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Do Regulatory T-Cells Play a Role in AIDS Pathogenesis?

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Abstract

The impairment of adaptive immune responses to HIV and abnormalities in the immune regulatory function mechanisms during HIV infection have been regarded as key issues in AIDS pathogenesis since the early years of the pandemic. However, the multiple mechanisms underlying this impairment are still not fully understood. New emerging information shows that alterations in the number and/or function of regulatory T-cells may contribute to HIV pathogenesis. Thus, pharmacologic manipulation of regulatory T-cells as well as blocking the activity of other immunomodulatory molecules, such as indoleamine 2,3-dioxygenase, glucocorticoid-induced tumor necrosis factor receptor and PD1, might provide a valuable approach to redirect the immune system towards an efficient antiviral response.

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Natural regulatory T-cells and immune suppression

The delicate balance between negative and positive signals is fundamental for effective immune responses able to eliminate pathogens without inducing autoimmune reactions. Regulatory T-cells (T_{reg}) are a subset of CD4+ T-cells that limit the expansion and activation of autoreactive CD4+ T-helper cells and prevent autoimmune diseases[1-5] (Fig. 1a).

Regulatory T-cells can be well characterized through a variety of different markers. Expression of high levels of the alpha chain of interleukin (IL)-2 receptor (CD25) has been used as a marker for the detection and isolation of T_{reg}[1]. In addition, reduced expression of the receptor for IL-7 (CD127) has been recently identified as a reliable phenotypic characteristic of T_{reg}[6].

Other surface molecules, such as glucocorticoid-induced tumor necrosis factor receptor (GITR), OX40 and HLA-DR, are also preferentially expressed by T_{reg}[1], but the nuclear protein forkhead box protein 3 (FOXP3), a transcription factor of the forkhead family, is considered, at present, the most accurate marker for T_{reg}[7-9]. The expression of FOXP3 has been recently found to confer to CD4+ T-cells suppressive T_{reg} activity through cooperation with the transcription nuclear factor of activated T-cells (NFAT)[10]. Expression of FOXP3 is strictly associated with immunosuppressive capacity, and ectopic expression of FOXP3 can confer suppressive activity also to non-T_{reg}[8,9]. The negative regulator of T-cell function, cytotoxic T-lymphocyte antigen 4 (CTLA4), is also expressed at constitutively high levels by T_{reg} and its expression is dependent on the FOXP3-NFAT transcription factors[10,11].

Unfortunately, all these markers, including FOXP3, can be transiently expressed by recently activated hu-

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man T-helper cells, complicating the precise identification of the T_{reg} subpopulation[12]. However, because of their strict functional association with immunosuppressive activity, both CTLA4 and FOXP3 can be considered markers of negative regulation of immune responses[12].

It has been suggested that CTLA4 directly mediates T_{reg} function, particularly through the induction of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) in antigen-presenting cells (APC)[13,14] (Fig. 1a). The IDO catalyzes the rate-limiting step of the degradation of the essential amino acid tryptophan (trp) into the kynurenine (kyn) pathway, resulting in depletion of the essential amino acid and concomitant accumulation of immunosuppressive catabolites (such as piconilic acid) in the extracellular environment[15]. Thus, IDO activation results in reduced levels of tryptophan and in a consequent block of T-cell proliferation.

Secretion of anti-inflammatory cytokines, such as transforming growth factor (TGF)-β and IL-10, has also been involved in T_{reg} function[16,17]. Interestingly, TGFβ can also function as an inducer of T_{reg} activity[18]. Indeed, TGFβ1 positively regulates the expression of FOXP3 in T-cells, which in turn results in the induction of a suppressive phenotype[18].

Although the physiologic function of T_{reg} is central for maintaining self-tolerance, as exemplified by the natural models of FOXP3 depletion, the scurfy mice, and the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome in humans, the negative regulatory activity of these cells can also be counterproductive as T_{reg} can suppress immune responses against tumors and viral infections. In contrast to natural T_{reg} of thymic origin, the existence of a class of so-called inducible T_{reg} has also been suggested. Under certain conditions, these cells can be generated in the periphery[19,20], and no clear phenotypic or functional difference has been described so far that distinguish natural T_{reg} from inducible T_{reg}. Thus, the terminology T_{reg} includes both types of CD4+CD25+FOXP3+ T_{reg}.

However, the concept that new T_{reg} can be generated from extra-thymic sites has fascinating and clinically important implications for the treatment of chronic inflammatory conditions such as cancer and persistent infections. Indeed, abnormal T_{reg} activity has been suggested as an underlying mechanism that prevents immune responses able to clear viral infection and eliminate local and metastatic neoplastic cells[20,21].

Are regulatory T-cell numbers increased or decreased in HIV/SIV infection?

The number of circulating T_{reg} has been reported to be decreased in chronically HIV-infected patients, and direct infection and killing of T_{reg} by HIV was suggested as a possible cause for their loss. These findings have led to the hypothesis that the lack of T_{reg}-mediated immunosuppression in HIV patients may favor immune activation and exacerbate HIV infection[22-25]. Accordingly, Kornfeld, et al. have shown that an increase in circulating T_{reg} occurs within 1-6 days after non-pathogenic SIV infection in African Green Monkeys, supporting a role for T_{reg} in tempering the negative consequences of chronic hyperactivation[26].

However, a different interpretation of these findings was proposed, based on the observation that the expression of T_{reg} markers FOXP3 and CTLA4 is increased in tonsils from HIV-infected patients, suggesting that altered trafficking and/or accumulation of T_{reg} into tissues could account for the decreased T_{reg} frequency in blood[27-29]. The accumulation of T_{reg} in lymphoid tissues occurs early in infection, as suggested by evidence obtained in the SIV_{mac251} macaque model of HIV infection of humans[30].

The dynamics of T_{reg} redistribution in lymphoid compartments other than lymph nodes, such as gut-associated lymphoid tissue, as well as the causes and consequences of HIV-driven T_{reg} redistribution have still not been completely explored.

Recent data in the SIV_{mac251} macaque model suggest that T_{reg} accumulation, measured as increased expression of FOXP3 and CTLA4, occurs in spleen, mesenteric lymph nodes, and gut of chronically SIV-infected macaques with high viral load (our unpublished observations). Spleen and gut, as well as lymph nodes, are known sites of active viral replication and immune activation[31-37]. Therefore, it appears that the accumulation of T_{reg} goes hand in hand with viral replication and immune activation in the same locale.

The accumulation of T_{reg} at sites of viral replication may limit hyperactivation and tissue damage, but may also prevent development of efficient adaptive antiviral immune responses and favor viral replication. Even more intriguing is the possibility of a vicious circle in which both mechanisms take place, i.e. HIV induces immune activation, driving T_{reg} into lymphoid organs, where their suppressive CTL activity allows for the establishment and maintenance of viral reservoir and production of new viruses.

An alternative hypothesis is that HIV may directly drive the expansion, migration and/or survival of T_{reg},

as suggested by the finding that exposure to reverse transcription-deficient HIV virions promotes survival of CD4+CD25+FOXP3+ T-cells[28]. Evidence for this hypothesis is also provided in two models of feline immunodeficiency virus (FIV) infection[38] and acute SIV infection[30]. The FIV induces phenotypic and functional activation of CD4+CD25+ T-cells, which acquire immunosuppressive capacity. Acute SIV infection is associated with an expansion of T_{reg} that occurs prior to development of fully mature effector T-cells. Thus, all together, these data support the notion that HIV may have evolved strategies to usurp basic immunoregulation mechanisms to favor its persistence.

Regulatory T-cells prevent efficient anti-HIV/SIV immune responses but may limit pathogenic immune activation

A role for T_{reg} in HIV pathogenesis is postulated, based on a number of reports that demonstrate that removal of CD4+CD25+ cells from peripheral leukocytes of HIV-infected patients or SIV-infected macaques *in vitro* results in an increase in HIV/SIV-specific immune response[23,24,39,40]. Moreover, data obtained in primary SIV infection in macaques also support a detrimental role of T_{reg}, since the frequency of T_{reg} inversely correlated with the magnitude of the SIV-specific CTL response[30].

However, the opposite hypothesis (i.e. that T_{reg}-mediated immunosuppression may be desirable in the setting of HIV infection) has also been postulated, based on the fact that the decreased frequency of circulating T_{reg} during HIV infection correlates with hyperactivation[25]. According to this hypothesis, the loss of T_{reg} would contribute to hyperimmune activation and expansion of the activated CD4+ T-cells, the main target for HIV infection. A caveat in this hypothesis, as mentioned before, is that the number of T_{reg} does not decrease but rather accumulates in lymphoid tissues of HIV-infected individuals. Patients with long-term nonprogressing disease with no signs of immunodeficiency despite up to 15 years of HIV infection have low numbers of T_{reg} cells in multiple lymphoid compartments, further supporting the notion that T_{reg} prevent efficient anti-HIV responses[28].

Data obtained in the nonpathogenic SIV infection in African green monkeys, however, also suggested a protective role of T_{reg} during acute infection, since TGFβ1, FOXP3, and IL-10 are induced very early after infection and are thought to limit virus-driven inflammation[26]. Therefore, the data provided so far by correlative studies conducted in SIV and HIV infection do not allow for a definitive conclusion on the detrimental or beneficial role of T_{reg} in infection.

In vivo studies have been performed to directly address the relative importance of immune activation and negative regulation. Rhesus macaques were treated with anti-CD40L and CTLA4-Ig to block CD28 co-stimulation (Fig. 1b) in order to decrease the adaptive immune response to SIV and limit immune activation[41]. In the first four weeks after infection, these treatments resulted in a lower and slightly delayed peak of plasma virus levels. Interestingly, the peak of viremia directly correlated to the fraction of cycling CD4+ T-cells (Ki67+) on the tenth day after infection, thus supporting a possible detrimental role for immune activation in the early phases of infection[41]. However, animals treated with the co-stimulatory blockers showed highest viral loads in the post-peak phase and more rapid progression to AIDS[41]. The fast disease progression was correlated with reduced anti-SIV cellular and humoral response[41].

The soluble CTLA4-Ig used by Garber, et al. may have not only blocked the CD28-B7 interactions (Fig. 1b), but also could have elicited a direct immunosuppressive effect through the induction of IDO[42,43]. The levels of IDO were not measured in that study[41], but IDO may be a key mediator in preventing anti-HIV/SIV responses. Indeed, in a mouse model for HIV-associated encephalitis, blockade of IDO with 1-methyl tryptophan (1MT) was proven to improve anti-HIV cell-mediated immunity[44]. Antiretroviral-induced immune reconstitution may simply be normalization of T_{reg} and not on account of the marginal increase in CD4 numbers in the periphery.

The opposite approach was also tested in macaques by administration of an anti-human-CTLA4 blocking antibody (Fig. 1c). These macaques were chronically infected with SIV_{mac251} and treated with antiretroviral therapy (ART). The CTLA4 blockade resulted in a decrease of TGFβ and IDO expression and, surprisingly, it also decreased SIV-RNA levels in tissues. These events were associated with augmented SIV-specific CD8+ T-cell responses in the treated macaques[40]. This latter study suggests that at least in chronic infection, blocking T_{reg} or negative regulation is not detrimental and may even confer immunologic and virologic benefit.

Altogether, the findings suggest that the balance of positive and negative regulators of immune responses is probably fundamental. On the one hand, T_{reg}-mediated immunosuppression may prevent efficient anti-SIV/HIV responses early during acute infection but, on the other hand, T_{reg} may limit viral replication within days from infection by inhibiting CD4+ T-cell activation and proliferation.

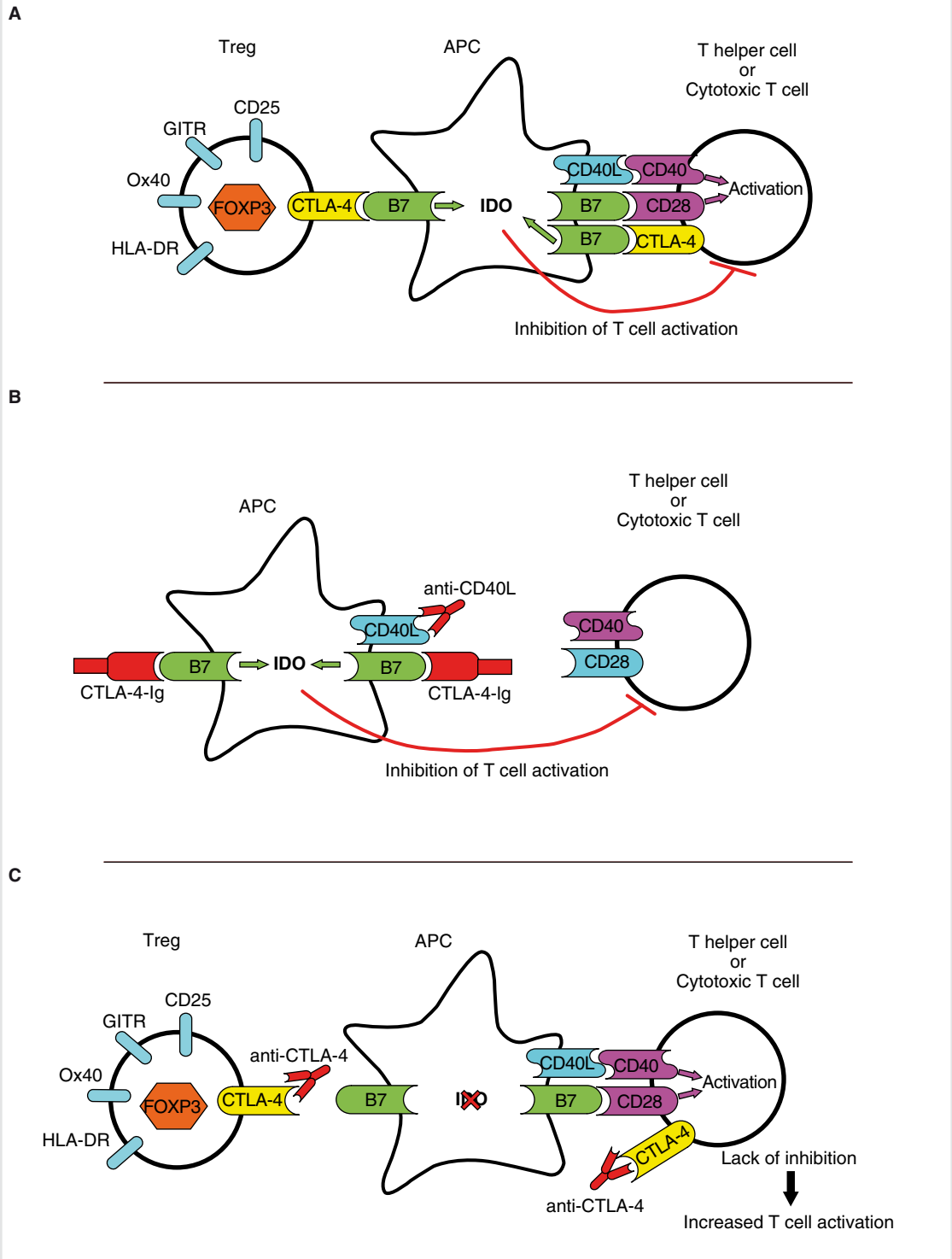

Figure 1. ***A:*** *physiologic balance between immune activation and immune suppression. T-lymphocytes are activated by APC through CD40-CD40L and B7-CD28 interaction; T_{reg} inhibit T-cell activation by inducing IDO in APC through CTLA4-B7 interaction; CTLA4 is also transiently expressed on T-cells after activation.* ***B:*** *co-stimulation blockade inhibits T-cell activation. Antibodies against CD40L and soluble CTLA4-Ig prevent co-stimulation of T lymphocytes; CTLA4-Ig binds to B7 and induces APC to express IDO, which in turn inhibits T-cell activation (Garber, et al., J Exp Med 2004).* ***C:*** *CTLA4 blockade enhances T-cell activation. Antibodies against CTLA4 prevent IDO induction in APC by CTLA4 expresses on T_{reg} and activated T-cells, thus favoring T-cell activation* (Hryniewicz, et al., Blood 2006).

Specific inhibition of anti-HIV/SIV immune cells or general immunosuppression

The mechanisms of antigen-specific or non antigen-specific inhibition by T_{reg} are still far from being understood. Removal of CD25+ T_{reg} from peripheral leukocytes of HIV-infected patients *in vitro* results in increased response not only to HIV antigens, but also to alloantigens and recall antigens such as cytomegalovirus[23,24]. Thus, although most studies have focused on the effect of T_{reg} on the response to a particular antigen of interest, such as HIV/SIV, it is not yet clear whether the increased T_{reg} activity observed in chronic infections, and HIV/SIV in particular, may result in a general suppression of T-cell responses to antigens other than HIV.

Multifunctional T-cell responses are impaired during HIV/SIV disease, and this impairment is detected also in patients who do not show a reduction in the number of circulating CD4+ T-cells[45-47]. These defects include reduced IL-2 production and T-cell proliferation in response to recall antigens, as well as impaired cytotoxic activity, not limited to HIV-specific T-cells[46-49]. The suppressive activity of T_{reg} appears to be non-antigen specific *in vitro*, as responses to mitogens can be enhanced by removal of CD4+CD25+ cells from PBMC of either HIV-infected or uninfected patients[24]. However, T_{reg} activity may be targeted to specific antigens *in vivo*, namely those antigens recognized by the T-cell receptor of T_{reg}.

The activated phenotype of natural T_{reg} populations in various models of infection also suggests the existence of antigen-specific T_{reg}. Supporting this hypothesis, T_{reg} purified from HIV-infected patients produce large amounts of IL-10 in response to p24 antigen[39]. Similar data (specific activation of T_{reg} in response to microbial antigens) have also been obtained in *H. pylori*-infected individuals and in HCV-infected patients[50,51]. Moreover, recent evidence from the murine model of leishmania infection has shown that T_{reg} are able to respond specifically to foreign antigens[52], further suggesting the possibility that HIV infection may induce HIV-specific T_{reg}.

During HIV infection, the accumulation and activation of T_{reg} in lymphoid tissues may contribute to a local immunosuppression that goes beyond the inhibition of anti-HIV responses. In particular, infected patients show a progressive loss of responsiveness to recall antigens first, then alloantigens, and finally mitogen stimulation, making it possible to speculate on the progressive acquisition of suppressive specificities during the course of infection[48]. This raises the possibility that HIV/SIV infection may lead to a progressive, uncontrolled tolerance towards nonself antigens.

Studies on the actual specificity of T_{reg}, both in physiologic conditions and during chronic infections, are still not conclusive, and more investigation is required to explore this possibility.

Potential clinical interventions

The ultimate effect of the imbalance of T_{reg} that occurs in HIV/SIV is still a controversial issue. As mentioned above, it has not been clearly established whether the T_{reg}-mediated down-regulatory activity is beneficial or deleterious, or if it has different outcomes in different stages of the disease. In addition, the molecular bases of T_{reg}-mediated immunoregulation are still under investigation. Thus, the modalities of possible interventions, as well as their potential outcome, are extremely difficult to predict.

Because of their ambiguous phenotype, which cannot be clearly defined by one marker, depletion of T_{reg} *in vivo* appears hard to achieve. More realistic is the interference with their regulatory activity, which can be obtained by blocking the immunosuppressive molecules that mediate it.

An attempt to inhibit CTLA4 inhibitory activity in human has been made in patients with malignant melanoma[53-58]. Administration of an antihuman-CTLA4 blocking antibody proved to effectively reduce the tumor mass in approximately 20% of patients[55,57]. Interestingly, reduction of tumor size was coupled to the development of autoimmune syndromes[53,55,57,58]. The CTLA4 blockade, used in combination with ART in SIV-infected macaques, reduced viral RNA levels in the lymph nodes during the time the animals were on ART, and was associated with an increase in antiviral responses that was nevertheless insufficient to modify the extent of viral rebound after cessation of ART[40]. These findings are in line with the demonstration that blocking co-stimulation by the administration of soluble CTLA4-Ig (in combination with anti-CD40L) worsened the course of SIV infection[41].

It is noteworthy, however, that the co-stimulation blockade in that study had a slightly positive outcome in the early phase of acute infection[41]. Thus, it is possible that positive and negative stimulation of immune responses may have contrasting effects, depending on the stage of disease.

An important observation in the study by Hryniewicz, et al. is that despite the anti-CTLA-mediated re-

duction in tissue viral RNA during ART, the rebound of plasma viral load after cessation of ART in animals treated with ART and anti-CTLA4 did not differ from animals treated with ART alone[40], suggesting that CTLA4 blockade may be effective only if associated with efficient control of viral replication by ART. An alternative explanation is that the balance between effector and suppressor functions is highly dynamic. The short treatment with CTLA4 blockade might have only temporarily shifted that balance.

Tryptophan catabolism by IDO was suggested as a major mediator of T_{reg} activity[13]. Moreover, HIV can directly induce IDO in macrophages[59,60] and plasmacytoid dendritic cells (Boasso, et al., manuscript submitted) without requiring mediation by T_{reg}. Thus, targeting IDO-mediated immunosuppression is an intriguing possibility. A powerful antagonist of IDO, 1MT, has been used *in vivo* in mouse models for enhancing both antitumor and anti-HIV responses[15,44]. Whether this approach is feasible in humans requires careful consideration.

Conclusions

The impairment of the adaptive immune responses to HIV and alteration of immune regulatory function mechanisms during HIV/SIV infection have been regarded as a key issue in AIDS pathogenesis since the early years of the pandemic. However, the multiple mechanisms underlying this impairment are not fully understood. It is now emerging that alterations in the number and/or function of T_{reg} may contribute to HIV pathogenesis. Thus, pharmacologic manipulation of T_{reg} as well as blocking the activity of other immunomodulatory molecules, such as IDO, and other molecules not described here, including GITR and PD1 (blocking GITR has not been shown to abrogate T_{reg} function, although the use of GITR agonistic antibodies has been shown to override T_{reg}-mediated suppression, possibly by making T-effectors insensitive to T_{reg}-mediated suppression)[61-63], could provide valuable approaches to redirect the immune system towards an efficient antiviral response. However, the biology of natural and acquired T_{reg} and their role in HIV disease need to be further explored in order to provide basic information on the modality and the time of intervention.

The objective of immunotherapy for HIV is to improve the adaptive immune response with the ultimate goal of eliminating HIV-1-infected T-cells. Immunomodulatory signals offer new targets to improve the manipulation of negative immune modulators that may affect both the anti-HIV immune responses as well as responses to other pathogens in HIV-1-infected individuals.

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