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PD-1 and CTLA-4 Inhibitory Cosignaling Pathways in HIV Infection and the Potential for Therapeutic Intervention

Daniel E. Kaufmann* and Bruce D. Walker†

The balance between proinflammatory mechanisms and the dampening of excessive immune activation is critical for successful clearance of a pathogen without harm to the host. In particular, molecules of the B7:CD28 family play a critical role in regulating T cell activation and peripheral tolerance. Chronic pathogens like HIV, which is characterized by ongoing viral replication despite detectable virus-specific T cell responses, and cancer cells have exploited these pathways to attenuate Ag-specific T cell immunity. This review summarizes evidence that molecules of the B7:CD28 family, PD-1, CTLA-4, and their ligands, play an active and reversible role in virus-specific T cell exhaustion associated with HIV infection in humans and in the SIV model in macaques. We discuss the potential for immunotherapeutic interventions based on manipulation of these inhibitory networks, the promising data obtained with blockade of the PD-1 pathway in animal models, and the challenges to such therapies. *The Journal of Immunology, 2009, 182: 5891–5897.

Effective virus-specific T cell responses are a primary reason for lack of immune control of persisting pathogens. In HIV infection, this lack of pathogen clearance leads to continuous viral replication and disease progression in the large majority of infected individuals, with the rare exceptions being subjects who control virus in the absence of therapy (1). Impaired immune function upon persistent Ag exposure is a feature that HIV shares with various other chronic infections, including hepatitis C virus, and cancer.

Some of the mechanisms leading to T cell exhaustion, defined as the progressive loss of key effector functions of Ag-specific T cells leading to ineffective T cell responses (2), are beginning to be elucidated. Studies of gene expression profiles of exhausted CTL in murine models (3) and humans (4) suggest that T cell exhaustion is due to both active suppression and passive defects in metabolism and cell signaling. The understanding of active inhibitory mechanisms leading to impaired cellular immunity is of particular relevance for the development of novel immunotherapeutic strategies. Evidence show that pathogens successfully evade immunity by exploiting negative regulatory pathways that play an important role in maintaining peripheral tolerance and avoiding excessive immune activation under physiologic conditions.

In this article we review recent studies that examined the involvement of two major inhibitory networks of the B7:CD28 family, the programmed death-1 (PD-1; CD279) and CTLA-4 (CD152) pathways, in immunodeficiency virus-specific immune dysfunction in humans and nonhuman primates. We also discuss specific therapeutic challenges relating to the balance between effective antiviral immunity and the pathogenic effects of hyperactivation.

The B7:CD28 family of cosignaling molecules

The outcome of a T cell response is shaped by the balance between costimulatory and coinhibitory signals, and an expanding array of cosignaling molecules are now recognized as having crucial roles in regulating T cell activation and tolerance. In particular, pathways of the B7:CD28 family provide both critical positive second signals that promote T cell activation and negative second signals that attenuate T cell responses and are crucial for regulating tolerance and autoimmunity (for review, see Refs. 5–7). The costimulatory molecule CD28 and two inhibitory molecules, CTLA-4 and PD-1, are functionally important members of the CD28 family. CD28 and CTLA-4 share the same ligands, B7-1 (CD80) and B7-2 (CD86), whereas PD-1 interacts with B7-H1 (PD-L1; where PD-L is PD ligand) and B7-DC (PD-L2; where DC is dendritic cell). Simultaneous recognition of the cognate MHC-peptide complex by the TCR (signal 1) and CD80 or CD86 by CD28 (signal 2) results in T cell activation, proliferation, and differentiation, as well as cytokine production, PD-1 and CTLA-4 are inducibly expressed on T cells following a TCR signal, and subsequent coligation of the TCR with one of these coinhibitors results in cell cycle arrest and termination of T cell activation. The importance of the PD-1 and CTLA-4 pathways in the physiologic regulation of T cell activation is demonstrated by the autoimmune diseases occurring in CTLA-4 and PD-1 knockout mice (5, 6) and further...
illustrated by the inflammatory side effects that can result from a therapeutic blockade of CTLA-4 in vivo, both in animal models and in humans (8, 9).

Despite these similarities, the regulatory roles of the CTLA-4 and PD-1 pathways differ (for review, see Ref. 10) and distinct effects of these pathways on T cells are increasingly recognized. These differences are probably due to several factors, including the following: 1) the differential expression patterns of CTLA-4 and PD-1 and their ligands among cell subsets and tissues; 2) differences in downstream signaling; and 3) distinct and potentially synergistic mechanisms of action, resulting in distinct changes in the T cell transcriptional profile (11). Recent data from HIV infection of humans (12) and lymphocytic choriomeningitis virus (LCMV) infection of mice (13) indicate a role for PD-1 in apoptosis. CTLA-4 increases T cell motility and overrides the TCR-induced stop signal required for stable conjugate formation between T cells and APCs (14). CTLA-4 is also constitutively expressed on regulatory T cells and plays a crucial role in their regulation and function (15, 16).

Although cosignaling molecules of the CD28 family are traditionally termed "receptors" and molecules of the B7 family are called "ligands," PD-L1, CD80, and CD86 can mediate "reverse signaling" in cells expressing these molecules, providing potentially important feedback mechanisms in APCs. Additionally, PD-L1, CD80, and CD86 can be expressed by T cells and may modulate their function. Finally, recent findings demonstrate a specific interaction between PD-L1 and PD80 inhibiting T cell activation and cytokine secretion (17). These data illustrate the complexity of the crosstalk between immunoregulatory pathways and the importance of further studies in the perspective of future immunotherapeutic strategies.

**PD-1 and HIV-specific CD8 T cell dysfunction**

The important role of the PD-1 pathway in CTL exhaustion was initially demonstrated in the murine LCMV model. Barber et al. (18) analyzed the kinetics of PD-1 expression during both acute and chronic LCMV infection and found that PD-1 was similarly expressed on early effector CD8 T cells after infection with both an LCMV strain that is rapidly cleared by the immune system (Armstrong) and an LCMV strain that leads to chronic infection and persistent viremia (clone 13). Concurrent with the resolution of viremia, PD-1 was rapidly down-regulated on virus-specific CD8 T cells in infection with the Armstrong strain. In contrast, PD-1 expression continued to increase on virus-specific CD8 T cells in chronically infected mice, and the high level of expression was sustained. Blocking the interaction of PD-1 with its receptor PD-L1 in vivo augmented LCMV-specific T cells, enhanced cytokine production, and led to a reduction in LCMV viral load. In a subsequent study (19), blockade of the PD-1 pathway, in combination with therapeutic vaccination, synergistically enhanced functional CD8+ T cell responses and improved viral control in mice chronically infected with LCMV. These studies suggest that manipulation of the PD-1 pathway could be a promising tool in treatment for chronic infections and possibly for cancer.

These results quickly led to the investigation of the role of the PD-1 pathway in HIV infection. A first series of studies (12, 20, 21) demonstrated that HIV-specific CTL expressed high levels of PD-1 and that PD-1 expression correlated with HIV-specific CTL dysfunction, as CTL expressing high amounts of PD-1 had impaired proliferative responses to the cognate Ag in vitro.

In cohorts of untreated subjects, PD-1 expression correlated with viral load and disease progression (20, 21). Longitudinal analysis of HIV-infected subjects before and after the initiation of antiviral therapy showed that control of viral load resulted in reduced PD-1 expression on HIV-specific CTL. Blockade of the PD-1 pathway by anti-PD-L1 Ab resulted in enhanced HIV-specific CTL proliferation.

One study (12) also examined the relationship between PD-1 expression and apoptosis and showed that PD-1-expressing CTL were more susceptible to both spontaneous and Fas-mediated apoptosis. Cross-linking of the PD-1 molecule with anti-PD-1 Ab preferentially triggered apoptosis in the CD8 cells expressing high levels of PD-1. The observation that the restoration of HIV-specific CTL proliferation was relatively modest suggested that only a minority of the HIV-specific, PD-1-expressing CTL have their function critically inhibited by PD-1 and are therefore effectively enhanced by a PD-1/PD-L1 blockade. A recent study in the LCMV model gives a likely explanation for the relatively limited effect obtained by blockade of the PD-1 pathway by demonstrating that exhausted CTL are reconstituted by multiple inhibitory receptors during chronic infection (22). These data still need confirmation in human chronic infections.

Whether blockade of the PD-1 pathway in HIV infection leads mostly to a quantitative increase in virus-specific CTL or results in a qualitative improvement in HIV-specific responses is a crucial issue that remains largely to be addressed. The question remains whether blockade of the PD-1 signal results in the following: 1) a change in the fraction of precursors that are induced to proliferate; 2) an increase in the number of cell cycles undergone by the same fraction of cells; or 3) a change in the phenotype of the cells on a single-cell basis.

In the studies described above (12, 20, 21), blockade of the PD-1 pathway resulted in an increase of the CTL capable of producing cytokines in response to restimulation with the cognate Ag at the end of the period of in vitro expansion in 6-day proliferation assays without a shift in the pattern of cytokines produced. As PD-L1 blockade did not significantly increase the fraction of cytokine-secreting CTL in assays performed with PBMC directly ex vivo, these findings may reflect altered T cell proliferation rather than a qualitative change in T cell function. The increase in telomere length observed in HIV epitope-specific CTL proliferating in the presence of PD-L1 blockade supports the hypothesis of a qualitative improvement of virus-specific CTL upon blockade of the PD-1 pathway (23). However, it is possible that this observation is a consequence of a preferentially increased proliferation of CTL with short telomeres. The potential effect of the PD-1 pathway on CTL killing capacity is a crucial effector function that needs to be tested in future studies.

The mechanisms of PD-1 regulation in activated and exhausted cells are still poorly defined. In a longitudinal study of HIV-infected subjects followed from the time of acute infection (24), PD-1 expression declined on CTL specific for epitopes that had undergone mutational escape along with an increase in CTL polyfunctionality as measured by the capacity to produce multiple cytokines, whereas an increase in PD-1 expression and monofunctionality was observed over time for CTL directed against conserved epitopes. These data indicate that repeated Ag-specific TCR stimulation plays an important role in modulating PD-1 expression in HIV infection. However, other
mechanisms contribute to Ag-independent up-regulation of PD-1. The accessory HIV protein Nef was recently shown to up-regulate PD-1 through a p38 MAPK-dependent mechanism during infection in vitro (25). Furthermore, a recent study showed that the common γ-chain cytokines IL-2, IL-7, IL-15, and IL-21 induce expression of PD-1 and its ligands (26), suggesting that the cytokine microenvironment, which differs among different tissues and body compartments, may play a significant role in PD-1 regulation in vivo. TCR-independent PD-1 up-regulation may significantly contribute to the high PD-1 expression that was observed on the bulk CD8 T cell population and that correlated with markers of disease progression (20). An important limitation of most human studies is the sole investigation of the peripheral blood compartment, whereas lymph node studies showed a significantly higher level of PD-1 expression on both HIV-specific T cells and bulk CD8 T cell populations than the corresponding peripheral blood subsets (27). Of particular interest will be the investigation of the PD-1 pathway in the GALT, given the central role of GALT involvement in HIV pathogenesis.

Further studies are required to better understand the regulation of PD-1 expression and function in activated and exhausted cells. Recently, NFATc1 was identified as an important factor in the regulation of PD-1 expression, thus providing a molecular mechanism responsible for the induction of PD-1 upon T cell stimulation (28). Critical questions remain as to what differentiates the regulation of PD-1 expression and function in exhausted compared with functional, activated cells. It will also be important to determine whether PD-1 expression is modulated by binding to its ligands PD-L1 and PD-L2, whose expression levels may vary over time during infection or in different tissues.

**PD-1 and HIV-specific CD4 T cell dysfunction**

Defective HIV-specific CD4 T cell responses are a hallmark of HIV infection, and virus-specific CD4 T cell help is considered to be important in restricting viral replication. Compared with CTL, few studies have investigated the role of the PD-1 pathway in CD4 T cell dysfunction.

There are some important similarities in PD-1 expression and function between HIV-specific CD4 and CD8 T cells. PD-1 is likewise up-regulated on the total CD4 T cell population in HIV-infected individuals (20), and PD-1 levels correlate directly with viral load and inversely with CD4 count. Blockade of the PD-1 pathway with the PD-L1 Ab also augmented HIV-specific CD4 T cell proliferative responses with a striking effect in some individuals (20, 27). D’Souza et al. (27) investigated PD-1 expression on HIV-specific CD4 T cells and showed strong analogies with the CTL studies described above. PD-1 was up-regulated on HIV-specific CD4 T cells compared with CMV-specific CD4 T cells in the same subjects, and PD-1 levels on HIV-specific CD4 T cells correlated with HIV viral load. PD-1 levels on CD4 T cells were also higher in lymph nodes than in peripheral blood. There was also a strong correlation between PD-1 levels in CD4 and CD8 Gag-specific T cells, further illustrating analogies between these two cell subsets with regard to the PD-1 pathway. Further studies may however show distinct functions of PD-1 between the CD4 and CD8 T cell subsets.

**PD-1 ligands and immune dysfunction in HIV infection**

Experiments in murine models have shown a crucial role for PD-1 ligands in protection from autoimmunity and excessive inflammatory responses. PD-L1 knockout mice infected by a chronic strain of LCMV die from immunopathology, whereas wild-type mice become chronically infected but survive (18). Conversely, expression of PD-L1 on tumor cells negatively impacts cancer survival in humans (29). Up-regulation of PD-L1 can attenuate pathogen-specific immune responses, such as in Schistosoma mansoni infection (30). In chronically LCMV-infected mice virus-infected splenocytes expressed high levels of PD-L1, suggesting a role in ineffective CTL responses (18) and that up-regulation of PD-L1 in lymphoid organs contributes to viral persistence (31).

Studies in the setting of HIV infection likewise suggest a role of PD-L1 up-regulation in progressive immune dysfunction. PD-L1 expression was found to be significantly elevated on monocytes and B cells in the peripheral blood of HIV-infected individuals compared with HIV-negative controls (32), and PD-L1 levels correlated with markers of disease progression, directly with viral load and inversely with CD4 counts. Control of viral load by antiviral therapy reduced PD-L1 expression on PBMC. PD-L1 was also found to be up-regulated on myeloid DC in HIV-infected subjects with progressive infection (33) but expressed at lower levels in antiretroviral therapy-treated subjects and controllers/long-term nonprogressors. Exposure of myeloid DC to HIV in vitro resulted in up-regulation of PD-L1 (33, 34). Two studies provide possible explanations for these findings. HIV-encoded TLR ligands up-regulated PD-L1 on DC and monocytes (35), and exposure of monocytes to HIV in vitro resulted in PD-L1 up-regulation by an IFN-α-dependent mechanism (34). Taken together, these results suggest that both viral factors and inflammatory cytokines may lead to the induction of PD-L1 on APCs, which could contribute to the functional impairment of PD-1 expressing HIV-specific CTL. Furthermore, PD-L1 can act bidirectionally, and therefore PD-L1-mediated modulation of APC and T cells may also have a significant impact in HIV infection. Progress in understanding the regulation of PD-1 ligand expression may also provide new therapeutic targets in the PD-1 pathway. A recent study showed that the oncogenic kinase nucleophosmin/anaplastic lymphoma kinase critically regulated PD-L1 levels on a lymphoma cell line and that a small molecule anaplastic lymphoma kinase inhibitor abrogated PD-L1 expression by tumor cells (36). This study suggests that inhibition of PD-L1 expression by a specific drug may enhance the efficacy of future immunotherapeutic protocols against chronic infections and cancer. Studies of PD-1 ligand expression and function in lymphoid tissues are, as for PD-1, necessary for a better understanding of the PD-1 pathway in HIV infection.

**CTLA-4 and HIV-specific T cell dysfunction**

The negative regulator of the B7-CD28 family, CTLA-4, has also been shown to impact T cell responses against persistent Ag, both in animal tumor models (37) and in cancer immunotherapy trials in humans (8, 9). Human trials that used a blocking anti-CTLA-4 Ab demonstrated a reduction in tumor mass and clinical benefit in a substantial minority of treated subjects. Studies of the role for CTLA-4 in chronic infections have given mixed results. Whereas CTLA-4 adversely affected pathogen clearance in Helicobacter pylori (38), Leishmania (39, 40), and...
Trypanosoma (41) infections of mice, CTLA-4 was shown not to be involved in CD8 T cell exhaustion in chronic murine LCMV infections (18). The possible involvement of CTLA-4-mediated immunoregulation in chronic viral infections of humans was suggested by the identification of CTLA-4 gene polymorphisms associated with hepatitis B virus viral clearance (42).

In HIV infection, early studies showed that CTLA-4 was moderately overexpressed in the total CD4 population with progressive disease and that its expression correlated inversely with CD4 count (43). CTLA-4 was also strongly expressed in HIV-specific CD4 T cells at the time of acute HIV infection (44). Our study of HIV-infected subjects at different stages of HIV infection (45) showed that CTLA-4 was selectively up-regulated on HIV-specific CD4 in all categories of HIV-infected individuals, with the exception of controllers/long-term nonprogressors who controlled viremia in the absence of antiretroviral therapy. In contrast to PD-1 (12, 20, 21), CTLA-4 was not highly expressed on HIV-specific CTL. CTLA-4 expression correlated with markers of disease progression, directly with viral load and indirectly with CD4 T cell counts. CTLA-4 was higher in HIV-specific CD4 cells that produced only IFN-γ than in polyfunctional cells producing both IL-2 and IFN-γ. In vitro blockade of CTLA-4 augmented HIV-specific CD4 T cell proliferation. However, whereas most HIV-specific CD4 T cells coexpressed CTLA-4 and PD-1, blockade of the two pathways produced variable results, with subjects responding to both, either, or neither. Depletion of CD25+ cells did not abrogate the impact of CTLA-4 blockade on HIV-specific CD4 T cell proliferation, suggesting that, in the in vitro assays used, CTLA-4 mediated HIV-specific CD4 T cell dysfunction mainly through blockade of the CTLA-4 molecules on effector cells. However, Ab blockade of CTLA-4 in vitro may not give a complete picture of regulatory T cell function in tissues. Studies also showed that besides their opposite effects on T cell function, CTLA-4 and CD28 differentially affected susceptibility of CD4 T cells to infection by macrophage-tropic R5 strains. Whereas CD28 costimulation led to low CCR5 expression and decreased susceptibility to HIV infection (46, 47), CTLA-4 signaling resulted in high CCR5 expression and enhanced susceptibility to viral infection (47). The studies demonstrated that cosignaling molecules can directly modulate susceptibility of CD4 T cells to viral infection.

The role that altered expression of the CTLA-4 ligands CD80 and CD86 may play in HIV pathogenesis is poorly understood. Exposure of monocytes and T cells to HIV in vitro lead to up-regulation of CD80 and CD86 on monocytes and T cells (34), and stimulation with HIV-1-derived TLR7/8 ligands was shown to up-regulate CD80, CD86, and PD-L1 on monocytes and DC (35). Other studies showed that exposure to infectious and noninfectious HIV virions induced DC activation and differentiation, including expression of high levels of HLA-DR, CD80, CD83, and CD86 (48, 49). In contrast to these consistent data obtained upon HIV exposure in vitro, ex vivo analyses in HIV-infected individuals have given a more complex picture. Expression of CD86 mRNA, but not CD80 mRNA, was decreased in PBMC from HIV-infected subjects, contrasting with an increase in PD-L1 expression (32). Lower expression of CD80 and CD86 was observed on DC from lymphoid tissue in individuals during acute HIV infection compared with subjects during acute EBV infection (50). A low induction of CD80/CD86 expression was also noted on B cells from HIV-infected individuals, correlating with reduced co-stimulatory function (51). In contrast, a higher expression of CD80 and CD86 was noted on CD4 and CD8 T cells of HIV-infected individuals (52). Interpretation of these data is complicated, because CTLA-4 shares its ligands with the costimulatory molecule CD28, and the specific interaction of CD80 with PD-L1 can inhibit T cell function (17).

Investigations of the PD-1 and CTLA-4 pathways in the SIV model

Studies of SIV infection in monkeys, which is a much closer model to human HIV disease than murine LCMV infection, have brought important insight into the role of the PD-1 and CTLA-4 pathways in T cell dysfunction. Patterns of PD-1 expression on virus-specific CTL in SIV-infected rhesus macaques were consistent with those in human studies (53, 54), and likewise SIV-specific CD8 and CD4 T cell proliferation could be enhanced by blockade of the PD-1 pathway. Of importance, PD-1 levels were higher in lymph nodes and in GALT, the main sites of viral replication, than in peripheral blood (54). Also consistent with data in humans (24), PD-1 expression gradually declined on CTL specific for epitopes that had undergone mutational escape (53). One study (54) compared the temporal expression of PD-1 on SIV-specific T cells following pathogenic SIV infection or vaccination with a DNA/modified vaccinia virus Ankara vaccine. Compared with persistent high PD-1 expression on CTL in pathogenic SIV infection, vaccine-induced CTL expressed lower levels of PD-1 that decreased further as the T cells differentiated into memory cells. These two studies strongly suggested that the macaque/SIV model is well suited for preclinical studies assessing the safety and therapeutic benefit of blocking the PD-1 pathway. Interestingly, examination of nonpathogenic SIV infection in sooty mangabeys indicated that their typically lower immune activation was associated with an early increased level of PD-1 expression on T cells of lymphoid tissue, suggesting that PD-1 up-regulation on bulk T cell subsets may exert a protective effect (55).

An important recent study (56) has evaluated the safety and immune restoration potential of a blocking anti-PD-1 Ab in SIV-infected macaques. The treatment was well tolerated and led to rapid increase in virus-specific CD8 T cells with improved functional quality, both in peripheral blood and in GALT. PD-1 blockade also resulted in expansion of virus-specific CD4 T cells and memory B cells and increases in envelope-specific Ab. These improved immune responses were associated with significant reductions in plasma viral load and increased survival of SIV-infected macaques. Furthermore, blockade was effective both early after infection (week 10) and at a later chronic disease stage (week 90), even when severe lymphopenia was present. These results obtained by blockade of a single inhibitory pathway are impressive given the multiplicity of inhibitory molecules expressed by exhausted CTL in the LCMV model (22) and are promising for potential future studies in humans. In contrast, although CTLA-4 expression was found to be up-regulated in CD4 T cells of lymphoid tissues in SIV infection (57), anti-CTLA-4 blockade failed to show a benefit in terms of plasma viral load or survival in acutely or chronically SIV-infected macaques (58, 59). Whereas an increase in HIV-specific CD4 and CD8 T cell responses was noted in one study (58), another study did not show an expansion of SIV-specific
CTL and observed an increase in CD4 T cell activation and viral replication at mucosal sites (59). Further studies are needed to understand these contrasting results between blockades of the PD-1 and CTLA-4 networks. Differences between expression and function of the PD-1 and CTLA-4 molecules in the CD4 and CD8 T cell subsets could contribute to these distinct effects. Because CTLA-4 is crucial for regulatory T cell function and findings in HIV infection showed that CTLA-4 is preferentially up-regulated on virus-specific CD4 T cells but not CD8 cells, (45), CTLA-4 blockade might preferentially expand SIV-specific CD4 T cells and increase CD4 activation, thus providing additional targets for viral infection without improvement in CTL function to offset this detrimental effect.

Unresolved issues on the path to therapeutic manipulation of inhibitory pathways in HIV infection

There is currently a strong interest in the potential for clinical interventions targeting immunoregulatory networks to enhance immunity against cancer cells and persistent viruses or to boost the efficacy of preventive and therapeutic vaccines. It is important to note that significant differences exist between the murine and human immune system (for review, see Refs. 60 and 61), and studies in primates, in particular of the SIV model in monkeys, are therefore essential before considering interventions in humans.

A number of important issues will need to be addressed before applying such strategies to HIV infection. First, does the intervention lead to a qualitative improvement of T cell responses in vivo, both in peripheral blood and lymphoid tissues? This is a complex question, because there is still a surprising lack of clear understanding as to what constitutes an effective immune response against HIV (62). Second, can blockade of a single inhibitory network like the PD-1 pathway result in a significant clinical benefit, given that multiple inhibitory molecules and complex defects contribute to T cell exhaustion in chronic infections (3, 22)? The recent study of PD-1 blockade in SIV-infected macaques discussed above has yielded promising results in this regard (56). Third, would blockade of the inhibitory pathway of interest be well tolerated or lead to excessive inflammation and autoimmunity? In patients with cancer, systemic therapy with blocking Ab to CTLA-4 is associated with tumor regression but also with systemic inflammation, including colitis and hypophysitis (8, 9). Early data on PD-1 blockade have been positive, with a good tolerance of a humanized anti-PD-1 Ab in a clinical trial of subjects with advanced hematologic malignancies (63) and few side effects of PD-1 blockade in SIV-infected macaques (56, 64). Fourth, would such an immune intervention bring an additional benefit to individuals receiving the current optimal standard of care, namely suppression of HIV replication on antiretroviral therapy? Fifth, what is the potential use for a blockade of inhibitory molecules as HIV vaccine adjuvants? Blockade of the PD-1 pathway or the inhibitory cytokine IL-10 have shown promise when used in combination with therapeutic vaccination in murine models of chronic infection in a setting where the natural T cell response to the pathogen is exhausted (19, 65). In uninfected macaques, the combination of an SIV-gag adenovirus vector vaccine with PD-1 blockade enhanced CTL responses (64), suggesting that blockade of this pathway could also augment the efficacy of preventive vaccination by blocking the PD-1 signal in nonexhausted, recently activated cells. There is now a strong rationale for evaluating the protective efficacy of combined vaccination and PD-1 blockade in the SIV infection model. Sixth, would the benefit of inhibitory cosignaling blockade be transient, requiring frequent administration of a blocking Ab or a small molecule targeting the pathway, or would the effect be sustained for relatively long periods of time? This is important, because administration of a mAb is a costly and relatively cumbersome strategy and because repeated dosing may increase the risk of toxicity. Seventh, as multiple inhibitory molecules contribute to T cell impairment in chronic viral infections, including HIV, could simultaneous blockade of multiple pathways result in synergistic efficacy without excessive toxicity? Both combined PD-L1/IL-10 blockade (66) and LAG-3/PD-L1 blockade (22) have given promising results in murine experiments. Identification of convergent intracellular signaling pathways impaired in exhausted cells might also provide additional therapeutic targets with far more efficient interventions. Enhancement of stimulatory pathways combined with a blockade of inhibitory mechanisms might also provide additional benefits. Lastly, a specific concern in HIV infection as compared with cancer is the major role played by systemic immune activation in disease progression. Multiple studies suggest that continuous immune activation is a crucial factor in progressive destruction of the immune system (for review, see Ref. 67). Inhibitory pathways, while contributing to HIV-specific T cell exhaustion, may attenuate the systemic immune hyperactivation and the ensuing immunopathogenesis, as in the animal models described above (18, 68). This is suggested by data in sooty mangabeys, which maintain high levels of plasma viremia and yet do not progress to disease (55). Therefore, it is not possible to rule out that the benefit of blocking inhibitory molecules in terms of T cell responses would be offset by the consequence of enhanced activation, such as increased activated target cells for viral replication. This hypothesis is supported by some data on CTLA-4 blockade in SIV-infected macaques (59).

Conclusions

Although access to effective antiretroviral therapy has revolutionized HIV patient care and has had an outstanding impact on prognosis for this infection, attempts to generate effective HIV-specific immune responses in HIV-infected or uninfected individuals have thus far failed. However, rare individuals can control HIV replication, likely through immune-mediated mechanisms (1), and vaccine-elicited protection against SIV has been achieved in animal models (69, 70). Despite the huge challenges, there is therefore hope to enhance immune responses against HIV and ultimately to develop an effective HIV vaccine (62). There is a critical need for detailed studies of how effective immune responses function against this virus and of the underlying mechanisms of immune impairment in chronic infections. Progress has been made in our understanding of T cell exhaustion in settings of Ag persistence. Evidence that molecules like PD-1 and CTLA-4 mediate a reversible dysfunction of HIV-specific T cells raises the possibility of therapies designed to revive T cell activity. Studies of SIV infection in monkeys suggest that the SIV/macaque model is well suited to investigate the role of immunoregulatory networks in lentiviral infections despite some notable differences (60). We believe that preclinical studies showing a clear and significant benefit in SIV trials are a prerequisite before considering therapeutic
manipulation of inhibitory molecules in the setting of HIV disease. Blockade of regulatory pathways in HIV infection, combined with antiretroviral drugs and/or therapeutic vaccination, might offer new therapeutic approaches in the near future. Although immediate success is uncertain, the significance of these studies goes beyond HIV and extends to other chronic infections and cancer.

Disclosures
The authors have no financial conflict of interest.

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