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Editorial

Editorial: **Is HIV-1 induction of macrophage expression of PD-L1 and PD-L2 its weakest or strongest link to disease? HIV-1 plays both sides by augmenting and limiting T cell activation to survive in vivo**

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For a long time, discussions about how best to control HIV-1 disease progression have called for halting HIV-mediated immune activation, as this is considered to be a driving force of viral replication, resulting in added viral targets and eventual immune decay. If HIV-1 infection only led to activation, we would expect that infection would unrelentlessly amplify itself toward a very short disease course, as activation would likely overwhelm antiviral responses and ultimately the host. However, we know that HIV-1 viremia can be sustained without overt symptoms for many years in vivo. The recent paper by Rodríguez-García et al. [1] expands a developing understanding of how HIV-1 infection may orchestrate control against immune activation by shutting down rather than promoting T cell responses via modulation of monocytes/macrophages.

What would the virus gain by sacrificing T cell activation and the resulting viral replication? The establishment of an anti-T cell activation signal on long-lived macrophages would counter antiviral CD8 responses that arise throughout infection and at the same time, act to provide a "viral friendly" environment for reservoir formation in macrophages and latent CD4 populations, as these cells are also receptive to negative signals. The balance between HIV infection and immune activation has been a central focus of immunological research to better understand and eventually elicit the control of HIV infection in vivo. Although seminal works have established that CD8 T cells can elicit a powerful control of HIV infection and replication, natural history studies have also documented that CD8 responses can control the magnitude of virus but cannot mediate total clearance. Lack of eradication is proposed to be the result of multiple persistence mechanisms including the intrinsic capacity for immune escape via RT-mediated epitope changes and the existence of viral reservoirs [2]. We now must add early induction of PD-1 ligands to its arsenal.

The enrichment of HIV-1-specific CD8 T cells in infected persons bearing changes consistent with immune exhaustion, such as PD-1 up-regulation, has raised the idea that HIV-1 evades T cell control by driving this compartment toward nonresponsiveness [3]. Negative regulatory molecules such as PD-1 have been identified as mediators of this T cell shutdown [4–6]. Would the early induction of PD-L1 ensure exhaustion phenotypes earlier than otherwise achieved by sustained CD8 T cell responses? The article by Rodríguez-García et al. [1] advances the hypothesis that the monocyte/macrophage, by its interaction with HIV-1 and up-regulation of PD-L1/L2, is a central player in the shutdown of T cell response via PD-1 on lymphocytes (**Fig. 1A**). This work follows the observations of Said et al. [7], who recently proposed that viral replication in vivo can promote PD-1 expression on monocytes, which when binding with monocyte PD-L1, can induce high levels of IL-10. Novel contributions by Rodríguez-García et al. [1] include the description of PD-L1 and PD-L2 expression in a human MDM system in response to HIV-1, microbial translocation production, and IL-10. They found that LPS led to an increase in PD-L1 levels from a low baseline and a minor increase in PD-L2 expression from a much higher baseline. Furthermore, inactivated HIV-1 particles increased PD-L1 and PD-L2 expression. A key, novel finding was different regulation of the two ligands by IL-10. Specifically, IL-10 increased PD-L1 expression in the presence and absence of

Abbreviations: PD-1=programmed death 1, PD-L1/L2=programmed death ligand 1/2, RT=reverse transcriptase, Treg=T regulatory cell

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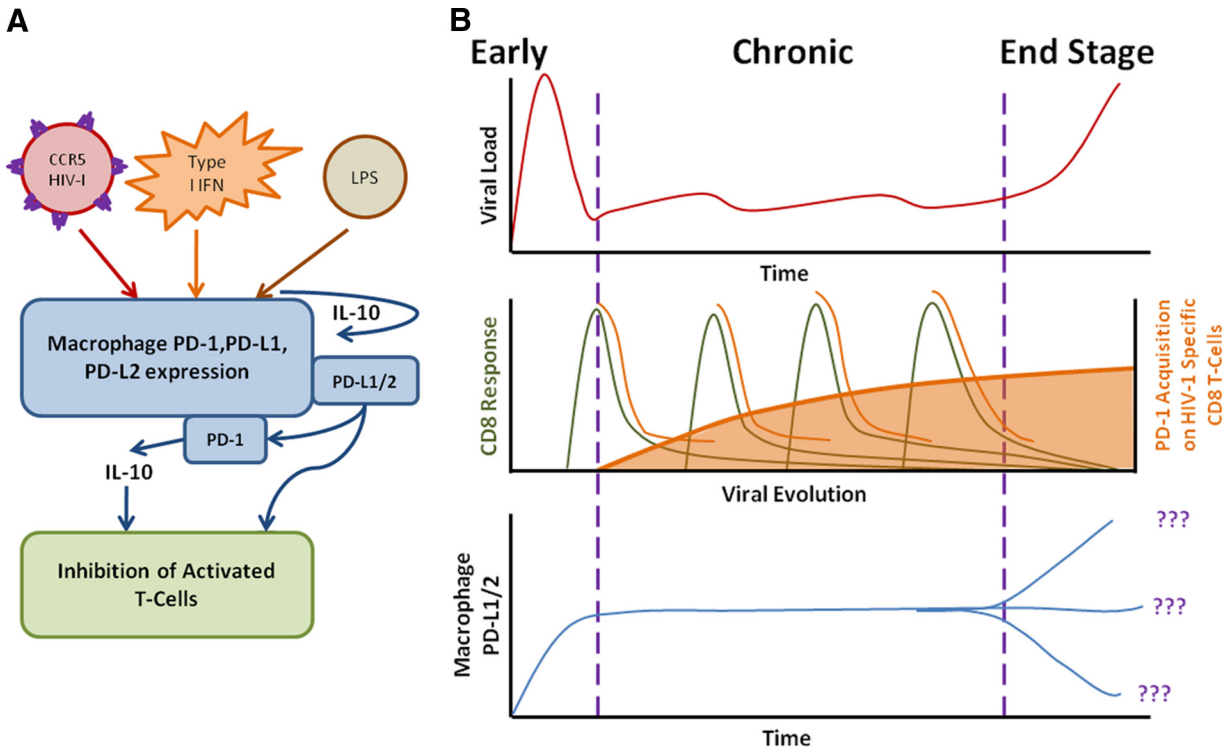

Figure 1. HIV infection and PD-L1/L2 induction models. (A) Schematic representation of viral, host, and bacterial stimuli that can act on macrophages to induce PD-1, PD-L1/L2, and cross-talk (IL-10) to affect T cell responses during chronic HIV-1 disease. Shown are HIV or HIV-associated stimuli inducing PD-1 and PD-L1 on monocyte/macrophages, PD-1-PD-L1 ligation induction of IL-10 production, and how PD-L1/L2 and IL-10 cross-talk to inhibit T cell activation [1, 7, 8]. (B) Model of early/chronic/end-stage disease, where viral replication during emergence of sequential CD8 T cell responses, as a result of immune escape/PD-1 expressing activated CD8 T cells and macrophage PD-L1/PD-L2 expression, is illustrated at each stage of pathogenesis.

inactivated HIV, and PD-L2 expression was unchanged. However, PDL-2 was increased by IL-10 blockade [1]. They do not show IL-10 induction by viral particles alone or examine the effect of PD-1 or PD-L1 blockade on IL-10 levels. However, the paper did show IL-10 induction by LPS, suggesting a physiological role of microbial translocation in contributing to this HIV-1-mediated inhibition of T cell activation. The link between HIV-1 particles and modulation of monocyte PD-L1 expression remains unknown, as this work does not define a ligand responsible for this effect but does establish that CCR5 ligation (likely as a result of its role in mediating macrophage infection) may be important as only CCR5 HIV-1-induced PD-L1 expression. The effect of independent CCR5 ligand (i.e., MIP-1β) remains untested. Although the paper discusses at length the potential implications of a dual induction of PD-L1 and PD-L2, the role of PD-L2 as compared with PD-L1 in mediating T cell control and/or IL-10 production remains undefined. Their work also stresses that HIV-1 induction of PD-L1 is distinct from LPS (i.e., LPS did not induce PD-L2, whereas it clearly induced IL-10). Future investigations will need to address if the effects of HIV-1 on PD-L1 are different among myeloid cells, DCs, macrophage activation phenotypes, concurrent T cell activation microenvironments, and/or tissue sites, to name a few of the variables that may strengthen/weaken its relevance in vivo.

Aside from HIV-1, additional mechanisms of PD-L1 induction in monocytes during HIV-1 disease have been described, such as LPS and type 1 IFN (Fig. 1A). Interestingly, the induction of type 1 IFN by plasmacytoid DCs upon interaction with HIV-1 particles, as shown by our group and others [9], may contribute to an early mechanism of PD-L1 induction in vivo. By contrast, increasing LPS levels in association with an established chronic infection are also expected to increase PD-L1 expression and IL-10 production by macrophages. The convergence of multiple PD-L1 induction mechanisms from early to chronic disease, as illustrated for viral (HIV-1), host (IFNs), and bacterial (LPS) factors, supports a central role for the macrophage as a constant source of negative regulatory pressure on T cell activation.

Although not addressed in the work discussed, PD-L1/L2 is but one of several negative regulatory factors that may contribute to shutting down T cell responses in vivo. As aptly reviewed by Rouse and Sehrawat [3], the immunopathology of viral infections may be intertwined with the activation of multiple negative regulatory mechanisms that may ensure the persistence of viral infection. The interplay among HIV-1 modulation of innate cells, PD-

L1, and IL-10 has yet to be reconciled with additional negative regulatory elements such as TGF-β, IL-35, resolvins, protectins, galectin-1 and -9, lymphocyte activation gene 3, T cell Ig domain and mucin domain 3, CTLA-4, suppressor of cytokine signaling expression, Tregs, myeloid suppressor cells, etc., all candidates to orchestrate a balance of sustained viral replication in the absence of what could be antiviral immune activation and tissue destruction.

A natural prediction from these observations, together with the high levels of PD-1 on circulating HIV-1-specific CD8 T cells, is that if a therapeutic intervention were to inhibit PD-L1, the functional capacity of anti-HIV CD8 T cells would be unleashed [8]. Indeed, blockade of the PD-L1-PD-1 and IL-10 axis in vitro in PBMC from HIV-1-infected persons and blockade of PD-1 in vivo in a SIV-infected macaque model have produced promising results [6, 8]. However, caveats exist that limit the extension to HIV-1 in vivo, including the limited range of PBMC to reflect in vivo dynamics and the limitation that SIV infection models may reflect a faster progression to disease rather than the multiyear human disease course. It also remains undetermined if targeting PD-L1 rather than PD-1 may differ in vivo, as we do not know to what extent the immune activation or viral replication results observed in the SIV system were a result of direct release of T cell activation or the inhibition of negative regulatory sequences within macrophages such as IL-10 production. Upregulation of PD-1 has also been observed in CD4 T cells of HIV-infected individuals, raising the possibility that this approach may also unleash greater bystander immune activation within uninfected and infected CD4 T cells [6, 8]. This concern is discussed in Velu et al. [8] when noting a transient rise in viral load in two of five animals treated during early chronic infection and all animals treated during late chronic infection. In addition, PD-1 blockade may release exhausted CD8 T cell responses that may include not only those that are HIV-specific but also other types of anergized responses (Fig. 1B). As discussed by Sharpe et al. [10], PD-1 interactions have a key role in immune tolerance and controlling inappropriate inflammation, which if blocked, may increase the risk for autoimmunity. Also of note, a decrease in circulating monocytes (and eosinophils) was observed in the SIV study to indicate that PD-1 has other functions in vivo. However, Velu et al. [8] reference the safety of PD-1 blockade by the clinical results from a HIV-unrelated human trial, where adverse events were observed only at the grade 1/2 level.

Having already considered PD-1 expression on T cells with regard to blockade of the PD-1 axis, one must also account for PD-L1 expression on additional cell types, including CD4 T cells [1, 11]. PD-L1 expression on CD4 T cells and macrophages may present a redundant mechanism for T cell inhibition. PD-L1 mice knockout studies have revealed increased responsiveness for PD-L1$-/-$ CD4 T cells upon stimulation, which again, may have implications in the context of HIV/SIV target cells and viremia [11]. From these studies, we interpret that HIV-1 infection may already be a step ahead in its objective to establish persistence by mediating the induction of PD-1 and its ligands to counter rapid disease progression in addition to acting against CD8 T cell immune pressure.

Regrettably, as long as the RT-mediated hypermutation of HIV can impact epitope diversity beyond that controlled by CD8 T cells (immune escape), it remains unproven that targeting negative regulatory pathways as a long-term strategy will lead to sustained CD8 T cell-mediated viral control. We are more optimistic of targeting these pathways at exposure/acute infection, as here, the induction of PD-L1/L2 may act to hold back newly emerging anti-HIV CD8 T cell responses and at the same time, promote IL-10 production. Therefore, is the initial PD-L1/L2 induction in macrophages a major viral vulnerability central to the establishment of productive infection? However, once chronic infection has been established, and additional redundant anti-inflammatory mechanisms are in place (i.e., IL-10, TGF-β, PGE-2, Tregs, etc.), is the role of PD-L1/L2 less critical? Finding the specific ligand in HIV-1 particles responsible for PD-L1/L2 induction will be an important first step toward answering these questions.

If HIV-1 can sustain these mechanisms in balance with T cell activation to ensure chronicity, what then may account for changes leading toward disease progression and AIDS-defining illnesses? What disturbs this multiyear yin-yang balance of immune activation and viral replication mediating immune suppression? This remains unknown. However, it is intriguing to speculate whether mechanisms regulating anti-inflammatory responses may represent an important turning point if dysfunctional in either direction. This may counter (or complement) the common view that disease progression represents the onset of AIDS-defining illnesses prompted by an opportunistic infection or cancer pathology event that the adaptive immune response cannot effectively control (promoting immune activation, viral replication, and CD4 T cell loss). Do macrophages hold a determinant role in disease progression, not because of changes in their ability to contribute to uncontrolled, proinflammatory responses but because of a loss in sustaining negative signals such as PD-1/PD-L1/2 in the presence of HIV? Or does the dysregulated increase of these immunosuppressive responses against T cell activation reach a point at which the immunosuppression itself increases susceptibility to AIDS-defining illnesses?

Above all, the PD-L1 response to HIV-1 in macrophages may represent a "stealth" viral strategy to prematurely co-opt a host mechanism that is otherwise activated *after* CD8 T cell activation as a pre-emptive strike against emerging CD8 T cell responses. We would argue that this may not only be a viral-mediated barrier to natural control but may also bear on CD8 T cell vaccine failures when attempting to expand memory responses in the context of high viral-mediated PD-1/PD-L1 expression on macrophages at sites of initial replication [12]. We expect these areas of investigation to

blossom in the near future, as work such as that by Rodríguez-García et al. [1] has finally started to stress the central role that macrophages may play with regard to preventing the CD8 T cell response from achieving its full potential for viral control.

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DISCLOSURE

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