Published in final edited form as:

Curr Opin HIV AIDS. 2014 September ; 9(5): 492–499. doi:10.1097/COH.0000000000000093.

Animal models for viral infection and cell exhaustion

Colleen S. McGary, Guido Silvestri, and Mirko Paiardini

Emory Vaccine Center and Yerkes National Primate Research Center, Emory University, Atlanta, GA

Abstract

Purpose of review—Despite eliciting an early antiviral T cell response, HIV-specific T cells are unable to prevent disease progression, partly due to their loss of effector functions, known as T cell exhaustion. Restoring this T cell functionality represents a critical step for regaining immunological control of HIV-1 replication, and may be fundamental for the development of a functional cure for HIV. In this context, the use of animal models is invaluable for evaluating the efficacy and mechanisms of novel therapeutics aimed at reinvigorating T cell functions.

Recent findings—While non-human primates continue to be a mainstay for studying HIV pathogenesis and therapies, recent advances in humanized mouse models have improved their ability to recapitulate the features of cell exhaustion during HIV infection. Targeting coinhibitory receptors in HIV- and SIV-infected animals has resulted in viral load reductions, presumably by reinvigorating the effector functions of T cells. Additionally, studies combining PD-1 blockade with suppressive ART provide further support of the use of coinhibitory receptor blockades in restoring T cell function by delaying viral load rebound upon ART interruption. Future *in vivo* studies should build on recent *in vitro* data supporting the simultaneous targeting of multiple regulators of cell exhaustion.

Summary—In this review, we describe the most recent advances in the use of animal models for the study of cell exhaustion following HIV/SIV infection. These findings suggest that the use of animal models is increasingly critical in translating immunotherapeutics into clinical practice.

Keywords

SIV; non-human primates; humanized mice; animal models; PD-1

Introduction

Chronic viral infections are characterized by the persistence of antigen, which often contributes to the progressive loss of T cell function known as T cell exhaustion (1, 2). Virus-specific T cells gradually lose their ability to proliferate and produce multiple cytokines, and thus become unable to effectively inhibit viral replication and eliminate the pathogen from the host (1). Initial studies from the murine model of lymphocytic choriomeningitis (LCMV) demonstrated that signaling through inhibitory receptors, such as

*Corresponding Author: Dr. Mirko Paiardini, Division of Microbiology and Immunology, Yerkes National Primate Research Center, Department of Pathology & Laboratory Medicine, Emory University School of Medicine, 929 Gatewood Rd, Atlanta, GA 30329, mirko.paiardini@emory.edu, phone: 404-727-9840 fax: 404-727-7768.

programmed death-1 (PD-1), is a key mechanism in limiting the function of CD8⁺ T cells (3). Since this description, increased levels of inhibitory receptors on virus-specific T cells have been found in the context of a number of chronic viral infections, including HIV-1 infection of humans and SIV infection of rhesus macaques (RMs), and these levels have been found to correlate with viral load and declining CD4 T cell count, two measures of disease severity (4–10). Therefore, numerous strategies have been explored to reverse this T cell exhaustion and increase T cell functionality in the context of HIV infection. Studies using non-human primate and humanized mouse models have become increasingly relevant for evaluating the therapeutic potential of these reversion strategies in controlling HIV/SIV replication. Moreover, as HIV-1 eradication becomes a key priority in contemporary HIV/AIDS research, these animal models will also be critical in testing pre-clinically the ability of novel immune-based interventions to promote a functional cure.

Here, we review the recent studies in animal models that investigated the impact of blocking PD-1 signaling, the best characterized co-inhibitory receptor, in controlling viral replication and restoring T cell function during HIV and SIV infection. We then discuss work that investigates these co-inhibitory blockades as therapeutic and possibly curative agents. Finally, we comment on recent *in vitro* data that supports the use of targeting multiple mechanisms of T cell exhaustion, through combination therapy, and the future of translating these therapies into animal models and clinical practice.

Cell exhaustion in animal models of HIV/SIV

Virus-specific CD8⁺ T cells are critical for the control of virus replication. Due to the inability of HIV-specific CD8 T cells to control HIV infection, as evidenced by the chronic viremia present in the majority of HIV-infected individuals, HIV-specific CD8⁺ T cells were the primary focus of initial studies investigating cell exhaustion during HIV infection. Early studies in the RM model of SIV infection confirmed that SIV infection elicits an early and vigorous SIV-specific CD8⁺ T cell response; however, these CD8⁺ T cells are unable to prevent disease progression, consistent with human HIV infection (11–14). Through the demonstration of loss of cytokine production (namely, IL-2 and IFN- γ), cytotoxic activity and ability to proliferate, SIV-specific CD8⁺ T cells were found to become “exhausted” during the chronic phase of SIV infection, and thus, validated the use of RMs in the study of HIV/SIV pathogenesis. Since then, the non-human primate model has been utilized to identify cellular and molecular mechanisms that regulate the function, and dysfunction, of T cells during SIV infection.

Studies in both humans and non-human primates have demonstrated that signaling through co-inhibitory receptors is one of the main mechanisms contributing to the induction of CD8⁺ T cell exhaustion during HIV/SIV infection. PD-1, one of the prototypic inhibitory receptors, is upregulated following TCR activation and signals a negative feedback mechanism to inhibit further T cell activation and proliferation (3, 15). HIV-specific CD8⁺ T cells have increased levels of PD-1 on their surface, which correlate with impaired CD8⁺ T cell function and measures of disease progression (4, 7, 9). Similarly, PD-1 expression is heightened on SIV-specific CD8⁺ T cells (8, 10). PD-1-expressing CD8⁺ T cells, in these RM studies, were found to have an impaired ability to proliferate and were increasingly

prone to apoptosis. However, the ability of PD-1 to be induced simply upon T cell activation (16, 17), as well as its expression on T cells from healthy individuals (18), has recently caused a renewed interest in the use of PD-1 expression as a marker for T cell exhaustion.

In an effort to delineate the role of PD-1 expression during SIV/HIV infection, Hong *et al.* longitudinally examined the co-expression of PD-1 and Ki-67, a marker of T cell proliferation, on RM T cells during SIV infection (19)*. Consistent with previous studies, SIV-specific CD8+ T cells were found to have reduced proliferative capacity after chronic SIV infection, as determined by the absence of Ki-67 expression, which correlated with PD-1 expression levels (19)*. Yet, increased PD-1 expression was not exclusive to SIV-specific cells. In fact, the frequency of non-proliferating CD8+PD-1+ T cells did not increase during chronic infection, while that of proliferating CD8+PD-1+ T cells did. This finding suggests a model where PD-1 identifies activated, and not simply exhausted cells, during SIV infection. Nevertheless, T cell exhaustion involves the gradual loss of multiple functions- not simply proliferation; therefore, the implications of this study are limited by a lack of coinciding data examining additional T cell dysfunctions as a result of SIV infection.

While the mechanisms regulating the exhaustion of CD8+ T cells have been well investigated, less work has been done to understand the regulation of CD4+ T cell dysfunction during SIV/HIV infection. Similar to CD8+ T cells, HIV-specific CD4+ T cells largely lose their chief functions- namely, proliferation and cytokine secretion- early in the course of infection (20, 21). This functional impairment also is associated with the increased expression of co-inhibitory receptors on CD4+ T cells (6, 22). Recent work has demonstrated that concurrent expression of multiple coinhibitory factors (PD-1, CTLA-4, and TIM-3) on CD4+ T cells is more strongly associated with lack of virus control than individual expression alone (23). Similarly, CD4+ T cells express elevated levels of PD-1 during SIV infection of RMs, which has been linked to the functional impairment of CD4+ T cells due to their increased proliferative capacity as a result of *in vitro* PD-1 blockade (10). The close phenotypic and functional attributes of T cells during HIV and SIV infection have made the SIV/RM model the leading animal model in evaluating therapies aimed at restoring T cell function, controlling virus replication, and ultimately slowing SIV disease progression.

PD-1 blockade during the natural history of HIV/SIV infection

The relationship between PD-1 overexpression and T cell exhaustion during HIV/SIV infection has made the use of *in vivo* PD-1 blockade a promising therapeutic option to facilitate T cell functional restoration (Table 1). The efficacy of PD-1 blockade was initially demonstrated in LCMV-infected mice, in which blocking the PD-1 receptor reinvigorated effector CD8+ T cells to eventually clear the chronic infection (3). Early *in vitro* studies extended the ability of PD-1 blockade to rescue the functions of both HIV- and SIV-specific CD8+ and CD4+ T cells, thus providing rationale for the first *in vivo* trials of PD-1 blockade in the rhesus macaque model of SIV infection (9, 10). Blockade of the PD-1 pathway *in vivo* resulted in increased SIV-specific T cell proliferation and cytokine production, SIV-specific B-cell responses, and remarkably prolonged survival of SIV-infected RMs (27). The therapeutic benefit of PD-1 blockade was further demonstrated in a follow-up study showing

that PD-1 blockade in SIV-infected RMs reduced immune activation by dampening pro-inflammatory signals and partially improving mucosal integrity (28). These studies have generated increased interest in determining the optimal dosing and staging of PD-1 blockade to best restore antiviral immunity, but also in evaluating its efficacy in inhibiting HIV-1 replication.

One early limitation to testing PD-1 blockade in SIV-infected RMs was the lack of an available recombinant macaque PD-1 antibody. In fact, the partially humanized PD-1 blocking antibody rapidly disappeared from the blood of the treated RMs as a result of xenogeneic antibody responses (27). To overcome this limitation, Amancha *et al.* recently developed a fully primate rPD-1-Fc fusion protein that is nonimmunogenic and able to block PD-1 ligation similarly to previous blocking agents (26)**. Administration of this PD-1 blockade resulted in enhanced proliferation of SIV-specific CD4+ T cells, as well as an increased frequency of polyfunctional SIV-specific CD8+ T cells. However, this restoration of T cell function was insufficient to affect virus levels in the PD-1-blockade treated RMs, similar to a separate study of PD-1 blockade on SIV-infected RMs (29). It is unclear whether this result was due to higher overall viral load set points in this cohort of SIV-infected RMs, compared to those tested by Velu *et al.* (27), or if the treatment regimen was not optimal for the full restoration of antiviral T cell functionality.

Despite the immunologic and virologic parallels between HIV infection in humans and SIV infection in RMs, the effects of PD-1 blockade on SIV replication cannot be always assumed to mimic its effects on HIV-1 replication. Studying HIV-1 infection *in vivo* has also relied on the use of humanized mouse models, given the limited species tropism of HIV (30). Yet, early humanized mouse models were limited in their ability to mount adaptive immune responses (31). The recent generation of improved humanized mouse models that exhibit many clinical manifestations of HIV-1 disease, in addition to generating HIV-specific T cell responses, has created a new model for evaluating novel therapies for HIV-1 directly *in vivo* (32–36). Two recent studies utilized these new models to investigate the efficacy of PD-1 blockade on HIV-1 replication *in vivo* (24, 25) (Table 1). Palmer *et al.* treated HIV-1-infected humanized BALB/c-Rag2^{-/-}Yc^{-/-} mice (Rag-hu), mice in which human CD34+ hematopoietic stem cells are engrafted to generate human T, B and dendritic cells, with a human anti-PD-L1 mAb (24, 37). Consistent with human HIV-1 disease, both CD4+ and CD8+ T cells expressed high levels of PD-1 after HIV infection in these animals, thus making them amenable to an impact by PD-1 blockade. Anti-PD-L1 treatment resulted in a significant reduction in HIV-1 plasma viral load and increased the levels of human CD4+ T cells in the HIV-infected mice, which suggests that blocking PD-1 signaling successfully restored HIV-specific T cell responses to a threshold able to suppress virus replication (24)**. Seung *et al.* similarly demonstrated a decrease in HIV-1 viral loads in chronically HIV-infected, BLT (Bone marrow-Liver-Thymus) humanized mice treated with anti-PD-1 antibody (25)*. However, the effect on viral load was exclusive to BLT mice whose CD8+ T cells expressed high levels of PD-1. This observation could be a result of increased levels of HIV-specific CD8+ T cells, which would support the ability of PD-1 blockade to restore antiviral functionality in these animals. However, neither study was able to directly investigate the frequencies of HIV-specific CD8+ T cells or the mechanism of viral

suppression in these animals (24, 25)**. Nevertheless, both studies successfully illustrate that PD-1 blockade can effectively reduce HIV-1 replication *in vivo* in humanized mice, thus providing further support to the use of co-inhibitory receptor blockades in restoring T cell functionality during HIV-1 infection.

Altogether, these recent studies agree with previous literature in animal models that supports the use of PD-1 blockade to help restore antiviral immunity in HIV-infected individuals. Extending the use of PD-1 blockade to the treatment of HIV-infected individuals, though, will require further studies in animal models to best determine the schedule, dosing, and specificity of PD-1 blockade for optimal restoration of T cell functionality and subsequent viral control. The safety of humanized PD-1-blocking mAbs has recently been demonstrated in the context of late-stage metastatic malignancies, thus paving the way for future clinical trials of PD-1 blockade during HIV infection (38–41). Additionally, these studies demonstrate the capacity of these animal models to model HIV-1 infection, and therefore, validate their use in evaluating the efficacy of novel immunotherapeutics.

PD-1 blockade during suppressive antiretroviral therapy

Despite the success of antiretroviral therapy (ART) in suppressing virus replication and dramatically decreasing the mortality and morbidity associated with HIV infection, a therapy that is able to eradicate the virus is still unavailable (42, 43). One of the major barriers to HIV eradication is the presence of a persistent reservoir of latently infected cells that are not eliminated by ART (44, 45). Therefore, identifying novel strategies that are able to reduce the size of the latent HIV reservoir, in addition to restoring antiviral immune function to T cells, represents a major priority in contemporary AIDS research.

Because a functional cure can only be achieved through restored immune control of the virus (in the absence of ART), interventions that reinvigorate exhausted T cells have become a major focus of HIV-1 cure research. The success of PD-1 blockade in restoring T and B cell functionality in SIV-infected RMs, while having a moderate impact on viremia, has provided support to the idea of combining this intervention with ART in order to improve the function of exhausted antiviral T cells (27). In essence, the rationale is that these reinvigorated HIV-specific CD8+ (and CD4+) T cells will have a renewed capacity to suppress virus replication, thereby preventing disease progression even upon ART interruption. Recent studies in SIV-infected RMs have shown that combining PD-1 blockade with ART results in a delayed viral rebound upon ART interruption (26, 46) (Table 2). Amancha *et al.* found that only when SIV-infected RMs were treated with both rPD-1-Fc and ART (PMPA/racivir) did animals show slower rebound in plasma viral loads as compared to ART-treated RMs alone (26)**. While drawing similar conclusions to the overall restoration potential of PD-1 blockade during SIV infection, Vargas-Inchaustegui *et al.* found slightly different responses based on treatment strategy (46)*. This study targeted PD-1 by using B7-DC-Ig, a fusion protein which acts primarily on cells expressing high levels of PD-1 on their surface (46, 48)*, and combined this blockade with ART in chronically SIV-infected RMs. After ART interruption, animals which had received PD-1 blockade only throughout ART had similar viral rebounds to those treated with ART alone; however, animals which continued to receive PD-1 blockade upon ART interruption

experienced reduced rebound viremia (46)*. In both studies, therefore, it can be speculated that improved functional T cell activities contribute to the slower rate of viral rebound. Nevertheless, the results of these studies are of somewhat difficult interpretation due to the lack of full viral suppression by ART prior to its interruption, which may contribute to persistent antigen exposure and T cell exhaustion despite ART.

A recent study by Mason *et al.* investigated the ability of PD-1 blockade to restore SIV-specific T cell function, and thus facilitate host control of viral replication, in the setting of full viral suppression in ART-treated, SIV-infected RMs (47)**. In this study, viral rebound was delayed in half of the PD-1-blockade-treated RMs, compared to untreated controls, following ART interruption. Remarkably, a quarter of the animals receiving PD-1 blockade continued to have undetectable viral loads for up to four weeks following ART interruption (Table 2). These results support a mechanism in which PD-1 blockade is responsible for the restoration of SIV-specific T cell functionality, allowing for partial control of virus replication and reduction of infected cells. However, all animals treated with PD-1 blockade eventually experienced viral rebound (47)***, as did the less suppressed animals discussed above (26, 46)***, suggesting that better control of viral replication will be necessary before PD-1 blockade can be hypothesized as a curative strategy. Achieving this level of immune control will likely require the careful combination of therapies that can reduce latent viral reservoirs with those that can reinvigorate antiviral T cell responses.

Combination therapy: the new frontier to restoring T cell functionality

Targeting multiple mechanisms involved in T cell exhaustion is an attractive strategy to more broadly reinvigorate the antiviral immune response in the setting of HIV infection. Apart from PD-1, several other coinhibitory molecules have been shown to regulate T cell responses, and be expressed at elevated levels, during chronic viral infections (5, 15, 49). Increased levels of TIM-3, LAG-3, CD160, and 2B4 have been found on HIV-specific CD8+ T cells during chronic infection; likewise, HIV-specific CD4+ T cells have elevated levels of CTLA-4 and TIM-3 on their surface (5–7, 50–54). Particularly in the context of CD8+ T cell dysfunction, greater co-expression of multiple coinhibitory receptors has been associated with more advanced stages of T cell exhaustion (5, 55). Additionally, inhibitory molecules are under the control of different signaling networks (56–58). Therefore, given the success of other coinhibitory receptor blockades in enhancing T cell proliferation and function *in vitro* (5, 6, 55, 59), future studies that test the efficacy of dual and triple co-inhibitory molecule blockades *in vivo* will be critical to determine if targeting multiple molecular networks can better rescue T cell function during HIV/SIV infection.

The induction of regulatory CD4+ T cells (Treg) has been proposed as an additional mechanism contributing to CD8+ T cell dysfunction during HIV infection (60). Increases in Tregs have been reported in both chronic HIV and SIV infections, potentially enhancing viral replication through the suppression of antiviral effector T cell functions (60–65). However, inhibiting Treg function using CTLA-4 blockade in SIV-infected RMs resulted in increased viral replication and was unable to restore virus-specific immune responses, but instead, resulted in increased T cell activation and more pronounced CD4+ T cell loss in these animals (66). This finding may be a result of CTLA-4 blockade acting not only on

Tregs, but also on activated CD4+ T cells expressing CTLA-4. Yet, murine models of chronic retroviral infections have found that transient, selective ablation of Tregs is able to reactivate previously exhausted CD8+ T cells (67). Therefore, a recent study by Dietze *et al.* investigated whether combining transient Treg depletion with two coinhibitory blockades (against PD-L1 and TIM-3) could restore CD8+ T cell function and reduce viral loads in a murine model of chronic retrovirus infection (68)*. These authors found that, while blocking inhibitory receptors had the more significant effect on restoring CD8+ T cell function compared to Treg depletion, the combination of the two therapies increased the CD8+ T cell response greater than any single treatment alone. While this particular combination approach may be difficult to translate into the treatment of HIV-infected individuals, this study provides important proof-of-concept that one can simultaneously target different mechanisms of cell exhaustion and synergize these approaches to better restore antiviral T cell responses.

Building on data demonstrating that blocking the IL-10 pathway can increase HIV-specific CD4+ T cell function (69–71), Porichis *et al.* evaluated the impact of combining PD-L1 blockade with IL-10R α blockade on restoring HIV-specific T cell function *in vitro* (72)**. Dual blockade significantly increased IFN- γ production by HIV-specific CD4+ T cells, compared to either treatment alone, suggesting that the simultaneous targeting of these two pathways can synergistically restore T helper cell function. However, it is unclear whether IFN- γ secretion will be advantageous in controlling HIV-1 viral replication. Furthermore, they found that the combined blockade could still increase cytokine production by HIV-specific CD4+ T cells from ART-treated subjects, though to a lesser extent than in T cells isolated from viremic individuals (72)**. Therefore, future studies will be necessary to expand these data and determine if these combined blockades can aid in restoring T cell function, as well as purging latent viral reservoirs, *in vivo*. Taken together, these early studies provide essential support for the next-generation of therapeutic strategies that combine blockades which target multiple regulatory pathways, and thus, more effectively combat T cell exhaustion in the setting of HIV-1 infection.

Conclusion

Restoring HIV-specific T cell functionality by reversing exhaustion represents a critical step in regaining immunological control of HIV-1 replication and could prove essential for the attainment of a functional cure. As the mechanisms regulating cell exhaustion during HIV infection become more clear, in particular with respect to the role of co-inhibitory receptors such as PD-1, non-human primate and humanized mouse models will become increasingly important for evaluating the efficacy of novel therapeutic strategies targeting these mechanisms. The latest data discussed here suggest that co-inhibitory receptor blockades, namely PD-1 blockade, can reinvigorate CD8+ T cells and delay viral rebound *in vivo*, and that these effects may be amplified by targeting multiple pathways of inhibition. Determining the optimal combination of blockades, in addition to schedule and dosing, should be of high priority in future animal studies.

Acknowledgments

This work was supported by Public Health Service (PHS) grants RR000165/OD011132 to the Yerkes National Primate Research Center. In addition, M.P. is supported by grants from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01-AI110334 and R21-AI104278. G.S. is supported by grants from the NIAID/NIH under award numbers R37 AI-066998 and R01-AI090797.

References

1. Wherry EJ. T cell exhaustion. *Nature immunology*. 2011; 12(6):492–499. [PubMed: 21739672]
2. Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. *Journal of virology*. 2004; 78(11):5535–5545. [PubMed: 15140950]
3. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006; 439(7077):682–687. [PubMed: 16382236]
4. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*. 2006; 443(7109):350–354. [PubMed: 16921384]
5. Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, et al. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. *The Journal of experimental medicine*. 2008; 205(12):2763–2779. [PubMed: 19001139]
6. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, Miura T, et al. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. *Nature immunology*. 2007; 8(11):1246–1254. [PubMed: 17906628]
7. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *The Journal of experimental medicine*. 2006; 203(10):2281–2292. [PubMed: 16954372]
8. Petrovas C, Price DA, Mattapallil J, Ambrozak DR, Geldmacher C, Cecchinato V, et al. SIV-specific CD8+ T cells express high levels of PD1 and cytokines but have impaired proliferative capacity in acute and chronic SIVmac251 infection. *Blood*. 2007; 110(3):928–936. [PubMed: 17440051]
9. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nature medicine*. 2006; 12(10):1198–1202.
10. Velu V, Kannanganat S, Ibegbu C, Chennareddi L, Villinger F, Freeman GJ, et al. Elevated expression levels of inhibitory receptor programmed death 1 on simian immunodeficiency virus-specific CD8 T cells during chronic infection but not after vaccination. *Journal of virology*. 2007; 81(11):5819–5828. [PubMed: 17376899]
11. Kuroda MJ, Schmitz JE, Charini WA, Nickerson CE, Lifton MA, Lord CI, et al. Emergence of CTL coincides with clearance of virus during primary simian immunodeficiency virus infection in rhesus monkeys. *Journal of immunology*. 1999; 162(9):5127–5133.
12. Hel Z, Nacsa J, Kelsall B, Tsai WP, Letvin N, Parks RW, et al. Impairment of Gag-specific CD8(+) T-cell function in mucosal and systemic compartments of simian immunodeficiency virus mac251- and simian-human immunodeficiency virus KU2-infected macaques. *Journal of virology*. 2001; 75(23):11483–11495. [PubMed: 11689630]
13. Vogel TU, Allen TM, Altman JD, Watkins DI. Functional impairment of simian immunodeficiency virus-specific CD8+ T cells during the chronic phase of infection. *Journal of virology*. 2001; 75(5):2458–2461. [PubMed: 11160749]
14. Xiong Y, Luscher MA, Altman JD, Hulsey M, Robinson HL, Ostrowski M, et al. Simian immunodeficiency virus (SIV) infection of a rhesus macaque induces SIV-specific CD8(+) T cells with a defect in effector function that is reversible on extended interleukin-2 incubation. *Journal of virology*. 2001; 75(6):3028–3033. [PubMed: 11222730]
15. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nature immunology*. 2009; 10(1):29–37. [PubMed: 19043418]

16. Kinter AL, Godbout EJ, McNally JP, Sereti I, Roby GA, O'Shea MA, et al. The common gamma-chain cytokines IL-2, IL-7, IL-15, and IL-21 induce the expression of programmed death-1 and its ligands. *Journal of immunology*. 2008; 181(10):6738–6746.
17. Salisch NC, Kaufmann DE, Awad AS, Reeves RK, Tighe DP, Li Y, et al. Inhibitory TCR coreceptor PD-1 is a sensitive indicator of low-level replication of SIV and HIV-1. *Journal of immunology*. 2010; 184(1):476–487.
18. Duraiswamy J, Ibegbu CC, Masopust D, Miller JD, Araki K, Doho GH, et al. Phenotype, function, and gene expression profiles of programmed death-1(hi) CD8 T cells in healthy human adults. *Journal of immunology*. 2011; 186(7):4200–4212.
19. Hong JJ, Amancha PK, Rogers K, Ansari AA, Villinger F. Re-evaluation of PD-1 expression by T cells as a marker for immune exhaustion during SIV infection. *PloS one*. 2013; 8(3):e60186. [PubMed: 23555918] * This study expands the definition of PD-1 in the context of SIV infection to both activated and exhausted T cells.
20. Palmer BE, Boritz E, Blyveis N, Wilson CC. Discordance between frequency of human immunodeficiency virus type 1 (HIV-1)-specific gamma interferon-producing CD4(+) T cells and HIV-1-specific lymphoproliferation in HIV-1-infected subjects with active viral replication. *Journal of virology*. 2002; 76(12):5925–5936. [PubMed: 12021325]
21. Younes SA, Yassine-Diab B, Dumont AR, Boulassel MR, Grossman Z, Routy JP, et al. HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. *The Journal of experimental medicine*. 2003; 198(12):1909–1922. [PubMed: 14676302]
22. D'Souza M, Fontenot AP, Mack DG, Lozupone C, Dillon S, Meditz A, et al. Programmed death 1 expression on HIV-specific CD4+ T cells is driven by viral replication and associated with T cell dysfunction. *Journal of immunology*. 2007; 179(3):1979–1987.
23. Kassu A, Marcus RA, D'Souza MB, Kelly-McKnight EA, Golden-Mason L, Akkina R, et al. Regulation of virus-specific CD4+ T cell function by multiple costimulatory receptors during chronic HIV infection. *Journal of immunology*. 2010; 185(5):3007–3018.
24. Palmer BE, Neff CP, Lecureux J, Ehler A, Dsouza M, Remling-Mulder L, et al. In vivo blockade of the PD-1 receptor suppresses HIV-1 viral loads and improves CD4+ T cell levels in humanized mice. *Journal of immunology*. 2013; 190(1):211–219. ** This study is the first of its kind to demonstrate the use of humanized mouse models for investigating the efficacy of novel therapies for HIV-1 infection, and importantly illustrates the potential of PD-1 blockade to reduce HIV-1 replication *in vivo*.
25. Seung E, Dudek TE, Allen TM, Freeman GJ, Luster AD, Tager AM. PD-1 blockade in chronically HIV-1-infected humanized mice suppresses viral loads. *PloS one*. 2013; 8(10):e77780. [PubMed: 24204962] * This study demonstrates the efficacy of PD-1 blockade in reducing HIV-1 viral loads in an improved humanized mouse model, and shows the specificity of this blockade in restoring T cell functionality by only impacting animals with high levels of PD-1 expression.
26. Amancha PK, Hong JJ, Rogers K, Ansari AA, Villinger F. In vivo blockade of the programmed cell death-1 pathway using soluble recombinant PD-1-Fc enhances CD4+ and CD8+ T cell responses but has limited clinical benefit. *Journal of immunology*. 2013; 191(12):6060–6070. ** This study describes the efficacy of the first fully primatized PD-1 blocking agent in SIV-infected rhesus macaques, and supports a model in which PD-1 blockade, in combination with ART, can partially restore T cell functionality and subsequently delay viral rebound following ART interruption.
27. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature*. 2009; 458(7235):206–210. [PubMed: 19078956]
28. Dyavar Shetty R, Velu V, Titanji K, Bosinger SE, Freeman GJ, Silvestri G, et al. PD-1 blockade during chronic SIV infection reduces hyperimmune activation and microbial translocation in rhesus macaques. *The Journal of clinical investigation*. 2012; 122(5):1712–1716. [PubMed: 22523065]
29. Finnefrock AC, Tang A, Li F, Freed DC, Feng M, Cox KS, et al. PD-1 blockade in rhesus macaques: impact on chronic infection and prophylactic vaccination. *Journal of immunology*. 2009; 182(2):980–987.

30. Nomaguchi M, Doi N, Kamada K, Adachi A. Species barrier of HIV-1 and its jumping by virus engineering. *Reviews in medical virology*. 2008; 18(4):261–275. [PubMed: 18386279]
31. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. *Nature reviews Immunology*. 2012; 12(11):786–798.
32. Berges BK, Wheat WH, Palmer BE, Connick E, Akkina R. HIV-1 infection and CD4 T cell depletion in the humanized Rag2-/-gamma c-/- (RAG-hu) mouse model. *Retrovirology*. 2006; 3:76. [PubMed: 17078891]
33. Brainard DM, Seung E, Frahm N, Cariappa A, Bailey CC, Hart WK, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. *Journal of virology*. 2009; 83(14):7305–7321. [PubMed: 19420076]
34. Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. *Blood*. 2006; 108(2):487–492. [PubMed: 16410443]
35. Tonomura N, Habiro K, Shimizu A, Sykes M, Yang YG. Antigen-specific human T-cell responses and T cell-dependent production of human antibodies in a humanized mouse model. *Blood*. 2008; 111(8):4293–4296. [PubMed: 18270327]
36. Watanabe S, Terashima K, Ohta S, Horibata S, Yajima M, Shiozawa Y, et al. Hematopoietic stem cell-engrafted NOD/SCID/IL2Rgamma null mice develop human lymphoid systems and induce long-lasting HIV-1 infection with specific humoral immune responses. *Blood*. 2007; 109(1):212–218. [PubMed: 16954502]
37. Traggiai E, Chicha L, Mazzuchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science*. 2004; 304(5667):104–107. [PubMed: 15064419]
38. Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2008; 14(10):3044–3051. [PubMed: 18483370]
39. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010; 28(19):3167–3175. [PubMed: 20516446]
40. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *The New England journal of medicine*. 2013; 369(2):134–144. [PubMed: 23724846]
41. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *The New England journal of medicine*. 2012; 366(26):2443–2454. [PubMed: 22658127]
42. Bhaskaran K, Hamouda O, Sannes M, Boufassa F, Johnson AM, Lambert PC, et al. Changes in the risk of death after HIV seroconversion compared with mortality in the general population. *JAMA : the journal of the American Medical Association*. 2008; 300(1):51–59.
43. Hogg R, Lima V, Sterne JAC, Grabar S, Battegay M, Bonarek M, et al. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet*. 2008; 372(9635):293–299. [PubMed: 18657708]
44. Eisele E, Siliciano RF. Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity*. 2012; 37(3):377–388. [PubMed: 22999944]
45. Richman DD, Margolis DM, Delaney M, Greene WC, Hazuda D, Pomerantz RJ. The challenge of finding a cure for HIV infection. *Science*. 2009; 323(5919):1304–1307. [PubMed: 19265012]
46. Vargas-Inchaustegui DA, Xiao P, Hogg AE, Demberg T, McKinnon K, Venzon D, et al. Immune targeting of PD-1(hi) expressing cells during and after antiretroviral therapy in SIV-infected rhesus macaques. *Virology*. 2013; 447(1–2):274–284. [PubMed: 24210124] * This study observed that

continuous administration of PD-1 blockade was critical for the maintenance of T cell functionality and reducing viral rebound in SIV-infected rhesus macaques.

47. Mason S. Dual approach to HIV-1 cure: Activation of latency and restoration of exhausted virus-specific T cell function. 6th HIV Persistence Workshop. 2013 Dec 5. Miami, FL 2013. ** This study demonstrated the capacity of PD-1 blockade to both restore SIV-specific T cell functionality and cause a significant delay in viral rebound in the setting of full viral suppression in SIV-infected rhesus macaques, thereby supporting the use of coinhibitory receptor blockades in combination with ART for an HIV-1 functional cure.
48. Mkrtichyan M, Najjar YG, Raulfs EC, Liu L, Langerman S, Guittard G, et al. B7-DC-Ig enhances vaccine effect by a novel mechanism dependent on PD-1 expression level on T cell subsets. *Journal of immunology*. 2012; 189(5):2338–2347.
49. Larsson M, Shankar EM, Che KF, Saeidi A, Ellegard R, Barathan M, et al. Molecular signatures of T-cell inhibition in HIV-1 infection. *Retrovirology*. 2013; 10:31. [PubMed: 23514593]
50. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, et al. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nature immunology*. 2002; 3(11):1097–1101. [PubMed: 12368911]
51. Leng Q, Bentwich Z, Magen E, Kalinkovich A, Borkow G. CTLA-4 upregulation during HIV infection: association with anergy and possible target for therapeutic intervention. *Aids*. 2002; 16(4):519–529. [PubMed: 11872994]
52. Peritt D, Sesok-Pizzini DA, Schretzenmair R, Macgregor RR, Valiante NM, Tu X, et al. C1.7 antigen expression on CD8+ T cells is activation dependent: increased proportion of C1.7+CD8+ T cells in HIV-1-infected patients with progressing disease. *Journal of immunology*. 1999; 162(12):7563–7568.
53. Price P, Keane N, Gray L, Lee S, Gorry PR, French MA. CXCR4 or CCR5 tropism of human immunodeficiency virus type 1 isolates does not determine the immunological milieu in patients responding to antiretroviral therapy. *Viral immunology*. 2006; 19(4):734–740. [PubMed: 17201668]
54. Sakhdari A, Mujib S, Vali B, Yue FY, MacParland S, Clayton K, et al. Tim-3 negatively regulates cytotoxicity in exhausted CD8+ T cells in HIV infection. *PloS one*. 2012; 7(7):e40146. [PubMed: 22792231]
55. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet JP, Bordi R, et al. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. *PLoS pathogens*. 2012; 8(8):e1002840. [PubMed: 22916009]
56. Che KF, Shankar EM, Muthu S, Zandi S, Sigvardsson M, Hinkula J, et al. p38 Mitogen-activated protein kinase/signal transducer and activator of transcription-3 pathway signaling regulates expression of inhibitory molecules in T cells activated by HIV-1-exposed dendritic cells. *Molecular medicine*. 2012; 18:1169–1182. [PubMed: 22777388]
57. Muthumani K, Choo AY, Shedlock DJ, Laddy DJ, Sundaram SG, Hirao L, et al. Human immunodeficiency virus type 1 Nef induces programmed death 1 expression through a p38 mitogen-activated protein kinase-dependent mechanism. *Journal of virology*. 2008; 82(23):11536–11544. [PubMed: 18799583]
58. Oestreich KJ, Yoon H, Ahmed R, Boss JM. NFATc1 regulates PD-1 expression upon T cell activation. *Journal of immunology*. 2008; 181(7):4832–4839.
59. Jin HT, Anderson AC, Tan WG, West EE, Ha SJ, Araki K, et al. Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(33):14733–14738. [PubMed: 20679213]
60. Weiss L, Donkova-Petrini V, Caccavelli L, Balbo M, Carbonneil C, Levy Y. Human immunodeficiency virus-driven expansion of CD4+CD25+ regulatory T cells, which suppress HIV-specific CD4 T-cell responses in HIV-infected patients. *Blood*. 2004; 104(10):3249–3256. [PubMed: 15271794]
61. Boasso A, Vaccari M, Hryniewicz A, Fuchs D, Nacs J, Cecchinato V, et al. Regulatory T-cell markers, indoleamine 2,3-dioxygenase, and virus levels in spleen and gut during progressive simian immunodeficiency virus infection. *Journal of virology*. 2007; 81(21):11593–11603. [PubMed: 17715231]

62. Epplé HJ, Loddenkemper C, Kunkel D, Troger H, Maul J, Moos V, et al. Mucosal but not peripheral FOXP3+ regulatory T cells are highly increased in untreated HIV infection and normalize after suppressive HAART. *Blood*. 2006; 108(9):3072–3078. [PubMed: 16728694]
63. Nilsson J, Boasso A, Velilla PA, Zhang R, Vaccari M, Franchini G, et al. HIV-1-driven regulatory T-cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS. *Blood*. 2006; 108(12):3808–3817. [PubMed: 16902147]
64. Nixon DF, Aandahl EM, Michaelsson J. CD4+CD25+ regulatory T cells in HIV infection. *Microbes and infection / Institut Pasteur*. 2005; 7(7–8):1063–1065. [PubMed: 15893493]
65. Tsunemi S, Iwasaki T, Imado T, Higasa S, Kakishita E, Shirasaka T, et al. Relationship of CD4+CD25+ regulatory T cells to immune status in HIV-infected patients. *Aids*. 2005; 19(9):879–886. [PubMed: 15905668]
66. Cecchinato V, Trynieszewska E, Ma ZM, Vaccari M, Boasso A, Tsai WP, et al. Immune activation driven by CTLA-4 blockade augments viral replication at mucosal sites in simian immunodeficiency virus infection. *Journal of immunology*. 2008; 180(8):5439–5447.
67. Dietze KK, Zelinskyy G, Gibbert K, Schimmer S, Francois S, Myers L, et al. Transient depletion of regulatory T cells in transgenic mice reactivates virus-specific CD8+ T cells and reduces chronic retroviral set points. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(6):2420–2425. [PubMed: 21262821]
68. Dietze KK, Zelinskyy G, Liu J, Kretzmer F, Schimmer S, Dittmer U. Combining regulatory T cell depletion and inhibitory receptor blockade improves reactivation of exhausted virus-specific CD8+ T cells and efficiently reduces chronic retroviral loads. *PLoS pathogens*. 2013; 9(12):e1003798. [PubMed: 24339778] * This study observed an enhanced restoration of T cell functionality in a murine model of chronic retrovirus infection by simultaneously blocking coinhibitory receptors and transiently depleting regulatory T cells, which supports a novel approach of combining therapies that target independent mechanisms of T cell exhaustion to treat chronic viral infections.
69. Brockman MA, Kwon DS, Tighe DP, Pavlik DF, Rosato PC, Sela J, et al. IL-10 is up-regulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. *Blood*. 2009; 114(2):346–356. [PubMed: 19365081]
70. Clerici M, Wynn TA, Berzofsky JA, Blatt SP, Hendrix CW, Sher A, et al. Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus. *The Journal of clinical investigation*. 1994; 93(2):768–775. [PubMed: 8113410]
71. Kwon DS, Angin M, Hongo T, Law KM, Johnson J, Porichis F, et al. CD4+ CD25+ regulatory T cells impair HIV-1-specific CD4 T cell responses by upregulating interleukin-10 production in monocytes. *Journal of virology*. 2012; 86(12):6586–6594. [PubMed: 22496237]
72. Porichis F, Hart MG, Zupkosky J, Barblu L, Kwon DS, McMullen A, et al. Differential impact of PD-1 and/or interleukin-10 blockade on HIV-1-specific CD4 T cell and antigen-presenting cell functions. *Journal of virology*. 2014; 88(5):2508–2518. [PubMed: 24352453] ** This study provides the first demonstration that combined blockade of PD-L1 and IL-10Rα synergistically improves T cell functionality following HIV infection in vitro, and advances the concept of targeting multiple regulators of T cell exhaustion to more effectively restore antiviral T cell functionality in vivo.

Key Points

1. Non-human primate and humanized mouse models continue to be utilized to investigate factors that regulate the function of T cells during HIV/SIV infection, and are invaluable for the translation of therapies targeting T cell exhaustion.
2. Improved humanized mouse models can effectively model T cell exhaustion during HIV infection, and recent studies have demonstrated their validity in investigating the efficacy of therapies directly on HIV-1 replication *in vivo*.
3. The use of coinhibitory receptor blockades in combination with fully suppressive antiretroviral therapy (ART) as a strategy for HIV-1 functional cure is supported by recent studies in SIV-infected rhesus macaques, in which animals undergoing such treatment experienced delayed viral rebound upon ART interruption.
4. Combination therapies, in which multiple mechanisms involved in T cell exhaustion are targeted, likely represent the next-generation of strategies aimed at fully restoring T cell functionality and viral control, and will likely require the future use of testing in animal models of HIV/SIV infection.

Table 1

Summary of the main findings from testing coinhibitory receptor blockades in the context of active HIV/SIV replication in animal models from the annual period of review.

		Main Effects				
Study	Intervention	Viral Load	T cell count	T cell functionality	Main advantages of the animal model	
Humanized mice:						
Palmer et al. (24)	Anti-PD-L1 mAb	Decrease	Increases in CD4 and CD8	<i>Did not measure</i>	<ul style="list-style-type: none">• Ability to directly measure impact of PD-1 blockade on HIV-1 replication	
Seung et al. (25)	Anti-PD-1 mAb	Decrease only in PD-1-high expressing animals	Increase in CD8	<i>Did not measure</i>	<ul style="list-style-type: none">• Can longitudinally assess immunological parameters in peripheral blood	
Rhesus macaques:						
Amancha et al. (26)	Primatized rPD-1-Fc fusion protein	No change	<i>Did not report</i>	Increase (CD8)	<ul style="list-style-type: none">• Ability to longitudinally assess impact of PD-1 blockade in gut tissues• Mechanisms of cellular exhaustion closely mimic those seen in HIV-1 infection of humans	

Table 2

Summary of the main findings from testing coinhibitory receptor blockades in the context of viral suppression by ART in non-human primate models from the annual period of review.

Study	Intervention	Main Effects			Main advantages of the animal model
		Viral Rebound	T cell proliferation	T cell functionality	
Rhesus macaques:					
Amancha et al. (26)	PD-1 blockade (see above) + ART	Delayed rebound (vs. ART alone)	Increase (CD8)	Increase (CD8; trend in CD4)	<ul style="list-style-type: none">• Ability to perform controlled ART interruption experiments• Combination ART can achieve full suppression of viremia
Vargas-Inchaustegui et al. (46)	PD-1 blockade + ART	Reduced rebound viremia only with continual blockade	Decrease in Tregs	Decrease in Gag-specific cytokine function	
Mason et al. (47)	PD-1 blockade + ART	Delayed rebound	Did not measure	Did not measure	