included in the CD4+ T-cells expressing high intensity of CD25[21].

It must also be considered that not all CD4+ Treg cells express CD25. In addition to the naturally occurring Treg population, other CD4+ Treg cell subsets have been described that would express different combinations of several of the aforementioned membrane antigens, with or without simultaneous expression of CD25[23]. Some of these cells have been called "induced T regulatory cells" and would be represented by two main subsets. On the one hand, Tr1 cells resemble natural Treg cells in several ways, although they do not express large amounts of CD25 on their surface (low-to-intermediate expression). Like natural Treg cells, they must be first activated. However, they require IL-10 for their formation, and once mature, they carry out their regulatory function mainly through the secretion of large amounts of this cytokine, moderate amounts of TGFβ, interferon (IFN)-γ or IL-5, or low amounts of IL-2. They are abundant in the intestine and their chief function may be to make animals or humans tolerant to the many antigens being part of their diet. These Tr1 cells have been obtained *in vitro* from peripheral blood CD4+ T-naive cells cultured with exogenous IL-10[24,25]. On the other hand, Th3 cells are induced following oral administration of antigens. They are also prevalent in the intestine, but unlike Tr1, their main cytokine to carry out their regulatory function is TGFβ. Like Tr1, they predominantly suppress immune responses to ingested antigens[26,27] (Fig. 1).

Regulatory T-cells in HIV infection

Immune responses mediated by T-cells are essential in the control of virus infections. After antigen encounter, a tightly regulated and orchestrated set of phenomena take part in the successful control and, in most cases, eradication of the inflicting pathogen. Among these phenomena, the successive expansion and differentiation of several types of T-cells with different functional abilities are essential to accomplish the task. However, after pathogen removal, the burst of expansion/differentiation vanishes and a pool of memory cells remains for future encounters with the same pathogen.

The HIV infection is the paradigm of chronic infectious disease and is associated with progressive loss of CD4+ T-cells and immune dysfunction. Diverse immune alterations are already present before a substantial decline of CD4 T-cells occurs[28]. Lack of immune competence against HIV and other pathogens, as well as a heightened immune activation, are among the most relevant alterations present in patients with chronic infection[29,30]. The precise mechanisms underlying these alterations are not completely understood. The recognition of the role of Treg cells in other infections has stimulated the search for a potential role of these cells in the HIV-induced immune dysfunction. So far, it is not clear if Treg cells play a detrimental effect in HIV infection by impairing T-cell responses and thus facilitating viral persistence, or conversely, if they exert a protective role by limiting immune activation, in which case a decline in its level would contribute to aberrant T-cell activation (Fig. 2). To add complexity to this issue, the study of Treg cells is hampered by the lack of consensus on what are the best markers to identify them. Most studies have based the quantitation of these cells on the expression of high levels of CD25 marker[19]. However, the addition of

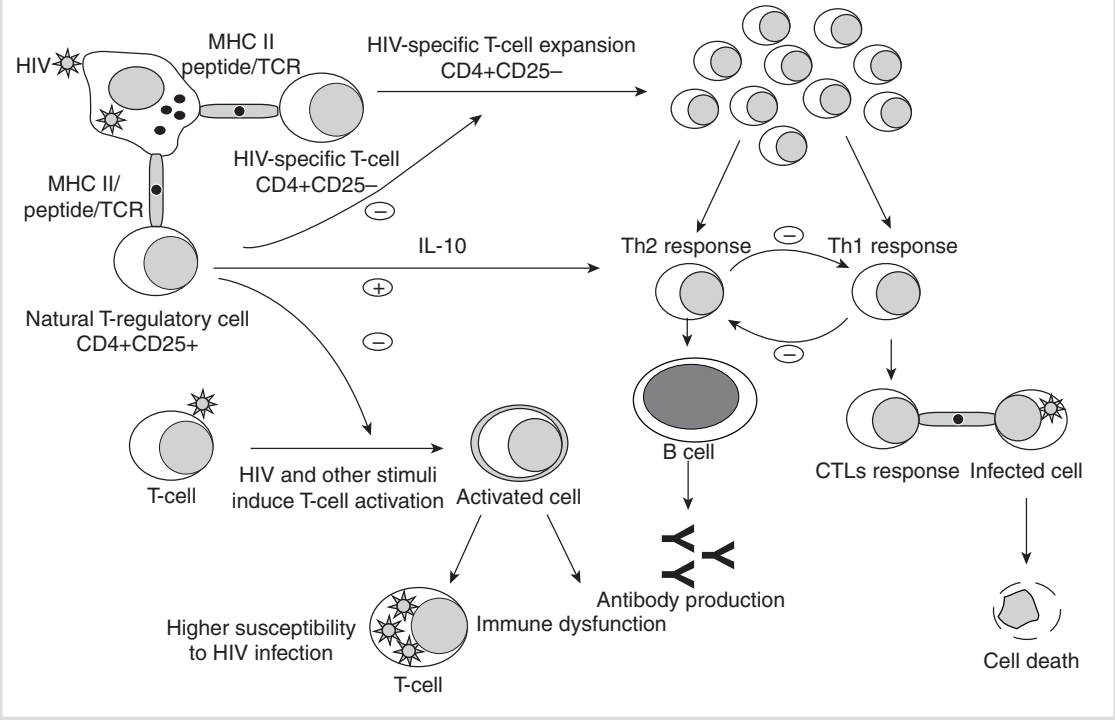

Figure 2. *Dual role for T regulatory cells (Treg cells) in HIV infection. Treg cells, either in an specific or non-specific manner, inhibit HIV-specific T-cell proliferation by direct cell contact and also produce cytokines such as IL-10 that polarizes the response toward a T-helper 2 (Th2) type. On the other hand, they also limit T-cell activation that is one of the main mechanisms involved in T-cell dysfunction and depletion.*

other markers such as human leukocyte antigen (HLA)-DR suggests that the regulatory subset may comprise a lower percentage of total CD4+ cells. Other markers such as cytotoxic T-lymphocyte associated protein 4 and glucocorticoid-induced TNF receptor have also been reported to be expressed by Treg cells[31,32], although they can also be expressed on T-effector cells, making the immunophenotyping problematic. A significant advance in this field was the identification of the transcription factor Foxp3 as a marker closely associated to Treg cell development and function[33]. The addition of this marker has enabled researchers in the field to more precisely define and quantitate levels of Treg cells in different diseases. In this regard, most studies in the field of HIV infection have been performed in the last three years. However, it must be kept in mind that Foxp3 is not the answer to all the questions related to Treg cells. Firstly, recent studies have questioned whether all Treg cells are Foxp3+ or all Foxp3+ are Treg cells[21]. Secondly, it has been suggested that transient expression of Foxp3 may be a normal consequence of T-cell activation[34], a phenomenon characteristic of HIV infection. In a recent paper, lack of expression of the IL-7-receptor (CD127) has been proposed as a new biomarker to identify Treg cells in humans[22].

Treg cells and HIV-specific immune responses

Several groups have analyzed the level and function of these cells in peripheral blood of HIV-infected patients, supporting the conclusion that Treg cells may contribute to HIV pathogenesis by altering the function of HIV-specific effector T-cell responses[35-38].

Weiss, et al.[35] analyzed the levels of CD4+CD25+ cells in HIV patients taking antiretroviral therapy. They found a significant increase in the frequency of these cells in peripheral blood that were able to potently inhibit proliferative responses to recall antigens as well as HIV antigens. Moreover, CD4+CD25+ regulatory cells responded to p24 antigen by secreting TGFβ and IL-10, although its suppressive activity was not dependent on these molecules. Similar conclusions were drawn from the study of Aandahl, et al.[36] when analyzing, in a group of untreated HIV patients, the ability of cytomegalovirus- and HIV-specific T-cells to

produce cytokines, although in their study, levels of CD4+CD25+ regulatory cells were not significantly elevated. In a study including both treated (with undetectable viral load) and untreated patients, Tsunemi, et al.[37] analyzed the relationship between levels of Treg cells and levels of Th1 and Th2 responses, and found a significant increase of Treg cells in peripheral blood of untreated patients with normal levels in the treated group. In both groups of patients, Treg cells were negatively correlated with Th1 and positively with Th2 frequency. Furthermore, in untreated patients, Treg cells were inversely correlated with CD4 counts. The authors conclude that increased levels of Treg cells are associated to more severe stages of infection and to polarization toward Th2 immune responses. In a recent study, Nilsson, et al.[38] analyzed the level and function of Treg cells in lymphoid tissues of untreated HIV patients with progressive or non-progressive disease. They found increased numbers of Treg cells (Foxp3+) in HIV progressors as compared to nonprogressors that were not correlated with immune activation. This increase in Treg cells in progressors impaired cellular immunity, as evaluated by production of different cytokines by CD8+ T-cells present in lymphoid tissues. Furthermore, in an *in vitro* model in which CD4 Treg cells were exposed to inactivated HIV, they demonstrate that gp120-CD4 interaction promotes the survival of these cells by inhibiting apoptosis, concluding that the selective expansion of Treg cells is driven by HIV.

Treg cells and immune activation

As counterpoint to the detrimental effect of Treg cells commented above, some authors have suggested that these cells could have a protective role in HIV pathogenesis by limiting T-cell activation, which is one of the main mechanisms involved in T-cell dysfunction and depletion. This idea has been specially advocated by Oswald-Ritcher, et al.[39] and by Eggena, et al.[40]. In the first study, the authors demonstrate in an *in vitro* system that Treg cells (CD4+CD25+Foxp3+) do express CCR5 and are highly susceptible to HIV infection. The HIV-positive patients had greatly decreased levels of CD4+CD25+Foxp3+ Treg cells as compared to HIV-seronegative controls, and those patients with the lower numbers of Treg cells had higher levels of T-cell activation and lower CD4 counts. The authors conclude that disruption of this cell population may contribute to hyperactivation of T-cells and to disease progression. Eggena, et al. analyzed a cohort of 81 antiretroviral-naïve, HIV-infected Ugandans, and found that the absolute numbers (but not the proportion) of Treg cells (defined as CD4+CD25+CD62L+) were decreased, and that they strongly correlated with both CD4 and CD8 activation. In a multivariate model, this association was stronger than with any other clinical factor examined, including CD4 count and viral load. They conclude that Treg cell depletion is a contributing factor to the high level of immune activation typically observed in untreated patients.

A double-edged role for Treg cells in HIV pathogenesis is drawn from the studies of Kinter, et al.[41] and Epple, et al.[42]. In the first study, there was a significant increase in the proportion of CD4+CD25+ Treg cells in HIV as compared to controls, and these cells were able to suppress both the proliferation and cytokine production of HIV-specific CD4 and CD8 cells, as well as the proliferation in response to polyclonal stimulation. Interestingly, the strongest HIV-specific suppressive activity was observed in patients with low levels of plasma viral load and high CD4:CD8 ratios and this activity was absent in patients with more advanced disease. These data suggest that Treg cell suppressive activity is maximal early in HIV infection and is lost with disease progression. Epple, et al. quantified Treg cells in the gastrointestinal mucosa of 26 HIV patients (13 of them treated and with undetectable levels of viral load), and found an increase in both the frequency and the absolute count of these cells only in the untreated patients. In contrast, neither the absolute numbers nor the frequency was significantly increased in peripheral blood. The authors conclude that active HIV replication induces accumulation of Treg cells in the gastrointestinal tract, and that this may reflect either an attempt to slow disease progression by inhibiting immune activation, or a higher susceptibility of the gastrointestinal tract to opportunistic infections.

Level of Treg cells and changes with HAART

Although most of the abovementioned studies have assessed the levels of Treg cells in HIV infection, the question of whether they are increased, decreased, or unchanged remains unresolved. Firstly, not all studies have found an altered frequency of these cells in HIV patients[36]. Secondly, some have reported increased[35,37,38,41,43] whereas others have reported decreased frequencies or absolute numbers[39], or even an increase and a decrease depending on whether cells are expressed as absolute numbers or as frequencies of total CD4+ cells[40]. At least two reasons account for these discordant results: firstly, it is obvious that results can dramatically change depending on whether data are presented as absolute numbers or as proportions, since total CD4 cells are diminished in HIV patients; secondly, the markers used to define this subset of CD4 cells vary in the different studies. Another important

aspect to consider is that changes observed in peripheral blood may not be representative of what is happening in other tissues such as intestinal mucosa or lymphoid nodes as some of these studies have demonstrated[38,42]. It must be pointed out that changes in peripheral blood may be the consequence of redistribution of Treg cells to other tissues with active viral replication[44]. Finally, changes in number and/or frequency of Treg cells may not always be paralleled by an increase in activity of these cells, since cells detected by surface markers staining may not be functional. In fact, some studies have found that despite an increase of CD25 expression on CD4 cells in peripheral blood, functional Treg cells decrease with disease progression and increasing viral loads[41].

Another important aspect of Treg cells in HIV patients is the modulation of this population with antiretroviral treatment and successful control of viral replication. Based on the hypothesis that Treg levels are modulated by HIV replication[35,38], one may expect that control of viral replication will induce opposite changes. Andersson, et al.[44] and Epple, et al.[42] analyzed the effect of HAART on the levels of Treg cells and both found a decrease when viral load was controlled with treatment. In the first study, levels of Treg cells were increased in lymphoid tissue and decreased in peripheral blood of untreated patients. Control of viral replication with HAART induced a decrease of these cells in lymphoid tissue and an increase in peripheral blood. The authors conclude that Treg cells migrate from peripheral blood to lymphoid tissue during periods of active HIV replication, and that this is reversed when viral replication is controlled with treatment. Similar results were obtained by Epple, et al. when they analyzed levels of Treg cells in gastrointestinal mucosa. However, in contrast to the work of Andersson, they found a slight increase in peripheral blood Treg cells that normalize after treatment.

Treg cells in HIV infection: a consensus model

On the basis of the abovementioned studies, we can conclude that several issues remain to be answered, some of them pertaining to pivotal questions and others more related to practical aspects of Treg cells. First, it has to be clarified if Treg cells have a protective or a detrimental role in HIV pathogenesis, or even a dual role, depending on disease stage. The answer to this question is of paramount relevance since any attempt to manipulate these cells as a new tool in HIV treatment necessarily requires understanding their role in disease progression. From a more practical point of view, studies directed toward the elucidation of the best markers to reliably measure the levels of these cells in peripheral blood or any other body compartments are also necessary to standardize results from different laboratories.

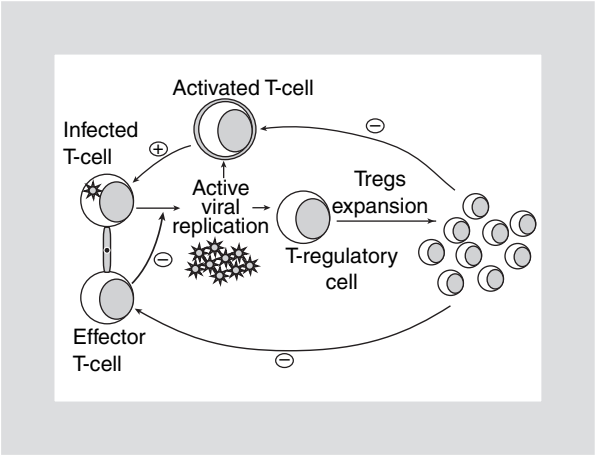

Figure 3. *A proposed model for Treg cells dynamics in HIV infection. Active viral replication is the main driving force of Treg cells expansion in HIV-infected patients. In the early stages of infection, patients with poor control of viral replication will have higher levels of Treg cells and as a consequence greater impaiment of virus-specific T-cell responses. In advanced stages of disease, the functionality of Treg cells may be compromised and its role limiting T-cell activation will fail, thus leading to higher levels of immune activation and immune dysfunction.*

In a presentation in the last retrovirus conference, Dr. Chougnet[45] presented a model of Treg cells in HIV infection, based on the published reports and on its own work, which could serve as a tentative consensus model of the dynamics of these cells (Fig. 3). According to this model, HIV directly induces Treg cell expansion and accumulation in lymphoid tissues, and such accumulation plays an important role in the inability of the immune system to control viral replication. In the early stages of HIV infection, higher viral loads will induce higher levels of these cells and thus a greater impairment of HIV-specific T-cells, whereas in patients with a better control of viral replication, lower levels of Treg cells will be generated and thus a better preservation of HIV-specific responses will be attained. In advanced stages of infection, one may speculate, based on some of the published studies[17], that a diminished functionality of Treg cells would tilt the balance toward incapacity of these cells to control T-cell activation and this would result in higher rates of CD4 depletion and disease progression.

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