In Vivo Blockade of the PD-1 Receptor Suppresses HIV-1 Viral Loads and Improves CD4+ T Cell Levels in Humanized Mice

Brent E. Palmer, C. Preston Neff, Jonathan LeCureux, Angelica Ehler, Michelle DSouza, Leila Remling-Mulder, Alan J. Korman, Andrew P. Fontenot and Ramesh Akkina

*J Immunol* 2013; 190:211-219; Prepublished online 3 December 2012; doi: 10.4049/jimmunol.1201108

http://www.jimmunol.org/content/190/1/211

**References**

This article cites 43 articles, 18 of which you can access for free at: http://www.jimmunol.org/content/190/1/211.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
In Vivo Blockade of the PD-1 Receptor Suppresses HIV-1 Viral Loads and Improves CD4+ T Cell Levels in Humanized Mice

Brent E. Palmer,* C. Preston Neff,† Jonathan LeCureux,† Angelica Ehler,* Michelle DSouza,* Leila Remling-Mulder,† Alan J. Korman,‡ Andrew P. Fontenot,* and Ramesh Akkina†

The programmed death-1 (PD-1) pathway limits the function of virus-specific T cells during chronic infection. We previously showed that blockade of the PD-1 pathway increases HIV-1–associated T cell function in vitro. However, the effect of PD-1 blockade on HIV-1 disease progression in vivo has not been examined. As in humans, HIV-1–infected humanized BALB/c-Rag22/−/γc−/− (Rag-hu) mice express elevated levels of PD-1 on T cells during chronic infection. To examine the effect of PD-1 blockade on disease progression, Rag-hu mice with chronic HIV-1 infection were treated with a blocking mAb directed against programmed cell death-1 (PD-1) ligand-1, the ligand for PD-1. Programmed cell death-1 ligand-1–treated Rag-hu mice exhibited a progressive decrease in the HIV-1 plasma viral load, with a 7-fold decrease by day 7, a 20-fold decrease by day 14, a 178-fold decrease by day 21, and a 269-fold decrease by day 28 postinitiation of treatment. By day 7, the percentage of CD4+ T cells was statistically higher in the treated compared with the untreated group, and this trend was sustained throughout the 28-d treatment period. Moreover, there was a strong inverse correlation between plasma viral load and the percentage of both CD4+ and CD8+ T cells in the treated mice but not the untreated mice. This study provides “proof of concept” that humanized mice can be used to examine the effects of immunotherapeutic interventions on HIV-1 infection. Furthermore, to our knowledge, these data demonstrate for the first time that blockade of the PD-1 pathway reduces HIV-1 viral loads. The Journal of Immunology, 2013, 190: 211–219.

Viruses-specific T cells are functionally compartmented during chronic infections. Although these T cells retain some functional attributes, their ability to proliferate and produce multiple cytokines (1, 2), both of which have been correlated with control of viral replication, is severely affected (3–5). It is now widely accepted that receptor-based inhibitory pathways limit the function of virus-specific T cells during chronic viral infection. Inhibitory receptors, such as programmed death-1 (PD-1), are expressed at elevated levels on both CD4+ and CD8+ T cells in subjects with chronic HIV-1 infection, and diminished function of these cells may contribute to ineffective control of HIV-1 replication (6–8). Disruption of the PD-1 pathway using mAbs that block the PD-1 programmed cell death-1 ligand-1 (PD-L1) interaction increases the proliferative and cytokine-producing capacity of HIV-1–specific T cells in vitro (6). Furthermore, blockade of the PD-1 pathway in vivo increased SIV-specific T cell function, decreased SIV viral loads (VLs) and opportunistic infections, and increased the life span of SIV-infected macaques (9). These findings suggest that mAbs that block the PD-1 pathway may have therapeutic benefit in HIV-1–infected subjects.

However, experimental studies designed to test the efficacy of PD-1–blocking reagents on HIV-1 disease progression, as defined by persistent HIV-1 VL and declining CD4+ T cell count, have been difficult to conduct because of the lack of suitable in vivo animal models. In this regard, recent advances in the development of new-generation humanized mouse models for HIV-1 infection now make these studies possible (10). These new mouse models are constructed by injecting human CD34 hematopoietic stem cells into either Rag2 common γ-chain knockout or NOD scid γ (NOD.Cg-Prkdcscid IItgmu1Wj/SzJ) mice, which are severely immunodeficient. Human CD34+ stem cells efficiently engraft in these mice and give rise to human T, B, and dendritic cells (DCs) and monocytes (11–13). Furthermore, CD4+ T cells, macrophages/monocytes, and DCs capable of supporting productive HIV-1 infection in vivo are generated continuously, and infected humanized mice exhibit many of the clinical manifestations, such as plasma viremia and decreasing CD4+ T cell counts, similar to those seen in HIV-1–infected humans (14, 15). In addition to acute infection, we showed that Rag-hu mice can sustain chronic HIV-1 infection lasting >1 y. HIV can be experimentally transmitted to these mice via multiple routes, including natural mucosal routes (16, 17).

These important attributes of next-generation humanized mice have paved the way to dramatically expedite novel immunotherapeutic and immune-reconstitution efficacy studies in vivo.
ently, these studies are performed using nonhuman primate models or in human clinical trials, both of which are expensive and laborious to conduct. In this proof-of-concept study we exploited HIV-1–infected Rag-hu mice with chronic viremia to evaluate the potential therapeutic effects of a proprietary human anti–PD-L1 mAb on HIV-1 VL and CD4+ T cell count in vivo. Our results demonstrate that HIV-1–infected Rag-hu mice treated with anti–PD-L1 Ab exhibited significantly lower VLs and elevated/restored levels of CD4+ T cells in contrast to infected, untreated control mice. The reduction in HIV-1 plasma VLs correlated inversely with the percentage of CD4+ and CD8+ T cells in treated, but not in untreated, HIV-1–infected Rag-hu mice. To our knowledge, this study is the first to show that humanized mice can be used to evaluate novel Ab-based immunomodulation strategies for the treatment of HIV-1 infection.

Materials and Methods

Generation of humanized Rag2-/-γc-/- mice

Humanized BALB/c-Rag2-/-γc-/- mice were prepared by engraftment with human fetal liver-derived CD34+ hematopoietic progenitor cells, as previously described (14). Briefly, newborn mice were conditioned by irradiating with 3.5 Gy and then injected intraperitoneally with 0.5–1 × 10^6 human CD34+ cells. Mice were screened for human cell engraftment at 10–12 wk postcondition. Peripheral blood was collected by tail bleed, and RBCs were lysed using the Whole Blood Erythrocyte Lysing Kit (R&D Systems, Minneapolis, MN). The WBC fraction was stained with Abs against the human pan-leukocyte marker CD45 (Caltag) and analyzed by FACS to determine the levels of human cell engraftment, as we previously described (14).

HIV-1 infection and measurement of VLs

Mice with ≥70% human cell engraftment levels were used in these experiments. They were infected with HIV-1 by i.p. injection of CCR5 tropic strain Bal-1 (1.0 × 10^6 IU) ≥12 wk after engraftment. Mice were observed daily, and blood samples were drawn weekly to assess plasma viremia. To detect HIV-1 in plasma of infected mice by quantitative RT-PCR, RNA was extracted from 25 to 50 μl EDTA-treated plasma using the QIAamp Viral RNA kit (QIAGEN, Valencia, CA). Quantitative RT-PCR was performed using a primer set specific for the HIV-1 LTR sequence and a corresponding LTR-specific probe, as described previously (14).

PD-L1 mAb treatment schedule

HIV-1 Bal-1–infected mice were monitored weekly to determine viremia. Consistent viremia was established in all infected mice by 4 wk. For Ab treatment, seven viremic Rag-hu mice were infected i.p. with 200 μg Bristol-Myers Squibb (BMS) human anti–PD-L1 mAb once every 3 d for 4 wk, and then treated intraperitoneally with 0.5–1 × 10^6 human CD34+ cells. Mice were screened for human cell engraftment at 10–12 wk postcondition. Peripheral blood was collected by tail bleed, and RBCs were lysed using the Whole Blood Erythrocyte Lysing Kit (R&D Systems, Minneapolis, MN). The WBC fraction was stained with Abs against the human pan-leukocyte marker CD45 (Caltag) and analyzed by FACS to determine the levels of human cell engraftment, as we previously described (14).

Flow cytometry

Whole blood was collected, and RBCs were lysed, as reported previously (14, 19). WBCs were stained with anti-human CD45-allophycocyanin, CD3-PE, CD4-PECy5.5, CD8-Alexa Fluor 405, and CD1–FITC. Cells were stained with the labeled Abs and analyzed using a Coulter EPICS XL-MCL FACs analyzer (Beckman Coulter, Fullerton, CA) or BD LSR II (Becton Dickenson). CD4+ T cell levels were calculated as a ratio of the entire human CD45 population (i.e., CD45+CD4+CD8-) to the total human CD45 population. To establish the baseline CD4+ T cell levels, cells from all mice were analyzed prior to infection (day 0). PD-1 expression was examined on CD45+CD3+CD4+ and CD45+CD3–CD8+ T cells and displayed as median fluorescence intensity (MFI) using FlowJo software (TreeStar, Ashland, OR). Maturation state and PD-1 expression levels were examined before and after treatment with anti–PD-L1. To obtain enough cells, blood from two groups (n = 5 and n = 6) of HLA-matched, HIV-1–infected Rag-hu mice was pooled and stained with anti-human CD45-PE, CD3-BV 65, CD4-V500, CD8–Alexa Fluor 405, CD27–allophycocyanin–H7, CD45RA–PECy7, and CD1–FITC and analyzed using a BD LSR II. Fluorescence-minus-one controls were used in all experiments.

Cytokine analysis

Plasma samples from Rag-hu mice preinfection and before and after anti–PD-L1 treatment were tested for cytokines using the Human Th1/Th2 Ultra-sensitive Cytokine kit (Meso Scale Development, Gaithersburg, MD). TNF-α, IFN-γ, IL-13, IL-10, IL-5, and IL-12 p70 levels in the plasma were assayed. Plasma samples were stored immediately after collection at -80°C for this assay. Assays were performed per the manufacturer’s instructions and analyzed using a Sector Imager 2400 (Meso Scale Discovery).

Statistical analysis

Statistical significances in the VL and the percentage of CD4+ T cells between mice that were treated or not with PD-L1 mAb were calculated by the Mann–Whitney U test. Correlations between HIV-1 plasma VL and the percentage of T cells were assessed by the Spearman test using Prism 3.0 software (GraphPad).

Results

PD-1 expression is elevated on T cells in HIV-1–infected Rag-hu mice

To determine whether HIV-1 infection resulted in elevated expression of PD-1 in Rag-hu mice, its expression on total CD4+ and CD8+ T cells from the peripheral blood of 12 uninfected and 14 HIV-1–infected Rag-hu mice was assessed 2–5 mo postinfection. As shown in Fig. 1, PD-1 expression (percentage positive and MFI) was elevated on both CD4+ and CD8+ T cells in HIV-1–infected Rag-hu mice compared with uninfected control mice. The median percentage of PD-1+ CD4+ T cells was significantly higher (p < 0.0001) in HIV–1–infected mice (73.5%; range, 21.5–89.7%) than in uninfected mice (10%; range, 1.6–59.4%). The same was true for CD8+ T cells: the median percentage of PD-1+ cells was 96.7% (range, 57.4–100%) in the HIV–1–infected mice and 51.1% (range, 31.5–74.6%) in the uninfected mice (p < 0.0001). The PD-1 MFI was also significantly higher (p = 0.0003) on CD4+ T cells from HIV–1–infected Rag-hu mice (median, 2436; range, 318–6316) compared with uninfected controls (median, 348; range, 226–1824). PD-1 expression was also significantly higher (p = 0.03) on CD8+ T cells from HIV–1–infected Rag-hu mice (median, 2195; range, 1513–5339) compared with uninfected mice (median, 1631; range, 1079–3148). Furthermore, the expression of PD-1 on total CD4+ and CD8+ T cells from HIV–1–infected mice was similar. However, the baseline PD-1 expression (MFI) on CD8+ T cells from uninfected Rag-hu mice was higher than on CD4+ T cells (4-fold higher) from the same animals. This may be due to a chronic low-level xenoreactivity of CD8+ T cells.

Anti–PD-L1 treatment reduces HIV-1 VL

It was shown that administering mAbs that block the PD-1 pathway increases virus-specific T cell responses in vitro and decreases SIV and lymphocytic choriomeningitis virus (LCMV) replication in vivo. However, the effect of PD-1 blockade on HIV-1 replication in vivo has not been examined. To determine the effect of PD-1 pathway blockade on HIV-1 replication, we used the Rag-hu mouse model of HIV-1 infection and used BMS humanized anti–PD-L1 mAb. Data on individual treated and untreated mice are shown in Fig. 2A. The median VL of both groups of mice pretreatment was not significantly different (p = 0.29). Seven days posttreatment, the plasma VLs in five of seven of the treated mice had decreased, whereas it had only decreased in one of six un-
treated mice. By 21 d, the VL of all treated mice had dropped below pretreatment levels, whereas four of six untreated mice had VLs that were higher than their pretreatment levels. At 28 d, all treated mice maintained lower VLs relative to their pretreatment values, whereas three of six untreated mice still had VLs that were higher than baseline. As shown in Fig. 2B, the treated Rag-hu mice had reductions in their mean VLs of 7-fold at 7 d, 20-fold at 14 d, 178-fold at 21 d, and 269-fold at 28 d following the initiation of anti PD-L1 treatment. Conversely, untreated mice had a 0.5- and 0.9-fold increase in the mean VL at 7 and 14 d, respectively, and a 3- and 2-fold increase in mean VL at 21 and 28 d, respectively, compared with the pretreatment levels. As shown in Fig. 2C, median HIV-1 plasma VLs were significantly lower in the treated mice compared with untreated mice at 7 d (p = 0.014), 14 d (p = 0.022), and 21 d (p = 0.015) posttreatment. By 28 d, the VL in the untreated mice started to decline; this is attributed to the loss of CD4+ T target cells, which were drastically reduced by this time point, consistent with observations in previous studies. Accordingly, there is no statistically significant difference (p = 0.064) in median VL between treated and untreated mice at this time point, although the levels in treated mice are still close to a log lower (6.2 × 10^3 versus 4.7 × 10^3). The VL was determined in treated mice for an additional 35 d after the cessation of treatment. During this time there was a relative increase in the VL, although it was still lower at day 56 compared with pretreatment. HIV-1 plasma VL was also examined in five Rag-hu mice treated with an isotype Ab (anti-diphtheria toxin mAb). As expected, no reduction in HIV-1 plasma VL was observed in isotype-treated Rag-hu mice (data not shown).

As shown in Fig. 2A, there was a loss of one mouse (#973) in the PD-L1–treated group before completion of the study. The CD4+ T cell counts and HIV-1 VLs of this mouse were not significantly different from the group of mice treated with anti–PD-L1 mAb. It is unlikely that the loss of this mouse was due to the Ab treatment, because none of the five uninfected Rag-hu mice treated with anti–PD-L1 died (data not shown). The death of this animal is more likely due to the sporadic, but well-documented, spontaneous morbidity that occurs among severely immunocompromised mice (20).

**Anti–PD-L1 treatment increases the levels of human CD4+ T cells**

To determine whether PD-L1 Ab has any effect on CD4+ T cell levels in HIV-1 viremic mice, we monitored their levels on a weekly basis during and after the treatment. Our results showed that CD4+ T cell counts increased in HIV-1–infected Rag-hu mice treated with anti–PD-L1 mAb. As shown in Fig. 3A, the percentage of human CD4+ T cells, normalized by human CD45, increased in each of the seven treated mice during the 28-d treatment period. Of the seven Rag-hu mice treated with anti–PD-L1, three (#970, #971, and #989) had large increases and three (#972, #987, and #866) had modest increases in CD4+ T cell percentages. As mentioned above, one of the seven HIV-1–infected Rag-hu mice (#973) died after 3 wk of treatment, although during this time the percentage of CD4+ T cells increased from 12 to 19%. Among the untreated HIV-1–infected Rag-hu mice, all but one exhibited a decline in the percentage of CD4+ T cells during the same 28-d period. As shown in Fig. 3B, the relative change in the percentage of CD4+ T cells at days 7 and 14 was fairly static compared with pretreatment values (1.4- and 1.5-fold higher, respectively). However, by days 21 and 28, the percentage was >2.5-fold and almost 3-fold higher, respectively, than pretreatment values. In contrast, the fold change in the untreated HIV-1–infected mice consistently decreased over the same period; by day 28, the mean percentage of CD4+ T cells had declined to half of the pretreatment levels. No change in the percentage of CD4+ T cells was observed in four uninfected Rag-hu mice during the same time period (Fig. 3A).

To determine the impact of cessation of PD-L1 treatment on CD4+ T cell levels, the PD-L1–treated group was followed until day 91: 63 d after the last anti–PD-L1 treatment. Surprisingly, as shown in Fig. 3C, the median percentage of CD4+ T cells remained elevated 63 d after the treatment was discontinued. As
The percentage of CD4+ T cells in PD-L1–treated mice was significantly higher than in untreated Rag-hu mice at 7 (p = 0.014), 14 (p = 0.014), 21 (p = 0.017), 28 (p = 0.015), and 35 (p = 0.015) days postinitiation of treatment. Five HIV-1–infected Rag-hu mice were also treated with anti–PD-L1; an increase in the percentage of CD4+ T cells in the absence of HIV-1 infection was detected (data not shown), suggesting that some level of T cell exhaustion exists in Rag-hu mice not infected with HIV-1. It is possible that an antixenograft response may lead to T cell exhaustion and limit the expansion of CD4+ T cells in the absence of HIV-1 infection. However, although PD-1 expression was elevated on CD8+ T cells from HIV-1–infected and uninfected Rag-hu mice, its level on CD4+ T cells was low and similar to that observed on CD4+ T cells from uninfected humans.

The percentage of CD8+ T cells also increased in mice treated with anti–PD-L1. The median percentage of CD8+ T cells in HIV-1–infected mice that were treated or not with PD-L1, as well as mice not infected with HIV-1, are shown in Fig. 3D. As seen in the CD4+ subset, the percentage of CD8+ T cells also increased during the 28-d treatment period. The percentage of CD8+ T cells is significantly higher in the PD-L1–treated mice compared with both untreated HIV-1–infected and uninfected Rag-hu mice at days 14 (p = 0.001), 21 (p = 0.004), and 28 (p = 0.015). Although the increases were statistically significant, they were not as dramatic as in the CD4+ T cell compartment. The lack of disparity may be due to the more subtle difference in PD-1 expression on CD8+ T cells from HIV-1–infected and uninfected Rag-hu mice.

**Human T cell levels are inversely correlated with HIV-1 plasma VLs**

The percentages of CD4+ and CD8+ T cells and HIV-1 VLs were assessed to determine whether an association exists between T cells and viral replication. When the percentage of T cells and HIV-1 VL from all time points during the treatment period were examined, a strong inverse correlation was found between VL and CD4+ (r = −0.66, p < 0.0001) and CD8+ (r = −0.64, p < 0.0001) T cells (Fig. 4). However, when the HIV-1 VL and CD4+ and CD8+ T cells in untreated mice were analyzed, no such correlation was found (r = +0.21, p = 0.26 and r = +0.02, p = 0.91, respectively). Not surprisingly, this suggests that viral replication
levels in untreated mice are directly correlated with the number of CD4+ T cells, implying that the more target CD4+ T cells there are to infect, the higher the plasma VL. Overall, these data suggest that reinvigorated T cells in PD-L1–treated HIV-1–infected mice suppress HIV-1 replication.

**PD-L1 treatment increases the percentage of naive and central memory T cells**

To better understand how anti–PD-L1 treatment increased CD4+ T cell counts and decreased HIV-1 plasma VLs, the expression of CD27 and CD45RA was used to determine the percentage of naive (CD27+, CD45RA+), central memory (CD27+, CD45RA–), effector memory (CD27+, CD45RA–), and terminally differentiated effector memory (CD27–, CD45RA–) CD4+ and CD8+ T cells. After 4 wk of treatment, the percentage of naive CD4+ T cells increased from 19 to 35%, whereas the naive CD8+ T cells increased from 23 to 38% (Fig. 5). In addition, there was an increase in central memory CD8+ T cells from 8% pretreatment to 14% 28 d postinitiation of treatment. Conversely, effector memory CD4+ T cells decreased from 41.5% pretreatment to 23.0% 28 d postinitiation of treatment, and CD8+ T cells decreased from 27.4 to 18.7%. Terminally differentiated effector memory CD4+ T cells decreased from 31.2 to 24.4%, and CD8+ T cells decreased from 38.75% pretreatment to 27.9% 28 d postinitiation of treatment. When CD4+ and CD8+ T cells are combined, naive and central memory cells had increased significantly at 28 d compared with pretreatment (p = 0.02 and 0.03, respectively), whereas effector memory and terminally differentiated effector memory cells trended lower (p = 0.08 and 0.3, respectively). PD-1 expression was also measured on CD4+ and CD8+ T cells before and during anti–PD-L1 treatment. PD-1 expression on CD8+ T cells remained consistent over time, whereas the MFI of PD-1 on CD4+ T cells increased from 1818 pretreatment to 3714 at 28 d postinitiation of treatment. These data suggest that naive and central memory T cell expansion contributed to increasing T cell numbers and a consequent enhanced control of HIV-1 replication.

**Th1 cytokine levels increase after PD-L1 treatment**

To further understand how PD-1 pathway blockade affects the course of HIV-1 infection in Rag-hu mice, peripheral blood was

---

**FIGURE 3.** Treatment of HIV-1–infected Rag-hu mice with anti–PD-L1 mAb increases the percentage of CD4+ and CD8+ T cells. (A) Percentage of CD4+ T cells normalized by human CD45 in the blood of PD-L1–treated HIV+, untreated HIV+, and untreated HIV– Rag-hu mice during the 4-wk treatment period. (B) Fold change from pretreatment values for all three groups of Rag-hu mice. (C) The median percentage of CD4+ T cells for HIV-1–infected PD-L1–treated and untreated Rag-hu mice. (D) Median percentage of CD8+ T cells for all three groups. The data are representative of three experiments. *p < 0.05 (Mann–Whitney U test).
drawn from 11 mice before infection, just prior to the first dose of anti–PD-L1 mAb, and 7 d after the initial dose of anti–PD-L1 mAb. The levels of human Th1 (IFN-γ, TNF-α, IL-12 P70) and Th2 (IL-13, IL-10, IL-5) cytokines in the plasma were measured (Fig. 6). The levels of both Th1 and Th2 cytokines in uninfected mice were low to undetectable. Postinfection, but before anti–PD-L1 treatment, the levels of all cytokines examined were detectable and elevated in comparison with preinfection levels, with IFN-γ

![FIGURE 4. HIV-1 plasma VL in PD-L1–treated mice correlates inversely with the percentage of CD4+ and CD8+ T cells in treated (top panels), but not untreated (bottom panels), HIV-1–infected Rag-hu mice. Spearman correlation was used to determine statistical significance of r and p values as defined in each panel.](http://www.jimmunol.org/)

![FIGURE 5. Increased percentages of naive and central memory T cells after anti–PD-L1 treatment. Blood was pooled from two groups of Rag-hu mice, each engrafted from the same donor and stained with anti-human CD45, CD3, CD4, CD8, CD27, CD45RA, and PD-1. The percentage of naive and memory subsets (A) and PD-1 expression (B) were determined before and after anti–PD-L1 treatment on CD4+ T cells (solid line) and CD8+ T cells (dashed line). *p < 0.05 (Mann–Whitney U test).](http://www.jimmunol.org/)
being the highest. The level of all Th1 cytokines increased 7 d after initiation of the anti–PD-L1 treatment, whereas there was little to no change in the level of Th2 cytokines. After treatment, there was a 6-fold increase in the amount of IFN-γ (148 to 880 pg/ml) and a 2-fold increase in the amount of TNF-α (9.6 to 17.5 pg/ml), both of which are produced by T cells (p = 0.002 for both comparisons). Although there was a 3-fold increase in IL-12 (5.1 to 15.9 pg/ml), which is produced by monocytes/macrophages and DCs, it was not statistically significant (p = 0.108). Conversely, there was no statistically significant change in the level of IL-10, IL-13, or IL-5 as the result of anti–PD-L1 mAb treatment. These data demonstrate that blockade of the PD-1 pathway induces the production of Th1, but not Th2, cytokines.

**Discussion**

Although the current antiretroviral therapies (ARTs) have achieved impressive suppression of HIV-1 VLs and prolonged life, there is no complete cure. This goal can only be achieved by eliminating all of the infected cells in the body by developing and testing novel and innovative methods, such as strongly reinvigorating the impaired immune system resulting from long-standing antigenemia associated with chronic viral infections. In these preliminary proof-of-concept studies conducted toward this end, we obtained strong in vivo evidence that interfering with the PD-1 pathway responsible for T cell exhaustion during chronic HIV-1 infection reduces VL and improves CD4+ T cell levels. The highlight of our present study is that the potential benefits of PD-1 blockade during HIV-1 infection were tested and verified in a physiologically relevant in vivo setting using a next-generation humanized mouse model that mimics key aspects of chronic HIV-1 infection.

Until recently, experimental studies centered on immune reconstitution and immunomodulation, using humanized mice. In these experiments, CD4+ and CD8+ T cells from HIV-1–infected Rag-hu mice had elevated expression of PD-1. Our results are consistent with previous studies that used a humanized BLT mouse model in which it was shown that HIV-1–specific T cells exhibited elevated levels of PD-1/PD-L1 complex expression. Increased naive T cells are a marker of increased thymic output and are important for immune reconstitution after ART; thus, it is likely that they fuel the expansion of CD4+ T cells after treatment with anti–PD-L1 (37, 38). Central memory cells were also shown to be an early indicator of immune reconstitution during ART (38, 39). We also found that the percentage of CD4+ T cells compared with pretreatment values and untreated HIV-1–infected Rag-hu mice. Interestingly, anti–PD-L1 treatment increased the percentage of naive and central memory T cells. Increased naive T cells are a marker of increased thymic output and are important for immune reconstitution after ART; thus, it is likely that they fuel the expansion of CD4+ T cells after treatment with anti–PD-L1 (37, 38). Central memory cells were also shown to be an early indicator of immune reconstitution during ART (38, 39). We also found a strong and statistically significant correlation between the percentage of CD4+ T cells and HIV-1 plasma VLs in treated, but not untreated, Rag-hu mice. The reduction in HIV-1 plasma VLs was dramatic in PD-L1 Ab-treated mice. In mice showing the highest viral suppression, HIV-1 plasma VLs decreased from >1 million to <1000 copies of HIV-1 RNA/ml. A mean 269-fold decrease was noted for the seven HIV-1–infected mice that received treatment. No such decrease in VLs was seen in untreated infected mice, indicating that the decrease in HIV-1 replication is due to blockade of the PD-1 pathway by the anti–PD-L1 mAb.

As noted above, Rag-hu mice support multilineage hematopoiesis with continuous generation of human T cells that mature in the mouse thymus. With regard to their immune competence, Traggiai et al. (11) demonstrated that the human TCR VB repertoire in Rag-hu mice was normal and that these T cells were able to respond to EBV in an HLA-dependent manner. Furthermore,
other investigators were able to detect HIV-1–specific T cell responses using intracellular cytokine staining assays in pooled cells from the spleen and lymph node of infected mice (L. Su, personal communication). Our present observation that HIV-1 plasma VLs decreased dramatically after anti–PD-L1 mAb treatment suggests that reinvigorated HIV-1–specific T cell responses are exerting pressure on HIV-1 replication in vivo. In addition, HIV-1 plasma VLs rebounded when the Ab treatment was discontinued, suggesting that PD-1 blockade was responsible for augmented HIV-1–specific T cell function resulting in control of HIV-1 replication. In addition, HIV-1 plasma VLs rebounded when the Ab treatment was discontinued, suggesting that PD-1 blockade was responsible for augmented HIV-1–specific T cell function resulting in control of HIV-1 replication. Taken together, these data suggest that Rag-hu mice treated with anti–PD-L1 Ab mount effective HIV-1–specific T cell responses that suppress HIV-1 replication.

Control of HIV-1 replication in humans has been associated with a strong Th1 response (40, 41). This is the case in HIV-1–infected Rag-hu mice, as well. Th1 cytokines, but not Th2 cytokines, were significantly elevated after the initiation of anti–PD-L1 treatment. IFN-γ and TNF-α were elevated 7 d after the initiation of treatment and before an increase in T cell numbers, demonstrating that anti–PD-L1 treatment enhanced Th1 cytokine production of antiviral T cells. Interestingly, IL-12p70 levels were also elevated, suggesting that the PD-1 blockade also induced Th1-promoting cytokine production by DCs and monocytes/macrophages. Furthermore, an increase in central memory CD4+ and CD8+ T cells and a decrease in effector memory CD4+ and CD8+ T cells were detected after treatment. Central memory CD8+ T cells were shown to control chronic LCMV infection in mice better than effector memory CD8+ T cells because of their superior capacity to expand (42). Preservation of the central memory compartment has also been associated with long-term control of HIV-1 and SIV replication (43, 44).

In the control infected, but untreated, mice, the high HIV-1 VLs are indicative of the establishment of infection and the presence of available cell targets (CD4+ T cells) for infection. In the PD-L1 mAb-treated mice, although the percentage of CD4+ T cells increased significantly during the treatment period (thus providing more potential target cells for infection), the VL actually decreased. In fact, in mice treated with the anti–PD-L1 mAb, the percentage of CD4+ T cells showed an inverse correlation with VL. No such correlation existed in untreated Rag-hu mice. Although not statistically significant, there was a direct correlation between HIV-1 plasma VL and CD4+ T cell count, suggesting that higher percentages of CD4+ T cells in these mice provided more targets for infection, which resulted in higher HIV-1 plasma VLs, as alluded to above. Not surprisingly, increased percentages of cytotoxic CD8+ T cells were inversely correlated with HIV-1 plasma VL in PD-L1–treated, but not untreated, mice. Therefore, these data collectively suggest that re-energized HIV-1–specific T cells are responsible for subduing HIV-1 replication in Rag-hu mice when the PD-1/PD-L1 pathway is blocked.

In summary, to our knowledge, this study shows for the first time that blockade of the PD-1 pathway reduces HIV-1 replication in vivo. No overt adverse effects were noted in these mice as the result of PD-L1 mAb treatment, even when treated every 3 d for 4 wk. These results also suggest that Rag-hu mice provide a good model to evaluate the immunomodulatory effects of receptor blockade in vivo on human cells and its effect on the progression of a chronic human viral disease. With regard to the clinical application of the Ab, this study opens up many new possibilities to answer important questions, such as how long the therapeutic effect can be sustained and whether Abs to other co-inhibitory molecules, such as CTLA-4, will have additive therapeutic benefits.

Disclosures

A.J.K. is an employee of BMS, from which the anti–PD-L1 mAb used in this study was obtained. The other authors have no financial conflicts of interest.

References

7. Shultz, L. D., M. A. Brehm, S. Bavari, and D. L. Greiner. 2011. Humanized mice model to evaluate the immunomodulatory effects of receptor blockade in vivo on human cells and its effect on the progression of a chronic human viral disease. With regard to the clinical application of the Ab, this study opens up many new possibilities to answer important questions, such as how long the therapeutic effect can be sustained and whether Abs to other co-inhibitory molecules, such as CTLA-4, will have additive therapeutic benefits.

References

7. Shultz, L. D., M. A. Brehm, S. Bavari, and D. L. Greiner. 2011. Humanized mice model to evaluate the immunomodulatory effects of receptor blockade in vivo on human cells and its effect on the progression of a chronic human viral disease. With regard to the clinical application of the Ab, this study opens up many new possibilities to answer important questions, such as how long the therapeutic effect can be sustained and whether Abs to other co-inhibitory molecules, such as CTLA-4, will have additive therapeutic benefits.


