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Role of PD-1 in HIV Pathogenesis and as Target for Therapy

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Abstract

Major advances in antiviral therapy (ART) have resulted in a dramatic decline in HIV-related deaths. However, no current treatment regimen leads to viral eradication or restoration of HIV-specific immune responses capable of durable viral control after cessation of ART. Thus, there is a need for novel interventions that could complement ART in order to eliminate virus or reach a state of “functional cure”. It has been shown in murine models and humans that the negative co-signaling molecule programmed-death 1 (PD-1) plays an active and reversible role in mediating T cell exhaustion in chronic infections. This review summarizes recent advances in our understanding of the PD-1 pathway in HIV infection, and the lessons learned from studies in the SIV model and cancer. We discuss the potential of immunotherapeutic interventions targeting PD-1 in order to augment immune responses or facilitate viral eradication. We also present the challenges to therapies targeting immunoregulatory networks.

Keywords

HIV; AIDS; chronic viral infection; T cell exhaustion; programmed-death 1 (PD-1); programmed death-ligand 1 (PD-L1); programmed death-ligand 2 (PD-L2); CD4 T cell; CD8 T cell; monocytes; B cell; viral reservoirs; viral eradication; elite controllers; immunotherapy; mucosal immunity

Introduction

Two major barriers for the eradication of HIV infection are the inexistence of an effective vaccine and the failure of antiretroviral therapy (ART) to clear the pathogen and restore immune responses capable of suppressing viral replication after cessation of treatment. In the setting of chronic viral infections that are not controlled by the immune system, such as HIV, persistent viral antigenemia leads to progressive loss of T cell functions such as T cell proliferation and cytokine secretion in a process called “T cell exhaustion” that leads to ineffective immune responses and defective viral clearance [1-4]. Identifying the underlying mechanisms of T cell dysfunction during HIV infection may provide crucial information for future vaccine design as well as novel targets for therapeutic interventions in order to improve current treatment strategies. Studies in both animal models and humans have demonstrated that suppression of immune functions through upregulation of inhibitory pathways contributes to an active and potentially reversible T cell impairment in the setting of chronic antigen exposure. Since the initial reports highlighting the role of one of these immunoregulatory molecules, programmed-death 1 (PD-1), as a major factor in T cell

exhaustion [5-7], significant progress has been made in our understanding of the complex network of molecular events that shape T cell dysfunction in HIV infection. Here we discuss recent advances in the understanding of the role of PD-1 in the immune dysregulation associated with HIV infection along with novel data suggesting that PD-1 may also be contributing to maintenance of latent HIV infection in memory CD4 T cells. We discuss manipulation of the PD-1 pathway as a potential therapeutic strategy to augment immune responses against HIV, improve general immune restoration, facilitate viral eradication or boost HIV vaccines. We comment on lessons learned from animal models and cancer trials, and present the challenges to therapies targeting immunoregulatory networks in HIV infection. Table 1 summarizes the main findings on the PD-1 pathway in HIV infection that are discussed in this review

PD-1 as a regulator of T cell T cell activation and peripheral tolerance

One of the main features of the immune system is its ability to maintain self-tolerance through complex and finely tuned immunoregulatory networks. These pathways include cytokines that regulate immune responses and receptors found on the surface of antigen presenting cells (APC) and T lymphocytes [8]. Upon antigen recognition, T cells receive two set of signals given by cell surface molecules [9]. The first signal derives from the T cell receptor (TCR) and confers the specificity of the immune response. The second signal derives from co-stimulatory molecules found in the immune synapse that can be either activating or inhibitory receptors [10]. Additionally, several cytokines (sometimes referred to as “third signal”) further modulate cell activity to make productive cellular responses and avoid death and/or tolerance induction [11]. The fate of the T cell response is shaped by the balance between co-stimulatory and co-inhibitory signals that regulate T cell activation and tolerance. PD-1 is one of the inhibitory molecules that contribute to this regulation. A member of the B7:CD28 family of coreceptors, PD-1 interacts with its ligands, B7-H1 (PD-L1) and B7-DC (PD-L2), to give a signal to the PD-1-expressing cell (direct signal) or to the cell expressing its ligands (reversed signaling) [12-14]. Whereas early models of the pathway defined PD-1 as expressed on T cells, PD-L1 broadly expressed in different tissues and PD-L2 selectively expressed on antigen-presenting cells (APCs), subsequent studies identified more complex phenotypic and functional patterns for the molecules of the PD-1 pathway. For example, PD-1 is expressed on monocytes and PD-L1 on activated T cells [10] and also regulate their functions. Thus, modulation of the PD-1 pathway *in vivo* is likely to affect a large number of cell types with complex consequences. Under physiological conditions, PD-1 is induced after T cell activation and serves as an inhibitory feedback mechanism to dampen the TCR signaling cascade and prevent excessive T cell activation, leading to inactivation of TCR signaling, cell cycle arrest, reduced cytokine production and decreased glucose metabolism. PD-1 also plays an important role in peripheral tolerance to self-antigens by promoting the development of regulatory T cells and inhibiting potentially pathogenic self-reactive T cells [10]. Of note, PD-1 is expressed on a significant fraction of functional T cells under physiological conditions in healthy individuals, including both CD8 T cells [15] and CD4 T cells (in particular a subset called T follicular helper cells that are important for development of antibody responses [16]).

The role of PD-1 in immune tolerance is illustrated by the development of autoimmune diseases in PD-1 deficient mice [17,18], and is an important consideration with regard to potential side effects of PD-1 blockade to treat human diseases. However, compared to CTLA-4, a related co-inhibitor of the B7:CD28 family that is also a target for immunotherapy of cancer [19], PD-1 seems to play a larger role in regulating immune defenses against infectious agents and to have a smaller impact on immune tolerance, as shown by the milder autoimmune phenotype of PD-1 compared to CTLA-4-deficient mice [20].

PD-1 in HIV-specific CD8 T cells—Several lines of evidence suggest that effective HIV-specific CD8 T cells play an important role in viral suppression in the rare subjects who control viral load in the absence of therapy (HIV controllers, reviewed in [21]). The role of the PD-1 pathway in mediating pathogen-specific CD8 T cell dysfunction in chronic viral infections was first demonstrated in the mouse model of LCMV (lymphocytic choriomeningitis virus) [22]. These landmark studies showed that PD-1 was expressed at high levels on virus-specific CD8 T cells in chronic infection and that preventing the interaction of PD-1 with its ligands with a blocking antibody resulted in improved T cell function and reduction in viral loads in the infected mice. These experiments thus showed the causal role of PD-1 in T cell exhaustion in chronic infections, and provided a proof of principle that inhibition of the PD-1 pathway has potential applications in the treatment of chronic infections. These results present strong analogies with the role of PD-1 in animal tumor models [23-25], consistent with the fact that in cancer, like in chronic infection, antigen persistence and T cell dysfunction worsen one another. A subsequent study showed that blockade of the PD-1 pathway in combination with therapeutic vaccination synergistically enhanced LCMV-specific CD8 T cell responses and had a greater impact on viral control compared to administration of the PD-1 blocking antibody or the vaccine alone [26]. These data suggest that manipulation of the PD-1 pathway may also have a role as adjuvant to enhance the efficacy of therapeutic or prophylactic vaccines.

Findings on the role of PD-1 in CD8 T cell exhaustion in chronically infected mice were quickly extended to major chronic viral infections in humans, including HIV [5-7,27], HCV [28,29] HBV [30,31] and in SIV infection in Rhesus macaques [32,33]. These findings in monkeys are important, as they provide an animal model close to HIV for preclinical studies of the PD-1 blockade. The first series of reports in HIV infection showed that PD-1 was expressed in high amounts on HIV-specific CD8 T cells [5-7,27] and that the expression of PD-1 on HIV specific CD8 T cells was correlated with parameters of disease progression, directly with viral loads and inversely with CD4 counts. Longitudinal analysis of PD-1 levels before and after antiretroviral treatment (ART) showed that control of viremia on successful therapy reduced the levels of PD-1 on HIV-specific CD8 T cells, indicating that antigen specific TCR stimulation is a determinant for PD-1 expression [5,6]. In accordance with that, studies in humans [34] and in SIV infection in monkeys [32,35] showed that PD-1 expression gradually declined on virus-specific CTLs targeting epitopes that had undergone mutational escapes. Conversely, another report [36] showed that CD8 T cells that bind to cognate HIV antigens with high affinity express more PD-1. Whether PD-1 blockade would have more or less of an impact on these high-affinity CD8 T cells *in vivo* remains to be determined and may have consequences on the quality and immunodominance pattern of virus-specific immune responses in HIV-infected subjects.

PD-1 blockade with a PD-L1 blocking antibody *in vitro* demonstrated the functional impact of this pathway by restoring proliferation of HIV-specific CD8 T cells and increasing the percentage of HIV-specific CTLs capable of producing cytokines at the end of the period of *in vitro* expansion [5,6]. As indicated by its name, PD-1 has also been implicated in cell death. Petrovas *et al* showed that PD-1 expression on virus-specific CTLs renders them susceptible to both spontaneous and FAS-mediated apoptosis [7]. Additional studies are required to determine if PD-1 blockade *in vivo* increases HIV-specific T cell survival.

Results of these studies suggest that blockade of the PD-1 pathway rescue function only in a fraction of the PD-1-expressing CD8 T cells. These findings suggest that other factors besides PD-1 contribute to the dysfunction of a large fraction of the exhausted HIV-specific CD8 T cells and thus make them unresponsive to blockade of the PD-1 pathway alone. Recent studies in the LCMV model confirmed this hypothesis by showing that several inhibitory molecules besides PD-1 are also upregulated on virus-specific CD8 T cells,

including the receptors 2B4, LAG-3, CD160 [37••] and TIM-3 [38]. The more dysfunctional the cells, the more inhibitory receptors they accumulate, consistent with the fact that exhaustion is a gradual process. These data suggest that blockade of multiple inhibitory molecules may be more effective than single blockade of the PD-1 pathway, and this was confirmed in the LCMV model in mice in which the combination of PD-1 and LAG-3 blockade [37••] and PD-1 and Tim-3 blockade [39•] augmented both the function of the virus-specific CD8 T cells and viral control. PD-1 inhibition can also synergize with blockade of an inhibitory cytokine like IL-10 in this mouse model [40]. Consistent with these findings, co-expression of inhibitory receptors was recently demonstrated on HIV-specific CD8 T cells [38,41•,42•], although with some phenotypic differences in the phenotypic patterns compared to mice. The majority of HIV-specific CD8 T cells co-express PD-1 and 2B4 (CD244), whereas about half of them also co-express CD160. This co-expression pattern had functional consequences: simultaneous blockade of the PD-1 and 2B4 pathways was more effective in restoring proliferation of HIV-specific CTLs in response to the cognate antigen *in vitro* compared to single blockade [41•]. It is currently unclear whether the low levels of LAG-3 observed on HIV-specific CD8 T cells in these reports illustrate virus-specific differences or reflect suboptimal sensitivity of the reagents used. Whether such combined approaches will be clinically relevant in HIV infection or result in excessive toxicity is unknown at the present time.

A general shortcoming of most studies in HIV infection is that the immunologic parameters used to assess virus-specific T cell responses have usually been selected on the basis of knowledge previously acquired in animal models or other human diseases. Although these studies have provided valuable information they will not by design, discover mechanisms that are not already known to be involved in regulating T cell immunity. The so-called “system biology” approaches can provide additional information by integrating complex data sets in order to discover new genes involved in the control of immune responses and to understand the molecular events elicited by PD-1 and other modulators of immune functions. This has been illustrated in a recent report [43••] in which gene expression at the messenger RNA (mRNA) level was established for the entire genome of HIV-specific CD8 T cells from viremic, chronically infected individuals and subjects who spontaneously control viral replication in the absence of therapy (HIV controllers). The determination of these “transcriptional profiles” allowed detailed molecular comparison of exhausted and functional virus-specific T cells. Using *in vitro* models, the authors were able to define the pattern of the genes regulated by PD-1, and thus to identify novel molecules that are potentially important for the activity of this pathway in HIV infection. In particular, they showed that PD-1 upregulated a transcription factor called BATF (for basic leucine transcription factor, ATF-like), which can have broad impact on T cell differentiation and function [44-47]. Inhibiting BATF expression with specific small interfering RNAs (siRNAs) restored proliferation and cytokine secretion in HIV-specific CD8 T cells *in vitro*. BATF knockdown also increased IL-2 secretion by HIV-specific CD4 T cells in viremic subjects, suggesting a significant role of BATF in CD4 T cell dysregulation [43••]. This study therefore offers a proof-of-principle that the combination of genetic studies and functional assays (“functional genomics”) can identify new molecular events involved in immune impairment in HIV infection. Such approaches are likely to be increasingly used in the near future, and can pinpoint potential new targets for therapeutic interventions.

To which extent PD-1 blockade leads to a qualitative improvement of HIV-specific CD8 T cells besides improving proliferative capacity remains to be determined. In particular, the potential effect of the PD-1 pathway on CTL killing capacity is of high relevance for clinical interventions and needs to be tested in future studies.

PD-1 and HIV-specific CD4 T cell responses—Even though dysregulation of HIV-specific CD4 T cell responses is a major feature of HIV infection, most of the studies in the field have been focused on HIV-specific CD8 T cells. CD4 T cells help orchestrate both the cellular and humoral arms of the immune response and may play a critical role in the control of HIV replication [48]. It is therefore imperative to further characterize the mechanisms that govern HIV-specific CD4 T cell dysfunction in order to rationally design effective vaccines and novel therapeutic interventions against HIV.

Earlier studies showed that PD-1 was upregulated on HIV-specific CD4 T cells [49,50] and, similar to HIV-specific CD8 T cells, that its expression was directly correlated with viremia and inversely with CD4 count [49]. Also consistent with the findings on CD8 T cells, blockade of the PD-1 pathway with a blocking anti-PD-L1 antibody restored HIV-specific CD4 T cell proliferation *in vitro* [6,49,50]. Further studies, however, demonstrated significant differences in the mechanisms regulating the CD4 and the CD8 T cell subsets. Besides PD-1, CTLA-4 is also a mediator of HIV-specific CD4 T cell dysfunction, yet it does not seem to play a major role in CD8 T cell impairment [50]. HIV-specific CD4 T cells do not express significant amounts of 2B4, CD160 or LAG-3 [42•]. A report showed that co-expression of PD-1, CTLA-4 and another molecule implicated in T cell exhaustion, TIM-3 [51•] on HIV-specific CD4 T cells correlated more strongly with viral load as compared to individual molecules, suggesting that like CD8 T cells, exhausted CD4 T cells accumulate inhibitory receptors as their exhaustion progresses. Interestingly, the authors found that in contrast to HIV-specific CTLs, a large fraction of HIV-specific CD4 T cells that co-express the three inhibitory receptors also express the costimulatory molecule CD28. Blockade of the PD-1 pathway concurrent with stimulation of CD28 resulted in greater proliferation *in vitro* than did either manipulation alone [51•]. These results thus show that combining blockade of an inhibitory pathway and activation of a stimulatory pathway can further enhance HIV-specific CD4 T helper cell responses. However, manipulation of CD28 currently appears an unlikely target for immune intervention, given the serious side effects caused by administration of a CD28 agonist in humans [52].

An important question not addressed in these early studies of the role of PD-1 in HIV-specific CD4 T cells was whether the impact of PD-1 blockade was limited to expansion of HIV-specific CD4 T cells or if this intervention could also revive CD4 T cell function independently of its impact on T cell proliferation. As a main role of CD4 T helper cells is to produce a variety of cytokines to orchestrate the immune responses, we therefore investigated in a recent report the impact of PD-1 blockade on cytokines production by HIV-specific CD4 T cells [42•]. Upon encounter of CD4 T cells with cognate viral antigens, blockade of the PD-1 pathway with an anti-PD-L1 antibody *in vitro* enhanced secretion of cytokines corresponding to several differentiated Thelper subsets that are known to mediate different kinds of functions *in vivo*. Although the impact of PD-L1 blockade on cytokine production and, to a lesser extent, cell proliferation was associated with markers of disease progression (CD4 count, viral load), restoration of cytokine secretion was also observed in most subjects with undetectable viremia [42•]. Yet, only clinical trials can prove that blockade of the PD-1 pathway in subjects on ART is capable of improving CD4 T cell responses beyond what is achievable by antiviral treatment. Such immune reconstitution may further impact the quality of the CD8 T cell and B cell subsets given the critical role of CD4 T cells in providing help to other lymphocyte subsets.

The PD1 pathway and antigen-presenting cells (APCs) in HIV infection—

Excessive immune activation is a hallmark of HIV infection. As a result, homeostatic immunoregulatory pathways, including PD-1/PD-L1, are up-regulated in an effort to reduce this ongoing inflammation. Expression of the PD-1 ligands, and especially PD-L1, has been shown to be elevated in HIV infection [53,54] compared to HIV-negative controls. ART

treatment was found to down-regulate PD-L1 expression on monocytes [55] and myeloid dendritic cells [56]. However, it also appears that HIV can itself modulate expression of molecules of the PD-1 pathway. HIV inactivated AT-2 virions can up-regulate PD-L1 expression through IFN- α production [57]. A recent study showed that incubation of macrophages with HIV AT-2 virions *in vitro* can cause up-regulation of PD-L1 and PD-L2 [58]. Surprisingly, they observed that incubation with IL-10 increased expression of PD-L1 but not PD-L2. Consistent with this study, HIV was shown to induce up-regulation of both PD-L1 and PD-L2 on macrophages and dendritic cells through the accessory HIV protein Nef [59]. They also showed that knockdown of the PD-1 ligands using siRNAs on antigen presenting cells (APCs) restored function of HIV-specific CD8 T cells. All these studies suggest that HIV can interfere with the function of APCs by up-regulating expression of the PD-1 ligands on APCs. These are significant findings, as impairment of APC functions will not only impact HIV-specific immune responses, but may globally affect T cell responses in the HIV-infected host.

Microbial translocation from the gut to the blood during HIV infection likely plays an important role in hyperimmune activation [60]. A recent study established a link between these microbial products and the general immune dysregulation caused by the PD-1 and IL-10 pathways during HIV infection [61••]. The authors demonstrated that microbial products induce expression of the PD-1 receptor on monocytes. Triggering of PD-1 expressed on monocytes by PD-L1 expressed on various cell types induced production of IL-10, an inhibitory cytokine, and led to reversible CD4 T cell dysfunction. These observations thus identified a new mechanism by which PD-1 contribute to immune impairment in HIV infection and explained how damage to the gut could upregulate two major inhibitory pathways (PD-1 and IL-10) besides causing immune activation. Therefore, it is apparent that during HIV infection there is multifactorial up-regulation of PD-1 and its ligands that contributes to the general immune dysregulation and not only to impaired HIV-specific responses.

Does PD-1 overexpression contribute to failure of immune restoration on antiviral therapy?—Combination antiretroviral therapy has dramatically changed the prognosis of HIV infection by improving immune responses against opportunistic pathogens. However, immunologic failure, defined as the failure to achieve and maintain an adequate CD4 response despite virologic suppression, occurs in a minority of subjects. A persistently low CD4 count while on suppressive ART is associated with a risk of AIDS and non-AIDS-related morbidity and mortality [62,63]. Recently, two studies have shown that high levels of PD-1 expression on the total CD4 and CD8 T cell subsets were associated with failure of immune reconstitution after successful viral control on ART [64,65]. Whether PD-1 is the cause or the effect of the observed low CD4 T cell counts needs to be determined. Should PD-1 up-regulation be a contributing factor to the persistent immune defect, manipulation of the PD-1 pathway could have a potential as adjuvant therapy to foster immune restoration in these patients.

Of note, subjects with immune reconstitution inflammatory syndrome (IRIS), which is defined as worsening or unmasking infections or tumors after ART initiation, showed increased PD-1 expression on their CD4 T cells [66]. In this acute setting, PD-1 upregulation should likely been seen as an attempt of the immune system to control excessive immune activation and not as a sign of exhaustion.

Beyond immune responses: HIV latency and the potential role of PD-1 as a target for viral eradication

Failure of current antiretroviral therapy to clear the pathogen is mainly attributed to persistence of HIV DNA as an integrated genome in long-lived or slowly dividing cellular reservoirs. It has been shown that despite complete or near complete inhibition of viral replication with standard therapies, replication-competent HIV persists indefinitely in infected individuals [67••]. The main hurdle to eradication of HIV is a small pool of latently infected memory CD4 T cells that harbor the virus and persist in spite of prolonged viral suppression on ART [67••]. Therefore, in order to design new therapeutic approaches that may eradicate HIV, or allow stopping therapy without recurrence of viral replication (“functional cure”), it is imperative to identify key mechanisms involved in the generation and maintenance of latently infected cells. Several strategies are currently under consideration to achieve these goals (for example, the use of the cancer drug vorinostat, a histone deacetylase - or HDAC - inhibitor). PD-1 has recently emerged as a potential target to facilitate HIV eradication. Within the memory CD4 T cell compartment, cells expressing high levels of PD-1 contain more proviral DNA than PD-1 low cells [67••]. Additional studies [68] suggest that blocking PD-1 triggers HIV replication and might therefore reactivate latent virus, giving the opportunity to the body to eliminate the resulting productively infected cells by cell death and/or clearance by HIV-specific immune responses. If these results are confirmed, further research will be needed to understand which genes modulated by the PD-1 pathway also regulate HIV latency.

Moving toward therapeutic interventions targeting PD-1 in HIV-infected patients

The past several years have seen the rapid development of novel therapies based on biologic response modifiers (or biologics) in several fields of clinical medicine, in particular autoimmunity and cancer. More specifically, the use of monoclonal antibodies to block inflammatory signals has had a major impact on some diseases, a classical example being TNF- α inhibitors in rheumatoid arthritis [69]. Conversely, blockade of inhibitory pathways to boost the immune system is becoming an attractive strategy in several human cancers. Development is currently more advanced in the oncology field than in chronic infectious diseases [70]. A recent trial of CTLA-4 blockade in metastatic melanoma showed significant impact on survival [19]. The safety and pharmacokinetics of two PD-1 blocking antibodies have been recently tested in clinical trials [71,72••]. Both studies administered a single intravenous infusion of anti-PD-1 at escalating doses in patients with metastatic malignancies. These treatments showed much better tolerance than CTLA-4 blockade and very promising antitumor activity in some tumors. Autoimmune side effects did occur in some subjects, however, including an inflammatory colitis [72••], indicating that careful monitoring of potential side effects is essential. Larger clinical trials are underway, and several pharmaceutical companies are currently developing antibodies against molecules of the PD-1 pathway.

Even though the results from the early cancer clinical trials are promising regarding safety of PD-1 antibodies, there are significant differences between the immunology of cancer and HIV infection. One main difference between the two diseases is the systemic immune activation observed with HIV that leads to a progressive destruction of the immune system and disease progression [73]. Therefore, separate trials have to be performed in order to assess whether blockade of the PD-1 pathway will improve or worsen this systemic inflammation. Blocking an inhibitory pathway might further increase T cell activation by interrupting a control feedback loop. It may also have the opposite effect through immune restoration and possibly through improved integrity of the gut mucosal barrier and control of low-grade replication of not only HIV, but also of other persistent viruses (eg, CMV) that contribute to the ongoing systemic inflammation [74].

Experiments in the Simian Immunodeficiency Virus (SIV) model of infection in rhesus macaques can be critical to assess potential benefits and risks of PD-1 blockade in HIV-infected humans. Indeed, several studies suggest that non-human primates are suitable for pre-clinical trials in order to assess both the role of PD-1 in immune dysfunction [32,33] and the safety and tolerance of therapeutic PD-1 antibodies [75••][76••]. A small pilot study of PD-1 blockade with an anti-PD-1 antibody in chronically SIV-infected, ART-naïve macaques [75••] produced encouraging data, showing that treatment was well tolerated and led to an increase of functionally improved SIV-specific CD8 T cells and CD4 T cells[75••]. Although the small size of the groups prevents definitive conclusions, one of the most interesting findings of this study was a marked benefit of PD-1 blockade on survival and AIDS-related symptoms, despite a relatively modest and transient impact on viral loads [75••]. These results suggest that beyond its effects on HIV-specific immune responses, PD-1 blockade may foster immune restoration and/or dampen harmful immune dysregulation. These observations are of obvious clinical significance for possible use of PD-1 blockade as adjuvant therapy in HIV infection. Additionally, PD-1 blockade increased expansion of memory B cells and the concentration of envelope specific antibodies. This result suggests that PD-1 blockade can have a positive impact on antibody responses as well. A more recent study by the same group showed that in SIV infected macaques with rapidly progressive disease, activated memory B cells expressing PD-1 are rapidly depleted [76••]. Blockade of the PD-1 pathway restored survival of activated memory B cells *in vitro* and enhanced the titers of both non-SIV and SIV specific antibody responses *in vivo* [76••]. Whether or not these results can be translated to HIV is currently unclear, as there are some species-specific differences, including more PD-1 expression on B cells in rhesus macaques than in humans. Finally, it has been shown that blockade of the PD-1 pathway in combination with SIV-gag adenovirus vector vaccine in non-infected macaques enhanced the CTL responses, suggesting a potential role of PD-1 blockade as an adjuvant for vaccine design [77].

Yet, it is important to note that there are differences between SIV and HIV infections, and ultimately only carefully designed trials in humans will provide definite answers on the potential role of manipulation of the PD-1 pathway in treatment of chronic diseases. A first ACTG phase I study testing the safety and efficacy of PD-1 blockade to reduce the latent HIV reservoir in ART-treated subjects is under review as of September 2011. Should this trial be approved, it would also provide important information on the impact of PD-1 on immune activation, HIV-specific cellular and humoral responses as well as its role on lymphoid tissue in the gut and in lymph nodes.

Conclusions—The discovery of effective antiviral drugs against HIV is certainly one of greatest medical achievements of our time. However, in spite of its efficacy, ART does not fully restore the immune system and does not elicit effective anti-HIV immunity. Individuals with long-term viral suppression still present some AIDS and no-AIDS related complications, chronic inflammation and because of latent reservoirs of replication-competent virus, cannot stop therapy without experiencing recurrence of viremia. Different adjuvant therapies, including immunomodulation, are being tested in clinical trials or are under consideration in order to address these remaining challenges. Over the past few years, PD-1 has emerged as an attractive potential target because it is not only responsible for HIV-specific T cell impairment, but plays a wider role in HIV pathogenesis. Thus, clinical trials of PD-1 blockade should be considered to address the following questions: i) First and foremost, is PD-1 blockade safe in HIV-infected patients? Great assets are here that the same PD-1 blocking antibodies considered for HIV immunotherapy are being tested in cancer trials and can be evaluated in the SIV model in non-human primates, which provides a wealth of important information for novel applications; ii) Does PD-1 blockade—with or without therapeutic intervention - enhance HIV-specific immune responses *in vivo*, and thus

foster immune control of HIV replication?; iii) Can PD-1 inhibition facilitate global immune reconstitution beyond what is achievable by ART alone – in particular further reconstitute the CD4 T cell pool and immune responses against other pathogens, including opportunistic infections? iv) By improving immune restoration, can PD-1 dampen –rather than increase – the harmful residual immune activation that persists in spite of suppressive ART? ; v) Does PD-1 blockade reduce the reservoir of latently infected CD4 T cells, thus facilitating viral eradication or functional cure? vi) Can PD-1 blockade be combined with manipulation of other inhibitory pathways with increased efficacy without additional toxicity? And finally vii) Can PD-1 blockade be used to increase efficacy of therapeutic or prophylactic vaccines, in particular as an immunomodulator at the local site of vaccine administration?

There is thus still a considerable amount of unknowns, and a better knowledge of the basic immunology of the PD-1 pathway and of its role of HIV pathogenesis, careful pre-clinical studies in the SIV model, and thoughtful consideration for the lessons learned from cancer trials will be important to define the right design of clinical trials of PD-1 blockade in HIV-infected people. Although immediate success is uncertain, blockade of the PD-1 pathway or of other regulatory molecules in combination with ART, and possibly therapeutic vaccination, might represent novel and effective therapeutic strategies in a near future.

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Summary table

The PD-1 pathway in HIV infection

	Reference
PD1 expression on HIV-specific T cells	
■ <i>PD-1 is upregulated on HIV-specific CD4 and CD8 T cells</i>	5,6,7,27,49,50
■ <i>PD-1 expression on HIV-specific T cells correlates with infection stage and markers of disease progression.</i>	5,6,27,42,49, 50
■ <i>PD-1 is down-regulated on HIV-specific CD8 T cells targeting epitopes that had undergone mutational escapes.</i>	34
■ <i>PD-1 is co-expressed with other co-inhibitory molecules on HIV-specific T cells</i>	38,41,42,50,51
Impact of PD-1 on HIV-specific T cells	
■ <i>Blockade of the PD-1 pathway enhances proliferation of HIV-specific CD4 and CD8 T cells</i>	5,6,7,27,42,49,50
■ <i>Blockade of the PD-1 pathway enhances secretion of diverse cytokines by HIV-specific CD4 T cells.</i>	42
■ <i>PD-1 expression on HIV-specific CD8 T cells renders them susceptible to both spontaneous and Fas mediated apoptosis</i>	7
■ <i>The gene expression profile elicited by PD-1 (transcriptional signature) is upregulated in HIV-specific CD8 T cells from subjects with progressive disease, but not from HIV controllers.</i>	43
■ <i>The master transcription factor BATF is up-regulated by PD-1 signaling and mediates a reversible dysfunction of HIV-specific CD4 and CD8 T cells.</i>	43
PD-1 pathway in global immune responses during HIV infection	
■ <i>High PD-1 expression on the CD4 and CD8 T cell subsets is associated with failure of immune reconstitution after successful viral control on ART.</i>	64,65
■ <i>PD-1 expressing memory CD4 T cells contain more proviral DNA than PD-1 low cells and likely represent an important viral reservoir.</i>	67
■ <i>PD-1 blockade triggers HIV replication in CD4 T cells in vitro.</i>	68
■ <i>Expression of PD-L1 on antigen-presenting cell subsets (monocytes, dendritic cells) is elevated in HIV infection.</i>	53,54,55,56
■ <i>HIV can directly up-regulate expression of PD-1 ligands on antigen-presenting cells (in vitro differentiated macrophages).</i>	57,58,59
■ <i>PD-1 is expressed at higher levels on monocytes in HIV infection and is upregulated by microbial products similar to those that translocate from the gut. Triggering of PD-1, on monocytes leads to IL-10 secretion that inhibits the proliferative response of antigen-specific CD4 T cells.</i>	61
Clinical interventions on the PD-1 pathway	
■ <i>Two Phase I clinical trials tested the safety and pharmacokinetics of two different PD-1 blocking antibodies in patients with metastatic malignancies. The treatment was well tolerated even though a few autoimmune side effects were observed in some subjects.</i>	71,72
■ <i>Expression and function of PD-1 in SIV infection in macaques has many similarities with findings on PD-1 in HIV-infection, making of SIV a good pre-clinical model for interventional trials.</i>	32,33
■ <i>In pre-clinical trials in the SIV-macaque model, administration of an anti-PD-1 antibody was well tolerated and led to increased SIV-specific CD8 and CD4 T cell function and improved survival.</i>	75
■ <i>In the same model, PD-1 was found to be upregulated on B cells administration of a PD-1 antibody restored survival of memory B cells in vitro and enhanced titers of both non-SIV and SIV-specific antibody responses in-vivo.</i>	76