Recombinant Human Interleukin-15 and Anti-PD-L1 Combination Therapy Expands a CXCR3⁺PD1^{-/low} CD8 T-Cell Subset in Simian Immunodeficiency Virus-Infected Rhesus Macaques

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Background. The PD1/PD-L1 pathway contributes to the pathogenesis of human immunodeficiency virus (HIV)/simian immunodeficiency virus (SIV) infection, and blockade of this pathway may have potential to restore immune function and promote viral control or elimination. In this study, we combined a checkpoint inhibitor anti-PD-L1 (Avelumab) and recombinant human interleukin-15 (rhIL-15) in SIV-infected rhesus macaques (RM).

Methods. The rhIL-15 was administered as continuous infusion in 2 cycles of 10 days in the context of weekly administration of anti-PD-L1 (Avelumab) in SIV-infected RM receiving combination antiretroviral therapy (cART). Safety, immunological parameters, and viral loads were monitored during the study.

Results. Administration of rhIL-15/anti-PD-L1 was safe and well tolerated. Treatment resulted in transient increases in proliferating (Ki67⁺) natural killer and CD8 T cells. In addition, treatment expanded a CXCR3⁺PD1^{-/low} CD8 T-cell subset with the ability to secrete cytokines. Despite these effects, no changes in plasma viremia were observed after cART interruption.

Conclusions. Expansion of the CXCR3⁺PD1^{-/low} CD8 T-cell subset with functional capacity and potential to traffic to sites of viral reservoirs in SIV-infected rhesus macaques had no demonstrable effect on plasma viremia after cART interruption.

Keywords. anti-PD-L1; checkpoint inhibitor; HIV/SIV infection; IL-15.

The viral reservoir elimination represents the biggest challenge to achieve more definitive treatment, either viral eradication or a sustained combination antiretroviral therapy (cART)-free virologic remission in human immunodeficiency virus (HIV)-infected patients. In chronic simian immunodeficiency virus (SIV)/HIV infection, persistent virus contributes to a sustained inflammatory environment characterized by accumulation of “activated/exhausted” T cells expressing a variety of immunomodulatory receptors including, PD1, CTLA-4, LAG3, CD244/2B4, TIGIT, CD160, and others [1–14]. In particular, PD1 interaction with its ligand PD-L1 modulates T-cell

receptor signaling leading to diminished effector function including cytokine secretion, proliferation, cytotoxicity, motility, and cell survival [15–20]. Recent evidence indicates that HIV-infected cells can express checkpoint receptors, facilitating their survival. These findings suggested that blockade of these receptors on both infected targets and on “exhausted” effector T cells may improve immune function and targeting of the viral reservoir [21–24].

Expression of PD1 has been observed in SIV/HIV-infected lymph nodes, particularly on follicular CD4 and CD8 T cells [3, 25]. PD-L1 expression in lymph nodes from HIV-infected patients were localized in germinal center B cells and extrafollicular cells with myeloid/macrophage morphology [26, 27]. Studies of in vitro and in vivo blockade of the PD1/PD-L1 pathway in SIV/HIV infection have shown transient effects on enhanced virus-specific responses and improved viral control [24, 27–33]. Therefore, ongoing preclinical studies of immune checkpoint blockade are focused on combination therapies to overcome these partial responses improving immune function against HIV. In particular, recombinant human interleukin-15 (rhIL-15) shows potential for inclusion in such combination immunotherapy regimes based on its biological effects including

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homeostasis (by promoting proliferation and survival) and enhancing effector function of memory CD8 T cells and natural killer (NK) cells [34–40].

The safety and biological effects of administration of IL-15 produced in several systems have been assessed in non-human primates. Interleukin-15 promoted proliferation of CD8 T cells and NK cells and, when administered by continuous intravenous infusion, appeared to be more efficacious than subcutaneous injection in expanding the memory CD8 T-cell pool [41–45]. In SIV-infected rhesus macaques (RM), when administered with cART showed transient proliferation of total memory CD8 T cells but did not lead to CD4 T-cell reconstitution in mucosal and lymphoid organs [46]. Other strategies to improve its biological activity and half-life include the studies of IL-15/IL-15Ra heterodimer that showed enhanced cytotoxic potential of NK and CD8 T cells in secondary lymphoid tissues [47]. The IL-15-IL-15Ra/Fc complex (ALT-803) showed enhanced SIV-specific responses and a transient control of viral replication [48, 49].

Altogether, these observations suggest that IL-15 given in combination with a checkpoint inhibitor may have potential to improve CD8 T-cell function. We previously showed that anti-PD-L1 (Avelumab) administration in SIV-infected RM in the context of cART was safe and well tolerated [27]. In this study, we hypothesized that rhIL-15-driven expansion of virus-specific CD8 T cells in the context of PD1/PD-L1 pathway blockade can potentiate immune function and viral control [27–32]. We evaluated the safety and biological effects of rhIL-15 administered in cycles of 10 days by continuous infusion in combination with weekly administration of anti-PD-L1 (Avelumab) over 24 weeks. The rhIL-15/anti-PD-L1 combination treatment was safe and well tolerated. The rhIL-15 infusions were associated with transient increases in proliferating NK cells and effector memory CD8 T cells. Multidimensional single-cell analysis showed an expanded CXCR3⁺PD1^{low} CD8 T-cell subset that secrete cytokines in response to SIV_{Gag} peptides. Despite these clear biological effects, indicative of delivery of bioactive levels of therapeutics, no impact was observed on rebound plasma viremia after cART discontinuation and during anti-PD-L1 administration.

MATERIAL AND METHODS

Animals and Study Design

The RMs were studied under an approved protocol by the Institutional Animal Care and Use Committee of Smithers Avanza (Study No. 2078-13161) in accordance with US National Institutes of Health guidelines, the US Department of Agriculture (USDA) Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals, and the US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals. Animals were ages ranging from males 2.1 to 4.1 years (n = 6) and females

2.7 to 3.0 years (n = 6). The RM were infected with 100 50% tissue culture infective dose units of SIV_{mac239}. At day –91 of the study (Figure 1B) and after reaching viral set point (day 70 post infection), animals started cART containing emtricitabine at 30 mg/kg and tenofovir at 20 mg/kg via daily subcutaneous injection (Gilead Sciences, Inc., Foster City, CA) and raltegravir at 400 mg/kg p.o twice/day (Hanna's Pharmaceutical Supply Co., Inc., Wilmington, DE). Animals were stratified in study groups using computer-generated random numbers after the first 3 weeks of cART therapy in the study groups control (n = 6) and treatment (n = 6) (Figure 1). At Day = 0, animals were administered sterile saline (n = 6) or a fully human anti-PD-L1 (MSB0010718C, Avelumab; provided by EMD-Serono, Rockland, MA, US) at a dose of 20 mg/kg per dose per week during 24 weeks as previously described to be safe and well tolerated [27]. Administration of saline or anti-PD-L1 treatments was performed by continuous infusion via the saphenous vein for 10 ± 2 minutes (Figure 1, doses depicted as arrows on the gray lines). The rhIL-15 (NCI-Frederick, Frederick, MD) was produced under current good manufacturing practice conditions in an *Escherichia coli* system as previously described [43]. At Day = 2 of the study, animals received the first cycle of 10 days continuous infusion of rhIL-15 (10 µg/kg per day) administered with surgically implanted infusion pumps as described [44]. A second cycle was administered during Days 37 through 47 of study (Figure 1, pink bars). At Day = 49 and after completion of the 2 cycles of rhIL-15 and 7 doses of anti-PD-L1, cART was discontinued and anti-PD-L1 treatment continued until completion of the 24 weeks of treatment. Blood was collected from all animals for plasma SIV ribonucleic acid (RNA) measurements, described by [50], and for hematology, clinical pathology, CD4 T-cell counts, and flow cytometry analysis (Supplementary Material and Methods).

Flow Cytometry

Whole blood was used for the analysis of T-cell subsets, B cells, NK cells, and monocytes.

Cytokine Secretion Assay

Frozen peripheral blood mononuclear cells (PBMCs) were used to assess phenotype and cytokine secretion from the control and rhIL-15/anti-PD-L1 treatment groups as described in Supplementary Material and Methods and Supplementary Table S1. The SIV_{mac239} Gag Peptide Pool was obtained through the NIH-AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH. Some mAbs were provided by the NIH-Nonhuman Primate Reagent Resource.

Statistical Analysis

Analysis of viral load (VL) Log₁₀ was performed using 2-sample *t* statistics. *P* < .05 was considered significant. The analysis of

Figure 1. Study design and viral loads during administration of recombinant human interleukin-15 (rhIL-15) and anti-PD-L1 (Avelumab) in rhesus macaques (RM) infected with simian immunodeficiency virus (SIV). (A) Design and timeline of the study. The SIV-infected RM receiving combination antiretroviral therapy (cART) were stratified to control (*n* = 6) and treatment groups (*n* = 6). The treatment group received weekly administration of anti-PD-L1 (MSB0010718C, Avelumab, 20 mg/kg; EMD-Serono) for 24 weeks, indicated as the arrows on the gray lines. The first dose was administered at Day = 0 of the study (week 1). The rhIL-15 was administered by continuous infusion (10 µg/kg per day), indicated as pink bars. The first cycle of rhIL-15 was administered 2 days after the first dose of anti-PD-L1, Days 2 to 12 of the study. The second cycle was administered at Days 37 to 47 of the study (between weeks 6 and 7). After completion of the second infusion of rhIL-15 and a total of 7 doses of anti-PD-L1, cART was discontinued and anti-PD-L1 (Avelumab) followed for additional weeks to complete 24 weeks of treatment. Viral loads were monitored through the study and for an additional 8 weeks after all treatment finished. (B) Viral load history. Log₁₀ viral loads since infection (Days -160 to day -7) and during study (Days 0 to 218). Black dashed line (Day -91) represents the initiation of cART, and red dashed line represents cART interruption (Day = 49). Pink bars represent rhIL-15 infusion cycles, first cycle (Days: 2 to 12), and second cycle (Days: 37 to 47). The gray lines with black arrows indicate the weekly doses of Avelumab. Dashed blue line represents the last dose of anti-PD-L1. Animal (ID: 05C, Log₁₀ viral load dashed red line) developed antidrug antibodies to anti-PD-L1. Viral rebound analysis was performed using a 2-sample *t* test, and *P* < .05 was considered significant.

fold change percentage and total cell numbers of lymphocytes, CD4 and CD8 T-cells and T-cell subsets, NK and B cells during the study (Supplementary Figures S1 and S2), 2-sample *t* statistic adjusted for multiple comparison was used to assess the difference between groups for the changes from the baseline.

P < .001 was considered significant, and *P* < .05 and *P* < .01 were treated as trend toward significance and possible significance, respectively. Comparisons between groups were performed by non-parametric Mann-Whitney test, and *P* < .05 was considered significant.

RESULTS

Administration of Recombinant Human Interleukin-15 by Continuous Infusion in Combination With Anti-PD-L1 (Avelumab) in Simian Immunodeficiency Virus-Infected Rhesus Macaques: Safety and Treatment Impact

We designed a preclinical study to test the biological effects of IL-15-dependent expansion of CD8 T cells in the context of PD1/PD-L1 blockade and its impact in anti-SIV responses. Previous observations from our group had shown that rhIL-15 administered by continuous infusion led to greater expansion of memory CD8 T cells [44]. We hypothesized that anti-PD-L1 administration during the rhIL-15 infusion will facilitate the expansion and function of SIV-specific memory CD8 T cells. The SIV_{mac239}-infected RMs were assigned in treatment ($n = 6$) and control ($n = 6$) groups as described in Material and Methods and Figure 1. The treatment group received weekly administration of anti-PD-L1 (Avelumab, 20 mg/kg) during 24 weeks (arrows on the gray lines indicate weekly doses) as previously described to be safe and well tolerated under cART [27]. The rhIL-15 was administered in 2 cycles of 10-day continuous infusion (10 μ g/kg per day) (depicted as pink bars in Figure 1). The first cycle of rhIL-15 was administered 2 days after the first dose of anti-PD-L1 (Days 2 to 12 of the study) to facilitate expansion of CD8 T cells in the context of PD1-PD-L1 blockade. After administration of 6 doses of anti-PD-L1, the second cycle of rhIL-15 was administered corresponding to Days 37 to 47 of the study (between weeks 6 and 7). Two days after completion of the second infusion of rhIL-15 and a total of 7 doses of anti-PD-L1, administration of cART was discontinued and anti-PD-L1 administration was continued for additional weeks to complete 24 weeks of treatment (Figure 1A, Study Design). In this treatment schedule, we hypothesized that cART interruption after the second cycle of rhIL-15 (at the peak of expansion) will give an advantage to the SIV-specific CD8 T cells, and in the context of PD-L1 blockade it will prevent premature exhaustion and promote viral control.

Administration of 2 cycles of rhIL-15 in combination with anti-PD-L1 (Avelumab) was well tolerated. Animals were monitored by physical examinations, body weight and food consumption, leukocyte counts, and clinical chemistry (Supplementary Figure S1 and Supplementary Tables S2 and S3). The rhIL-15-related events were observed only in 1 animal and included facial and neck swelling after the first cycle. Transient changes in the clinical chemistry panel were observed during the IL-15 cycles (Supplementary Table S2). No changes were noted in white blood cell count or leukocyte differential (Supplementary Figure S1).

No adverse effects associated with anti-PD-L1 administration were noted during suppressed viremia or during viral replication after cessation of cART. In the treatment group, 1 of 6 animals (animal identification [ID]: O5C) developed antidrug antibodies (ADA) for anti-PD-L1. The ADA was detected after

the fourth dose of anti-PD-L1 and remained through the study (data not shown and Figure 1B, Log₁₀ VL dashed red line).

Figure 1B depicts longitudinal plasma viremia levels from the time of infection (Days = -160 to -7) and days of the study treatment (Day = 0 to 218). The average mean Log₁₀ VL before initiation of study (Days = -160 to -7) in control and treatment groups were 4.02 and 4.71, respectively ($P = .106$). At Day -7, not all of the animals had suppressed viremia, and the mean Log₁₀ VL in control and treatment groups were 1.53 and 2.53, respectively ($P = .073$).

After 7 doses of anti-PD-L1 and completion of 2 cycles of rhIL-15 (Day 49 of the study), cART was stopped (Figure 1B). To assess the treatment impact, we compared the VL changes. The mean Log₁₀ VL before (Days -160 to -7) and after treatment (Days = 0 to 218) were 4.019 and 3.869 for the control group and 4.708 and 4.259 for the treatment group. The mean changes in Log₁₀ VL after treatment were -0.149 for the control group and -0.449 for the rhIL-15/anti-PD-L1 group with a 95% confidence interval (CI) of -.819 to .220, and the comparison between the groups was not significant ($P = .228$). The analysis showed similar results ($P = .311$) when the animal (ID: O5C) that developed antidrug (anti-PD-L1) antibodies was excluded (Figure 1B, Log₁₀ VL red dashed line). The mean changes of the Log₁₀ VL were -0.4299 for the treatment group and -0.149 for the control group (95% CI, -.876 to .315).

Cycles of continuous infusion of rhIL-15 in combination with the checkpoint inhibitor anti-PD-L1 (Avelumab) treatment were safe and well tolerated. Treatment had no effect on plasma viremia after discontinuation of cART during continued weekly administration of anti-PD-L1.

Administration of Recombinant Human Interleukin-15 by Continuous Infusion and Anti-PD-L1 Treatment Drives Proliferation of Natural Killer and CD8 T Cells

Administration of rhIL-15 has been evaluated as single intervention in SIV-infected and -noninfected macaques showing biological effects in NK and CD8 T cells [45, 46 and Supplementary 51]. In this study, in addition to the safety, we investigated biological effects of administration of rhIL-15 in SIV-infected RM receiving anti-PD-L1 treatment. We observed that continuous infusions of rhIL-15 (10 μ g/kg per day) during 10 days led to increased cycling of CD8 T and NK cells (Figure 2). In the CD8 T-cell subsets, the biggest effect was observed in the CD8 T-cell effector memory phenotype during the 2 cycles of rhIL-15 (Figure 2B). In CD4 T cells, a moderate effect was observed in the effector memory subset. The increase of cycling cells was associated with modest fold changes from the pretreatment baseline in CD8-naïve T-cell count after completion of the first cycle and total CD8 naïve T-cell count after completion of the second rhIL-15 infusion (Supplementary Figures S1 and S2 and Supplementary Tables S3 and S4). These data show that rhIL-15 administered as continuous infusion in the context of anti-PD-L1 treatment drives proliferation of lymphocyte subsets and NK and memory CD8 T cells as previously described when used as monotherapy [45, 46].

Figure 2. Administration of recombinant human interleukin-15 (rhIL-15) and anti-PD-L1 (Avelumab) promotes proliferation of natural killer (NK) and CD8 T-cell subsets. (A) Detection of proliferating cells was performed by whole blood staining of samples from the control ($n = 6$) and treatment ($n = 6$) groups using a cocktail of monoclonal antibodies for surface markers: CD3, CD20, CD4, CD8, CD95, and CD28 followed by intracellular staining of Ki67 (Supplementary Table S1, Panel 1). The top panel shows the gating strategy of T, B, and NK cells. CD4 and CD8 T-cell subsets were defined as naive (T_N CD95⁺CD28⁺), central memory (T_{CM} CD95⁺CD28⁺), and effector memory (T_{EM} CD95⁺CD28⁺). Ki67 for each lymphocyte subset was gated based on immunoglobulin G (IgG) control. (B) Expression of Ki67 in CD4 and CD8 T-cell subsets and NK cells. Graph represents median and interquartile range in control (black symbol) and treatment (red symbol) groups. Comparison between groups was performed using nonparametric Mann-Whitney test, and $P < .05$ was considered significant. *, $P < .05$; **, $P < .01$. cART, combination antiretroviral therapy; CM, central memory; EM, effector memory; FSC, forward scatter; SSC, side scatter.

Administration of Recombinant Human Interleukin-15 in Combination With Anti-PD-L1 Leads to Phenotypic and Functional Changes of a CXCR3⁺PD1^{low} CD8 T-Cell Subset

We further assessed the immunological changes induced by administration of rhIL-15 and anti-PD-L1 in T cells by flow cytometry (Supplementary Table S1, Panel 4). We studied the PBMCs from the time points as depicted in Figure 3A: Day -7

(baseline), Day 49 corresponding to the time point of discontinuation of cART after completion of the second IL-15 cycle (Day = 37 to 47), and Days 92 (anti-PD-L1 alone, week 14 of the study) and 204 (week 30, no-treatment).

Treatment induced a significant increase in the proportion of CXCR3⁺PD1^{low} and to a lesser degree CXCR3⁺PD1⁺ CD8 T cells, and there was a reduction in CXCR3⁺PD1⁺CD8 T cells

(Figure 3B, right panel). The proportion of CXCR3⁺PD1⁻ and CXCR3⁺PD1⁺CD8 T cells was reduced after viral rebound, although it remained significantly higher than the control group. In contrast, CD4 T-cell subsets showed no significant changes (Figure 3B, left panel).

We next assessed the treatment impact on the functional capacity of the CXCR3⁺ CD8 T cells and PD1⁺ CD8 T cells (Figure 4). The PBMCs from the time points corresponding to Days -7, 49, 92, and 204 of study (as described above) were stimulated with SIV_{Gag} peptides and dimethyl sulfoxide as control. A significant increase of CXCR3⁺ and PD1⁺ CD8 T cells secreting IL-2 was observed (Figure 4). The proportion of IL-2-secreting T cells decreased at Day 92, although it remained significantly different in the CXCR3⁺CD8 T cells to the control group (Figure 4A). The IL-2 secretion seemed to be an antigen independent effect of IL-15 because no difference was observed between the control and SIV_{Gag} peptides stimulation.

CD107a was significantly increased in CXCR3⁺ but not in the PD1⁺ CD8 T cells at the time point Day 49 of the study when

compared with the control group. This change was transient and decreased to basal levels by Day 92 of the study. CXCR3⁺ CD8 T cells secreting interferon (IFN) γ and tumor necrosis factor (TNF) α were observed at Day 49 and showed a trend to increase at Day 92 during viral rebound (Figure 4A and Figure 1B). The PD1⁺ CD8 T cells secreted cytokines after SIV_{Gag} peptide stimulation although there were less apparent differences between the groups (Figure 4B). These data suggest that rhIL-15/anti-PD-L1 treatment drives expansion of CXCR3⁺ CD8 T cells that exhibit capacity to secrete cytokines and potential to migrate to peripheral tissue. In contrast, similar secretion of IFN γ and TNF α was observed in the PD1⁺ CD8 T cells in control and treatment groups, suggesting a heterogeneity of the SIV-specific CD8 T cells in terms of CXCR3 and PD1 expression.

To better characterize the dynamics of this population during treatment, we performed multidimensional single-cell analysis using FlowSOM (Flow Self-Organizing Map, FlowJo) [Supplementary 52]. FlowSOM uses 2-level clustering and star charts. The results are visualized as cell clusters distributed in a

Figure 3. Administration of recombinant human interleukin-15 (rhIL-15) and anti-PD-L1 mainly drives expansion of CXCR3⁺PD1⁻ CD8 T cells. (A) Study timeline and the time points analyzed: Day -7 (baseline), Day 49 combination antiretroviral therapy (cART) discontinued after completion of 2 cycles of rhIL-15 and 7 doses of anti-PD-L1. Day 92 corresponds to anti-PD-L1 dose 14 and Day 204 corresponds to 6-week follow-up after completion of all treatments; the last dose of anti-PD-L1 occurred on Day 162 of the study (week 24). (B) Dynamics of PD1 and CXCR3 expression in CD4 and CD8 T-cell subsets during the study. Peripheral blood mononuclear cells (PBMCs) from control and treatment groups were thawed and rested overnight. Cells were stained for surface markers using Panel 4 (Supplementary Table S1). (Top Panel) CD4 and CD8 T cells were analyzed by gating live cells and singlets followed by CD3⁺ T cells. Gating for expression of CXCR3 and PD1 was performed based on FMO (Full minus one). (Bottom Panel) Frequency of CXCR3 and PD1 is plotted as median and interquartile range for each of group; black symbols for the control group and red symbols for the treatment group. Comparison between groups was performed using non-parametric Mann-Whitney test, and $P < .05$ was considered significant. *, $P < .05$; **, $P < .01$.

Figure 4. Recombinant human interleukin-15 (rhIL-15) and anti-PD-L1 combination therapy promotes expansion of a CXCR3⁺ CD8 T-cell subset with effector capacity. Peripheral blood mononuclear cells (PBMCs) from control (n = 6, black symbols) and treatment (n = 6, red symbols) groups were thawed and rested overnight. Rested PBMCs were stimulated with SIV_{Gag} (closed symbols) and dimethyl sulfoxide as control (open symbols) during 6 hours for functional analysis. After stimulation, cells were stained with a cocktail of monoclonal antibodies for surface markers and intracellular markers (Supplementary Table S1, Panel 4). (A) CXCR3⁺ and (B) PD1⁺ CD8 T cells were analyzed for cytokine secretion (IL-2, interferon [IFN]γ, and tumor necrosis factor [TNF]α) and the degranulation (CD107a surface expression). The frequency of cells is plotted as median and interquartile range. Comparison between SIV_{Gag}-stimulated groups was performed using nonparametric Mann-Whitney test, and *P* < .05 was considered significant. *, *P* < .05; **, *P* < .01.

minimal spanning tree (Figure 5A, each clustered is colored). Each cluster contains nodes (representing heterogeneous cell populations within the cluster). Inside each node, a pie chart indicates the mean fluorescence intensities of the selected markers. These charts visualize the mean intensities of the markers for all cells in the dataset assigned to a node. The height of each wedge within the chart indicates the intensity of expression of the selected marker. In this analysis, we evaluated the phenotype and functional markers CXCR3, PD1, IL-2, IFNγ, TNFα, and CD107a.

The same FlowSOM map was applied to the groups to allow longitudinal comparison. In the 8 clusters of CD8 T cells (Figure 5A), the dark green cluster (amplified for visualization in Figure 5B) confirmed the results presented in Figure 4A. In addition, this cluster was enriched compared with the baseline (Day -7) in the treatment group at Day 49 of the study (Figure 5B). The cluster is formed by CXCR3⁺-expressing CD8 T cells that are PD1^{-low} and express functional molecules such as IL-2, CD107a, IFNγ, and TNFα. This subset of CD8 T cells was still detectable at Day 92 of the study and returned to baseline levels at Day 204 of the study. A smaller cluster (Supplementary Figure S3, clear blue) was composed by CD8 T cells that express CXCR3⁺PD1⁺ and can also secrete cytokines. In contrast to CD8 T cells, minor changes were observed in CD4 T cells (Supplementary Figure S3).

These results suggest that rhIL-15 in the context of anti-PD-L1 treatment drives expansion of a CD8 T-cell subset with the potential to traffic peripheral tissues and secrete cytokines.

DISCUSSION

In this first study of combination treatment using rhIL-15 and the checkpoint inhibitor Avelumab in SIV-infected RM, we assessed the safety and immunological parameters of the treatment. The rhIL-15 induced transient proliferation of NK cells and CD8 T cells as previously described in studies using IL-15 administration as monotherapy in SIV-infected and noninfected RM [41–46], [Supplementary 51]. In contrast, we observed modest and transient changes in peripheral cell counts. These differences may be related to the rhIL-15 preparation, dose, mode of administration, and/or the effect of the checkpoint inhibitor.

Likewise, in patients with malignancies, rhIL-15 administered by continuous infusion led to proliferation of CD8 T cells and NK cells, and lower doses showed activation of CD8 T cells without cell expansion [Supplementary 53]. Patients experienced dose-related adverse events [Supplementary 53, 54].

In HIV-infected patients, a recent report of a phase 1 study using an IL-15 superagonist (ALT-803) showed increased activation in CD4, CD8 T cells, and NK cells and transient increase of HIV transcription [Supplementary 55].

The rhIL-15/anti-PD-L1 promoted expansion of the CXCR3⁺PD1^{-low} CD8 T-cell subset with effector capacity. It has been shown that CXCR3 promotes T-cell trafficking in the microenvironment of peripheral tissues and lymphoid

Figure 5. Recombinant human interleukin-15 (rhIL-15) and anti-PD-L1 combination therapy promotes expansion of a CXCR3⁺PD1^{low} CD8 T-cell subsets with functional capacity. Multidimensional single-cell analysis was performed by FlowSOM analysis. Eight thousand events of CD8 T cells from SIV_{Gag} peptide stimulated from each sample of the control (n = 6) and treated (n = 6) groups were concatenated. Compensated concatenated files were then analyzed using FlowSOM (FlowJo). The clustering was done based on the expression of CXCR3, PD1, interleukin (IL)-2, interferon (IFN)γ, CD107a, and tumor necrosis factor (TNF)α. The same FlowSOM map was applied to all samples to allow comparison between the groups and the time points. FlowSOM identified 8 clusters (colored). The nodes contain a pie chart showing the intensity of expression of selected markers. (A) Clustering of the control and rhIL-15/anti-PD-L1-treated animals on time points Days -7, 49, 92, and 204 of the study. (B) Days 49 and 92 of the treatment group are magnified to show the relative expression of CXCR3, PD1, IL-2, IFNγ, CD107a, and TNFα.

compartment facilitating the interaction of T cells with antigen-presenting cells, and IL-15 administration enhanced this property [45], [Supplementary 56]. Although we did not have access to tissues to assess accumulation of the CXCR3⁺PD1^{low} CD8 T-cell subset, these cells have the potential to traffic into peripheral tissues harboring the reservoir and exert effector function.

In addition, a new subset of virus-specific CD8 T cells that express PD1 and TCF1 in chronic infection have been described. These cells exhibit stem cell-like properties with self-renewal capacity and expand during PD1 blockade [Supplementary 57, 58]. If the CXCR3⁺PD1^{low} CD8 T cell subset expanded during treatment has stem-cell like properties needs to be addressed.

The expansion of CXCR3⁺PD1^{-/low} CD8 T cells during IL-15 cycle was transient and decreased when cART was interrupted, suggesting that treatment of SIV/HIV infection may require therapeutic strategies that promote more robust and long-lasting effects in CD8 T cells.

In addition to PD-L1, PD1 interacts with PD-L2. PD-L1 is expressed by a wide array of cells (hematopoietic, mesenchymal stem cells, and epithelial cells) [15]. In contrast, PD-L2 expression is more restricted to antigen-presenting cells and its expression can be induced [15], [Supplementary 59]. Although treatment expanded a subset of CD8 CXCR3⁺ virus-specific T cells with low PD1 expression, we cannot exclude that interaction with PD-L2 expressing antigen-presenting cells can have an effect.

One limitation of the present study is that the SIV RNA plasma levels were still detectable, before initiation of treatment. The viral reservoir is likely to be bigger and more challenging to be overcome by the IL-15-expanded CD8 T cells. Studies in HIV-infected patients had shown that residual viremia under successful cART is associated with slow decay of the reservoir [Supplementary 60]. Therefore, successfully suppressed residual viremia in combination with immunotherapies may be required to achieve viral control.

Currently, HIV-infected patients are receiving checkpoint inhibitors as clinical care for cancer and anti-HIV responses and viral effects are being monitored. Human immunodeficiency virus-infected patients treated with anti-PD1 (Nivolumab) for lung cancer showed reduction in cell-associated HIV deoxyribonucleic acid and increases in HIV-specific CD8 T-cell responses [Supplementary 61, 62]. In contrast, other studies reported inconsistent changes during treatment [Supplementary 63, 64].

The HIV/SIV reservoir is maintained by clonal expansion of infected cells [Supplementary 65, 66]. In an in vitro model of HIV latency in CD4 T cells, blockade of PD1 using pembrolizumab enhanced the effects of a latency reversal agent, bryostatine, without increasing T-cell activation [Supplementary 67]. These cells are heterogeneous in phenotype and function, suggesting that combination strategies targeting multiple cell subsets harboring HIV/SIV will be required [Supplementary 68].

CONCLUSIONS

We showed that combination treatment rhIL-15/PD-L1 led to the expansion of a virus-specific CXCR3⁺PD1^{-/low} CD8 T-cell subset with the capacity to secrete cytokines. This T-cell subset may be targeted as a potential source of more effective SIV-specific CD8 T cells with a potential to traffic to sites of viral reservoirs.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to

benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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