The Role of Regulatory T Cells in Chronic and Acute Viral Infections

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Regulatory T cells, a subset of CD4+ T lymphocytes, play a pivotal role in the maintenance of the balance between the tissue-damaging and protective effects of the immune response. These cells have immunosuppressive function and have been intensely studied in the context of autoimmunity, cancer, allergies, asthma, and infectious diseases. Their role in chronic and persistent viral infections is well appreciated. In acute viral infections, the function of these cells is still unclear. The host and pathogen factors that control the generation and activity of regulatory T cells and the role of these cells in modulating expansion, contraction, and development of immune memory in acute respiratory virus infection need to be further elucidated.

An appropriate immune response must strike a balance between maximizing recognition and clearance of infectious agents and minimizing immune-mediated pathology and autoimmune responses. One mechanism by which the immune system finds this balance is through the action of regulatory T (Treg) cells.

The idea of T cells that may be involved in suppression of immune responses was first introduced in the 1970s [1], but an inability to positively identify these cells led to the dismissal of this idea. Interest in the concept of a Treg cell was rekindled when T cells were identified that coexpressed CD4 and CD25 and that could suppress effector T cell responses [2]. It is now known that Treg cells are of paramount importance in peripheral tolerance and are involved in immune evasion by certain malignancies. They also play roles in transplantation, allergies, and pregnancy. However, it is their role in viral infections that is the focus of this review.

A major obstacle in Treg cell research has been the identification of specific markers that identify these cells. Treg cells are generally identified as having a CD4+CD25high phenotype. Other surface molecules expressed by the majority of Treg cells include cytotoxic T lymphocyte antigen 4, glucocorticoid-induced TNF receptor-related gene, CD45RO, OX40, CD62L, CD127lo, and various chemokine receptors, including CCR5, CCR8, CCR4, and CCR7 [3]. However, different subsets of Treg cells may vary in the markers that they express. In a recent study, CD39 and CD73 were found on Treg cells and are proposed to play a role in dampening T cell activation. The detection of CD39 on Treg cells and not on other T cells may prove to be a useful tool for Treg cell isolation [4].

To date, the most specific marker identified for the classification of Treg cells is expression of the transcription factor known as forkhead box P3 (Foxp3). Expression of Foxp3 is necessary and sufficient for the development of the suppressive function of Treg cells. Several studies have shown that ectopic expression of Foxp3 in otherwise nonsuppressive T cells can induce the Treg cell phenotype [5–9]. During development, Foxp3 expression is induced by a combination of high-affinity T cell receptor engagement and signaling through both CD28 and the common γ-chain of several cytokine receptors [10, 11]. The induction of Foxp3 expression and its subsequent impact on cell devel-
opment represents the initiation of differentiation into a dedicated regulatory cell lineage. This is accomplished by the ability of Foxp3 to enhance its own expression and to increase expression of the cellular markers CD25, CTLA4, and GITR, while suppressing such genes as T-bet, GATA3, and RORgt, which are required for the establishment of the Th1, Th2, and Th17 lineages, respectively [12–16]. In addition, expression of Foxp3 seems not only to be necessary for the development of Treg cells in the thymus but also to be required for maintenance of the suppressive activity in the periphery [14]. Recently, genomic studies have shown that Foxp3 can directly and indirectly act as both a transcriptional enhancer and a repressor and is involved in the establishment of epigenetic changes and the induction of microRNA expression [17, 18]. This results in the establishment of a Foxp3-dependent transcriptional network, effectively programming the cell into its suppressive phenotype. Although Foxp3 is the most specific marker for Treg cells, the nuclear location of Foxp3 limits its usefulness as a tool for ex vivo isolation of these cells. Thus, it is important to use several markers to ensure accurate identification of Treg cells.

CD4+CD25high T cells, so-called natural Treg cells, are the type best characterized, but other subsets of Treg cells exist. Peripherally induced Tr1 (IL-10–producing) and Th3 (transforming growth factor[TGF]–β-producing) Treg cells play a role in antigen-specific inhibition but are difficult to differentiate from natural Treg cells [19, 20]. Each subset has been shown to function by suppressing cytokine secretion and proliferation of effector T cells, but the mechanism by which suppression is mediated varies. Direct cell-to-cell contact between Treg cells and antigen-presenting cells can interfere with T cell priming [21]. Treg cells can also interact directly with target T cells to dampen activation [22] or block degranulation [23]. Other types of Treg cells include CD8+ regulatory cells and natural killer regulatory cells. However, the roles of these cells remain to be better elucidated. This review focuses on naturally occurring CD4+CD25Foxp3+ Treg cells (herein referred to as Treg cells) that develop in the thymus and on the role that these Treg cells play in viral infection.

ANTIGEN SPECIFICITY

Several studies have suggested that Treg cells may be antigen specific. Natural Treg cells are known to arise in the thymus and to possess T cell receptors specific for self-antigens [24–26]. However, these cells also suppress immune responses to infectious agents. The mechanism by which natural Treg cells, specific for self-antigens, recognize pathogen-associated peptides is unclear. Studies comparing the T cell receptor repertoires of Treg cells with those of conventional T cells suggest that the repertoires are only partially overlapping, with Treg cells exhibiting a diverse T cell receptor repertoire. This may account for some of the foreign antigen-specific nature observed in Treg cells [26]. There is some evidence that the suppressive function of Treg cells can be stimulated by weakly cross-reactive variants of the index peptide, which may allow pathogen-derived antigens to stimulate these cells [27, 28]. Nonspecific activation of Treg cells through Toll-like receptor signaling may also lead to suppression of responses to non–self-antigens [29].

Virus-specific Treg cells have been identified in several different models of infection. Treg cells specific for human papillomavirus 16 and 18 proteins demonstrate inhibitory function only in the presence of their cognate antigens [30]. Several studies document Treg cells playing a role in the suppression of proliferation, cytokine production, and cytotoxicity of effector CD8+ T cells in viral infections caused by cytomegalovirus, HIV, and hepatitis C virus (HCV) [31, 32]. However, once activated, Treg cells may act to suppress CD4+ and CD8+ T cell responses in a nonspecific manner [27, 33]. Furthermore, Treg cells expanded nonspecifically in vitro by stimulation with anti-CD3 antibody and IL-2 possess enhanced suppressive effect for a myriad of antigens [34]. These data support the notion that Treg cells are not uniquely specific for self-antigens and that they may be expanded when stimulated with cognate antigen or cross-reactive analogues of that antigen or when generated de novo in the periphery during the immune response.

THE ROLE OF TREG CELLS IN CHRONIC OR PERSISTENT VIRAL INFECTIONS

The role of Treg cells has been studied in the context of several chronic viral infections, including those due to herpes simplex virus type 1 (HSV-1), HSV type 2 (HSV-2), HCV, and HIV.

HSV infection. Various models of HSV-1 infection demonstrate the importance of Treg cells in viral clearance and lesion severity. The HSV-1–specific T cell response has been shown to be subject to control by Treg cells in both mice and humans. These studies demonstrated that Treg cells act both during the acute phase of infection and during the formation of memory cells and the recall response [35, 36]. A study investigating the control of HSV-1–specific T cell responses in rabbits revealed that Treg cells localize to the site of infection but are effective at modulating responses by T cells isolated from the peripheral blood [37]. In a model of HSV-2 infection in humans, in vitro depletion of CD4+CD25+ T cells from infected persons resulted in enhancement of proliferation and cytokine secretion by memory T cells to both HSV and cytomegalovirus antigens, highlighting the ability of Treg cells to suppress T cell responses in an antigen-nonspecific manner [38].

The above examples provide evidence of the suppressive effects of Treg cells on immune responses in a viral system, but the role of Treg cells is complex. In a murine model of herpetic stromal keratitis caused by HSV-1, Treg cells were associated...
with favorable markers of disease status. In that model, T<sub>reg</sub> cells minimized immunologic lesions. In the absence of T<sub>reg</sub> cells, greater CD4<sup>+</sup> T cell influx into the cornea occurred, resulting in increased immunopathology [35]. Together, these studies demonstrated the role of T<sub>reg</sub> cells in the delicate balance between clearance of the infectious agent and severity of disease.

**HCV infection.** The factors determining whether infection with HCV will lead to acute or chronic disease are poorly understood. Resolution of infection has been correlated with robust T cell responses to the virus. In cases of viral persistence resulting in chronic disease, HCV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in the blood appear to be diminished [39]. However, sustained cytotoxic T lymphocyte responses in the liver have been observed that may contribute to hepatic immunopathology [39, 40]. In patients with chronic HCV infection, inflammatory lesions lead to hepatic injury, which may necessitate liver transplantation. The abundance of natural T<sub>reg</sub> cells found in liver biopsy samples obtained from patients at the time of transplantation is inversely correlated with the histological severity score [32], indicating a role for T<sub>reg</sub> cells in preventing tissue damage. Moreover, persons with chronic infection harbor more T<sub>reg</sub> cells in their circulation than do uninfected persons [41]. This is mirrored by a high ratio of Foxp3<sup>+</sup> cells to other lymphocytes in the hepatic infiltrate of patients with chronic infection [42]. The elevated frequency of T<sub>reg</sub> Cells may help to explain the attenuation of T cell responses against HCV infection.

Consistent with this notion, in vitro T<sub>reg</sub> cell depletion results in enhanced HCV-specific CD8<sup>+</sup> T cell responses [41]. One study indicated that the suppressive action could be attributed to secretion of TGF-β and IL-10 [32], suggesting a Th3- or Tr1-type response. A separate study corroborated the finding of elevated levels of IL-10–secreting HCV-specific Tr1-type T<sub>reg</sub> cells that recognized the same epitopes as did circulating conventional CD4<sup>+</sup> T cells in patients with chronic infection [43]. Later studies confirmed that CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells suppress HCV-specific cytokine and proliferative CD8<sup>+</sup> T cell responses in patients with chronic infection but found this suppression to be independent of IL-10 and TGF-β [44, 45]. In most reports, an elevated level of T<sub>reg</sub> cells and increased suppressive capacity were associated with chronic disease. Thus, T<sub>reg</sub> cells appear to assist in maintenance of chronic viral infection and to attenuate the tissue-damaging response to infection.

**HIV infection.** Chronic immune activation is a hallmark of HIV infection and is correlated with disease progression and CD4<sup>+</sup> T cell loss [46–50]. Activation drives several immune dysfunctions, such as T cell anergy and activation-induced cell death [46, 48, 51, 52], and facilitates viral replication. The mechanisms contributing to T cell hyperactivation in HIV disease are not well understood. T<sub>reg</sub> cells have been shown to be decreased in number in the peripheral blood of HIV-infected persons, particularly those with advanced disease [9, 53–56]. This loss of T<sub>reg</sub> cells has been proposed to be a driving factor in the hyperactivation of the immune system. In support of this idea, HIV-driven depletion of T<sub>reg</sub> cells is associated with increased CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation [9, 53].

An alternative explanation for the loss of peripheral T<sub>reg</sub> cells is recruitment of these cells to sites of HIV infection and replication, such as the mucosa and lymph nodes. Support for this hypothesis comes from various studies demonstrating an increase in T<sub>reg</sub> cell frequency in the lymph nodes [54, 57, 58] and gastrointestinal mucosa [59] of HIV-infected persons. Conflicting data come from reports of elevated T<sub>reg</sub> cell frequency in the peripheral blood of HIV-infected persons [60–63]. However, some studies may be confounded by an incomplete use of phenotypic markers to adequately distinguish T<sub>reg</sub> cells from activated CD4<sup>+</sup> T cells. Furthermore, the inclusion of patients receiving antiretroviral therapy may have skewed results in some cases. Our own unpublished data indicate that T<sub>reg</sub> cells are depleted in patients with progressive HIV disease, whereas patients who have controlled infection (as measured by CD4<sup>+</sup> T cell count and viral load) have T<sub>reg</sub> cell frequencies similar to those among uninfected control subjects (figure 1).

Robust CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses are crucial for control of HIV replication; however, as in other viral systems, these responses are subject to modulation by T<sub>reg</sub> cells. Removal of T<sub>reg</sub> cells has been shown to lead to increased HIV-specific effector T cell responses in some studies [9, 31, 53, 56, 58, 61, 64, 65]. The majority of these studies examined the effect of T<sub>reg</sub> cell depletion on CD4<sup>+</sup> or CD8<sup>+</sup> T cell proliferation or IFN-γ production after stimulation with HIV antigens, but it was recently shown that T<sub>reg</sub> cells also suppress the HIV-specific cytolytic and nonlytic antiviral response of CD8<sup>+</sup> T cells [65]. The effect of T<sub>reg</sub> cell depletion varies among HIV-infected patients. T cells from persons with high plasma viral loads or low CD4<sup>+</sup> T cell counts proliferate poorly in response to stimulation, and T<sub>reg</sub> cell depletion does not rescue the proliferative capacity of these cells [64, 65]. These data suggest that T<sub>reg</sub> cells suppress HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses early during infection but play a lesser role in patients with progressive HIV disease. This effect may be attributed to HIV-driven peripheral depletion of T<sub>reg</sub> cells [9, 53–56], redistribution of T<sub>reg</sub> cells to tissue sites of HIV replication [54, 57–59], or other immunologic or virologic factors that contribute to the observed proliferative senescence. These observations are consistent with previous studies demonstrating the unresponsive nature of T cells rendered functionally anergic because of exhaustion in patients with chronic HIV infection [66–68].

Although the majority of T<sub>reg</sub> cell depletion studies have focused on responses in peripheral blood, the suppressive effects of these cells have also been demonstrated in the lymph nodes of HIV-infected persons [58]. In cells isolated from the lymph...
nodes, T<sub>reg</sub> cell depletion enhances cytolytic activity of CD8<sup>+</sup> T cells. Unlike data from peripheral blood, T<sub>reg</sub> cells isolated from lymph nodes of patients with increasing viral load maintain suppressive activity [58], supporting the notion that functional T<sub>reg</sub> cells accumulate in lymphoid tissue during advanced HIV disease, rather than lose functionality.

HIV-specific T cell responses have been detected in uninfected children born to HIV-infected mothers [69–71]. A recent report examined T<sub>reg</sub> cells in HIV-exposed uninfected infants and neonates. HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses could be detected in the umbilical cord blood samples from exposed, uninfected newborns, and these responses were augmented on depletion of T<sub>reg</sub> cells. Furthermore, high levels of T<sub>reg</sub> cells corresponded to low levels of immune activation in the umbilical cord blood from exposed, uninfected neonates [72]. The authors suggested that T<sub>reg</sub> cells maintain a low level of immune activation in utero, allowing for an environment in which HIV-specific T cell responses can effectively block vertical transmission of the virus [72].

Overall, the role of T<sub>reg</sub> cells in HIV infection is complex and involves many variables. This role is further complicated by the fact that T<sub>reg</sub> cells are susceptible to infection due to HIV, leading to their depletion and subsequent dysregulation of the immune response [9].

THE ROLE OF T<sub>REG</sub> CELLS IN ACUTE VIRAL INFECTIONS

The common theme with regard to the role of T<sub>reg</sub> cells in chronic or persistent infections is that T<sub>reg</sub> cells decrease the effectiveness of the adaptive immune response to viral agents, allowing for the maintenance of chronic infection, while ameliorating immunopathology. In sharp contrast to the plethora of research and data regarding the role of T<sub>reg</sub> cells in chronic infections, relatively few reports focus on acute viral infections, with the exception of influenza virus infection.

As in chronic infections, depletion of T<sub>reg</sub> cells results in increased CD8<sup>+</sup> T cell proliferation [44], IFN-γ production, and cytolytic activity [73] in response to influenza virus antigens. Interestingly, in a study looking at virus-specific responses
in patients with chronic HCV infection, T<sub>reg</sub> cell–mediated suppression of responses against HCV and influenza virus antigens was stronger in patients with chronic HCV infection than in healthy patients who had recovered from HCV infection [44], suggesting that chronic disease enhances the suppressive activity of T<sub>reg</sub> cells.

In a mouse model of ocular inflammation, T<sub>reg</sub> cells played a protective role in minimizing lesion severity. In this model, expression of influenza virus hemagglutinin (HA) neoantigen was induced in the retina by gene transfer. HA-specific T cells were then adoptively transferred into the mice, followed by subcutaneous vaccination with the cognate HA peptide, leading to development of uveitis. Donor pathogenic T cells exhibiting a proinflammatory cytokine profile migrated specifically into the eye expressing HA. The disease was attenuated by administration of HA-specific T<sub>reg</sub> cells and was up-regulated in their absence [74].

The in vitro generation of T<sub>reg</sub> cells from CD4<sup>+</sup>CD25<sup>-</sup>–naive or memory T cells has become a useful tool for studying T<sub>reg</sub> cell responses to pathogens. In a study designed to examine the antigen specificity of T<sub>reg</sub> cells, influenza virus HA–specific T<sub>reg</sub> cells were generated and then activated when exposed to cognate antigen. These T<sub>reg</sub> cells were able to suppress both specific and nonspecific bystanders. These findings led to the suggestion that T<sub>reg</sub> cells induced in the periphery during an encounter with influenza virus antigen are involved in controlling the spread of the immune response by suppressing effector cells and activated bystanders [33]. The origin of signals controlling the generation and fate of these T<sub>reg</sub> cells is not clear.

**CONCLUSIONS**

The importance of T<sub>reg</sub> cells in the maintenance of immune homeostasis has been demonstrated in several models of infection. The role that these cells play in chronic and persistent infectious diseases has been an exciting topic of research in recent years. However, there is little information on how these cells control the immune response to acute viral pathogens. The host and pathogen factors that control the generation and activity of T<sub>reg</sub> cells and their role in modulating expansion, contraction, and development of immune memory in acute respiratory virus infection need to be further elucidated. The antigen-specific nature of T<sub>reg</sub> cells poses more questions. Do these cells expand and contract when exposed to cognate antigen? What form of memory is maintained, and what are the signals for homeostatic control of these cells? The study of T<sub>reg</sub> cells in acute infection is also complicated by the small antigen-specific population in peripheral blood, which likely underrepresents the effect of T<sub>reg</sub> cells at the site of viral infection, as demonstrated by several studies showing compartmentalization of T<sub>reg</sub> cells at the site of viral infection or replication in the context of HSV-2 [37], HCV [32, 42], and HIV infections [54, 57–59]. The local effects of T<sub>reg</sub> cells are difficult to study in humans because of the limited accessibility to the sites of viral infection or replication. Furthermore, the suppressive capacity of these cells in peripheral blood may not reflect the activity of T<sub>reg</sub> cells at sites of infection [58], and disease outcome may be profoundly affected by T<sub>reg</sub> cell compartmentalization [75].

Understanding the role of T<sub>reg</sub> cells in the context of chronic and acute viral infections may eventually aid in designing treatments that will limit tissue damage and in maintaining immunity to these pathogens. Indeed, clinical trials are ongoing to evaluate the efficacy of T<sub>reg</sub> cells, either expanded to treat transplant recipients experiencing graft-versus-host disease [76] or depleted to enhance antitumor responses in patients with cancer [77, 78]. These practices may eventually be applied as treatment options in a clinical setting or during vaccine administration to improve vaccination success.

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