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Up-regulation of PD-L1 on Monocytes and Dendritic Cells by HIV-1 derived TLR Ligands

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Abstract

Increased PD-L1 expression has been reported in HIV-1-infected individuals, but the mechanisms leading to PD-L1-up-regulation remain to be elucidated. Here we demonstrate that HIV-1-derived TLR7/8 ligands can induce MyD88-dependent up-regulation of PD-L1 on pDCs, mDCs and monocytes. These data suggest a mechanism through which HIV-1-derived TLR ligands might contribute to the functional impairment of virus-specific PD-1+ T cells by inducing the up-regulation of PD-L1 on antigen-presenting cells.

Chronic viral infections are characterised by the persistence of antigen, leading to general immune activation and functional impairment of virus-specific T cells (1–6). More recently, it has been demonstrated that these functionally impaired T cells up-regulate PD-1 (1–4). Interestingly, blocking the interaction of PD-1 with its ligand, PD-L1 (B7-H1) that is expressed on a variety of cell subsets including DCs and monocytes, allowed PD-1+ T cells to regain their proliferative capacity (1–4,7). PD-L1 expression levels have been demonstrated to directly correlate with viral load in HIV-1 infected individuals (8). However, the mechanism by which persistent HIV-1 replication induces the up-regulation of PD-L1 on antigen-presenting cells (APCs) remains to be elucidated. Here we demonstrate that the up-regulation of PD-L1 on DCs and monocytes can be induced by HIV-1-derived TLR7/8 ligands (9,10) and is dependant on the TLR adaptor molecule MyD88.

Freshly isolated PBMCs from fifteen HIV-1 negative individuals were stimulated with either a TLR9 ligand (ODN02216 5μM, Invivogen), synthetic TLR7/8 ligands CL097 (Invivogen, 1μg/ml) and Imiquimod (Invivogen, 1μg/ml), or HIV-1-derived TLR7/8 ligands (U-rich HIV-1-derived ssRNA sequences, 15 μg/ml), as described previously (9). The study was approved by the MGH Institutional Review Board, and each study subject gave informed written consent. After 20h of stimulation with the respective TLR ligands, up-regulation of PD-L1, CD80 and CD86 was assessed using 7-color flow-cytometry. PBMCs were labeled with a CD86-Biotin antibody, washed and stained with an antibody-cocktail containing CD3/CD19/CD56 -Alexa 700, CD14-APC-Cy7, CD11c-APC, CD123-PeCy5, PD-L1-PeCy7, CD80-FITC (all BD Biosciences), and Streptavidin-Cascade Yellow (Molecular probes). After fixation in Fix&PermA (Caltag), samples were acquired on an LSRII flow-cytometer. Unpaired two-tailed student T-tests were employed to assess statistical significance of differences.

Incubation of human PBMCs with the previously described HIV-1 derived uridine-rich ssRNA resulted in the up-regulation of PD-L1 on APCs, including monocytes (expressing TLR7 and TLR8), mDCs (expressing TLR8) and, to a lesser extent, pDCs (expressing TLR7) (figure 1A). In contrast, no up-regulation of PD-L1 was observed on either of these APCs when cells were incubated with control oligonucleotides in which all uridines were replaced by adenosines (U-to-A variants) (figure 1A), indicating that the HIV-1-derived ssRNA oligonucleotides were detected by TLRs, as previously shown (9). This was further supported by the lack of PD-L1 up-regulation on bone marrow derived macrophages (BMDM) derived from mice deficient for MyD88 (MyD88KO), a crucial signalling protein for TLR7 and TLR8, while BMDM derived from wild-type mice up-regulated PD-L1 in response to the HIV-1-derived TLR ligands (data not shown). In line with the results using HIV-1-encoded TLR7/8 ligands, the synthetic TLR7 and TLR7/8 agonists Imiquimod and CL097, and the TLR9 agonist ODN02216 also induced up-regulation of PD-L1 on human APCs (figure 1A).

In addition to the up-regulation of PD-L1, CD80 and CD86, two ligands for CTLA-4, were also significantly up-regulated on pDCs in response to the HIV-1 derived TLR ligands (as shown for CD80 in figure 1B). In contrast to this predominantly U variant-specific up-regulation of CD80 and CD86 on pDCs, mDCs and monocytes also up-regulated these two molecules, but not PD-L1, in response to control U-to-A oligonucleotides (figure 1B), indicating the presence of additional receptors other than TLR7/8 on those cells capable of sensing HIV-1 derived oligonucleotides.

In chronic HIV-1 infection, expression levels of PD-L1 (B7-H1) correlate directly with viral load levels (8), indicating a potential direct effect of the virus on PD-L1 expression. However, the mechanisms by which PD-L1 is up-regulated in these settings are not well understood. Here, we demonstrate for the first time that stimulation of DCs and monocytes with viral TLR7/8 ligands, including HIV-1-derived ssRNA, can lead to the up-regulation of PD-L1, providing a potential mechanism involved in the described up-regulation of PD-L1 in chronic HIV-1 infection. This up-regulation of PD-L1 by HIV-1-derived oligonucleotides was specific for the uridine-rich TLR7/8 ligands and depended on MyD88, while the U-to-A variants of these oligonucleotides also induced the up-regulation of other co-stimulatory molecules including CD80 and CD86 on monocytes and mDCs, but not pDCs, indicating the presence of other RNA sensing receptors in these cells.

Viral infections, including LCMV, HCV and HIV-1, can lead to the up-regulation of PD-1 expression on virus-specific T cells (1–4), sometimes starting during primary infection (2). Interaction of PD-1 with its ligand, PD-L1, results in the functional impairment of the PD-1 + virus-specific T cells (7). Most importantly, the blockade of this interaction between PD-1 and PD-L1 has been shown to reconstitute T cell function (1,3,4), and to enhance immune control of LCMV viremia in *in vivo* (2). Activation of DCs or monocytes by TLR ligands can result in secondary activation of T cells *in vitro* (9), and pathogen-encoded TLR ligands have been proposed to contribute to the unspecific immune activation observed during chronic persistent infections, such as HIV-1 (11). The pDC response to viral infections might therefore have different effects on T cell immunity at different stages of infection. While virus-encoded TLR ligands enhance the ability of pDCs to prime virus-specific T cells in primary infection, persistent TLR-mediated stimulation of pDCs in chronic infection might contribute to unspecific immune activation, while PD-L1 expression on pDCs down-modulates the function of virus-specific effector T cells that preferentially express PD-1.

Further studies are needed to explore the regulatory network between pathogen-induced activation of APCs, resulting unspecific T cell activation, and expression of inhibitory receptors/ligands such as PD-1 on virus-specific T cell subsets. A better understanding of these pathways will be crucial for the design of immunotherapeutic interventions aimed at decreasing

the unspecific hyper-activation of the immune system that has been strongly associated with HIV-1 immunopathology, while increasing virus-specific T cell immunity that might contribute to control of viral replication.

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Figure 1. Up-regulation of PD-L1 and CD80 on monocytes, mDCs and pDCs following stimulation with HIV-1 derived TLR ligands

Figure 1A: Flow plots demonstrating a representative example of the expression of PD-L1 on CD14⁺ monocytes (defined as CD3^{neg}CD19^{neg}CD56^{neg}CD14^{pos}), CD11c⁺ mDCs (defined as CD3^{neg}CD19^{neg}CD56^{neg}CD14^{neg}CD11c^{pos}) and CD123⁺⁺ pDCs (defined as CD3^{neg}CD19^{neg}CD56^{neg}CD14^{neg}CD11c^{neg}CD123^{bright}) in unstimulated controls (first column), after 20h stimulation of PBMCs with a control RNA sequence (A-Variant of gag RNA₁₁₆₆, gag RNA_{1166(A)} (AAGAAAAGAGAAACAAAAGA), second column) and after stimulation with Gag RNA₁₁₆₆ ((UUGUUAAGUGUUCAAUUGU), third column) and gp160RNA2093 ((UUUUUGCUGUACUUUCUAUA), last column). All RNAs were

complexed to DOTAP. Strong up- regulation of PD-L1 was seen in all cell types evaluated only after the stimulation with the U-rich sequence. The respective Bar-Graphs demonstrate the up-regulation of PD-L1 on Monocytes, mDCs and pDCs after 20h of stimulation with the indicated ligands for at least three independent experiments (data represent the mean results and standard deviations). Stars represent p values compared to unstimulated controls (***: $p < 0.0005$; **: $p < 0.005$; *: $p < 0.05$). RNA 40: (GCCCCGUCUGUUGUGUGACUC). RNA 41: (GCCCCGACAGAAGAGAGACAC)

Figure 1B: Flow plots demonstrating a representative example of the expression of CD80 on CD14+ Monocytes (defined as $CD3^{neg}CD19^{neg}CD56^{neg}CD14^{pos}$), CD11c+ mDCs (defined as $CD3^{neg}CD19^{neg}CD56^{neg}CD14^{neg}CD11c^{pos}$) and CD123++ pDCs (defined as $CD3^{neg}CD19^{neg}CD56^{neg}CD14^{neg}CD11c^{neg}CD123^{bright}$) in unstimulated controls (first column), after 20h stimulation of PBMCs with a control RNA sequence (A-Variant of gag RNA₁₁₆₆, gag RNA_{1166(A)} (AAGAAAAGAGAAACAAAAGA), second column) and after stimulation with Gag RNA₁₁₆₆ ((UUGUUAAGUGUUUCAAUUGU), third column) and gp160RNA2093 ((UUUUUGCUGUACUUUCUAUA), last column). All RNAs were complexed to DOTAP. Significant up- regulation of CD80 was seen on pDCs only after the stimulation with the U-rich sequence, while mDCs and monocytes also significantly up-regulated CD80 in response to the U-to-A variants, indicating the presence of other RNA sensing receptors in these cells. The respective Bar-Graphs demonstrate the up-regulation of CD80 on Monocytes, mDCs and pDCs after 20h of stimulation with the indicated ligands for at least three independent experiments (data represent the mean results and standard deviations). Stars represent p values compared to unstimulated controls (***: $p < 0.0005$; **: $p < 0.005$; *: $p < 0.05$). RNA 40: (GCCCCGUCUGUUGUGUGACUC). RNA 41: (GCCCCGACAGAAGAGAGACAC).