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Plasmacytoid dendritic cells sense HIV replication before detectable viremia following treatment interruption

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Plasmacytoid dendritic cells (pDCs) are robust producers of interferon α (IFN α) and one of the first immune cells to respond to simian immunodeficiency virus infection. To elucidate responses to early HIV-1 replication, we studied blood pDCs in 29 HIV-infected participants who initiated antiretroviral therapy during acute infection and underwent analytic treatment interruption (ATI). An increased frequency of partially activated pDCs was observed in the blood prior to detection of HIV RNA. Concurrent with peak pDC frequency, there was a transient decline in the ability of pDCs to produce IFNα in vitro, which correlated with decreased interferon regulatory factory 7 (IRF7) and NF-kB phosphorylation. Levels of phosphorylated IRF7 and NF-kB inversely correlated with plasma IFNα2 levels, implying that pDCs were refractory to in vitro stimulation after IFNα production in vivo. After ATI, decreased expression of IFN genes in pDCs inversely correlated with time to viral detection, suggesting that pDC IFN loss is part of an effective early immune response. These data, from a limited cohort, provide a critical first step in understanding the earliest immune response to HIV-1 and suggest that changes in blood pDC frequency and function can be used as an indicator of viral replication before detectable plasma viremia.

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- 1 Plasmacytoid dendritic cells sense HIV replication before detectable viremia following
- 2 treatment interruption

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Abstract

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Plasmacytoid dendritic cells (pDCs) are robust producers of interferon α (IFN α) and one of the first immune cells to respond to simian immunodeficiency virus infection. To elucidate responses to early HIV-1 replication, we studied blood pDCs in 29 HIV-infected participants who initiated antiretroviral therapy during acute infection and underwent analytic treatment interruption (ATI). An increased frequency of partially activated pDCs was observed in the blood prior to detection of HIV RNA. Concurrent with peak pDC frequency, there was a transient decline in the ability of pDCs to produce IFNa in vitro, which correlated with decreased interferon regulatory factory 7 (IRF7) and NF-κB phosphorylation. Levels of phosphorylated IRF7 and NF-κB inversely correlated with plasma IFNα2 levels, implying that pDCs were refractory to in vitro stimulation after IFNα production in vivo. After ATI, decreased expression of IFN genes in pDCs inversely correlated with time to viral detection, suggesting that pDC IFN loss is part of an effective early immune response. These data, from a limited cohort, provide a critical first step in understanding the earliest immune response to HIV-1 and suggest that changes in blood pDC frequency and function can be used as an indicator of viral replication before detectable plasma viremia.

Introduction

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60 Plasmacytoid dendritic cells (pDCs) are important mediators of the early innate immune 61 response to viral infection through robust production of type I interferons (IFNs), particularly 62 IFNα (1, 2). pDCs recognize single-stranded RNA and unmethylated CpG-rich DNA through 63 activation of toll-like receptor (TLR)7 or TLR9 (3-5), respectively, which results in activation of 64 NF-κB and interferon regulatory factory 7 (IRF7) to induce cytokine production. The 65 constitutive expression of high levels of IRF7 by pDCs allows for rapid production of type I 66 IFNs (6). Type I IFNs contribute to the immune response through inhibition of viral replication 67 (7), as well as by mediating activation and survival of myeloid dendritic cells (mDCs), 68 macrophages, natural killer cells, and T cells (8). Loss of pDC function early after infection 69 could thus have critical effects on the propagation of the immune response. To date, little is 70 known about the pDC response during the earliest stages of HIV-1 infection, prior to detection of 71 viral RNA, or how this response contributes to subsequent immune control or disease 72 progression. 73 74 HIV-1 infection induces a marked alteration of pDC frequency and function after peak viremia 75 that correlates with disease progression. Frequencies of pDCs are lower in the blood of people 76 living with HIV-1 than in uninfected individuals and are only partially restored after 77 antiretroviral therapy (ART)(9-14). In viremic individuals, pDC frequency positively correlates 78 with CD4 count and inversely correlates with plasma viral load (VL) (15). pDCs from 79 individuals with HIV-1 have a reduced capacity to produce IFNα following in vitro stimulation 80 with TLR7 or TLR9 ligands, HIV-1, and other viruses (14, 16-18). HIV-1 infection affects IFNα 81 production by altering both the intracellular signaling capability of pDCs and the phenotypic

make-up of the pDC population in the blood. Exposure of pDCs from healthy donors to HIV-1 in vitro results in a delay and reduction of IFNα production compared to TLR7 agonists and other viruses (19, 20). This delay has been associated with increased phosphorylation of spleen tyrosine kinase (SYK) due to engagement of gp-120 with the pDC marker CD303 (19) and decreased nuclear translocation of IRF7 due to decreased phosphorylation of Akt (21). Furthermore, decreased production of IFN α has been associated with an increase of Tim-3⁺ exhausted pDCs present in the blood during HIV-1 infection (22), increased CD40 expression on blood pDCs during HIV-1 infection (23), and the mobilization of immature pDCs from the bone marrow after SIV infection (24-26). These studies were mostly performed with pDCs from healthy donors exposed to HIV-1 in vitro, or with pDCs obtained from individuals during the chronic stage of HIV-1 infection. Limited knowledge about the function of pDCs during the earliest stages of infection, prior to detection of plasma viral RNA, has been derived from non-human primate models. pDCs are the first major cell population recruited to the vaginal mucosa after SIV infection in rhesus macaques, where they accumulate beneath the endocervical epithelium and produce IFNα within one day of infection (27). A transient increase in the frequency of pDCs in the blood has also been reported to occur prior to peak viremia after intravenous SIV infection (25, 26, 28). Frequent sampling of plasma after intravenous SIV inoculation of African green monkeys (AGMs) revealed a low, transient increase in plasma IFNα levels two days post infection, prior to a subsequent robust increase in plasma IFN α levels that accompanies viremia (29). It is logistically difficult to determine if a similar increase in plasma IFNα levels occurs within days

of HIV-1 infection, however plasma IFN α levels are one of the first to increase during the initial

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cytokine storm that occurs after HIV-1 infection, similar to what has been described in SIV infection (28, 30). The importance of the early IFNα response has been shown by decreased SIV acquisition when rhesus macagues were treated with Type-I IFNs (31, 32) and more rapid disease progression when treated with an IFN-I receptor antagonist (31). However, the uncontrolled type I IFN response after acute HIV and pathogenic SIV infection is associated with chronic immune activation and disease progression even in treated individuals (29, 33-36). Although it is challenging to measure the early pDC response during the eclipse phase of acute HIV-1 infection (AHI), when virus is not yet detectable in the blood, it is possible to monitor the immune response to the initial low-level viral replication in the tissues after the ART interruption in aviremic individuals. The identification of post-treatment controllers, who maintain undetectable viral loads for years after treatment cessation (37), has led to an increased interest in identifying therapeutic interventions to induce control after analytic treatment interruption (ATI). ATI also allows measurement of the early innate immune response to HIV replication. Three ATI studies have been completed with participants in the RV254 cohort, a unique group of individuals living with HIV-1 in Thailand who initiated ART during AHI (Fiebig stages I-V) (38, 39): the single arm RV411 study of ATI after very early ART initiation during Fiebig I (40), the RV409 study of vorinostat, hydroxychloroguine, and maraviroc (VHM) with ART vs. ART alone (control arm) followed by ATI (Kroon, unpublished observations), and the RV397 study of

VRC01 broadly neutralizing antibody vs. placebo administration during ATI (41). Here, we

analyzed the mobilization, activation, and function of pDCs in the blood of participants in these

three ATI studies to identify markers of viral replication prior to detection of viremia by standard

commercial assays (≥20 copies/mL). Although these data are from three different clinical trials

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- 128 with limited numbers of participants, these participants are homogenous in age, virus clade, and
- time of ART initiation.

Results

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To study the innate response prior to detectable viremia, we analyzed longitudinal samples from participants enrolled in three separate ATI studies: RV411, RV409, and RV397 (Table 1, Supplemental Figure 1). Activation of innate cells, including pDCs, was measured prior to ATI in the participants of RV409 who received VHM, so only participants in the control arm of RV409, who received no therapeutic intervention, were included in the current analysis. All participants in all three trials experienced viral rebound during ATI. We examined the presence of pDCs in the blood after ATI to look for indications of pDC mobilization prior to detection of viremia. There was a transient increase in the frequency of pDCs (Lin-HLA-DR+CD11c-CD303+, Figure 1A) in the blood prior to the detection of plasma viral RNA (Figure 1B). In participants for which the absolute pDC count could be calculated, we found that the transient increase in pDC frequency approximated a similar increase in absolute pDC count (Supplemental Figure 2A). As pre-rebound samples from RV411 participants were limited to the baseline visit, when treatment was interrupted, and the last visit at which the participant was aviremic (VL<20 copies/mL), we used these time points to quantify changes in pDC frequency prior to detection of viremia in the three ATI cohorts. A significant increase in pDC frequency occurred between the baseline ATI visit and the last aviremic visit (median 7.6% vs median 10.8%, p < 0.001)(Figure 1C). As administration of VRC01 antibody to participants in RV397 could have altered their pDC response, we confirmed that there was a significant increase of pDC frequency in the blood of the participants who underwent ATI in the absence of therapeutic treatment (p < 0.001)(Supplemental Figure 2B) and that there was no significant difference in the fold change of pDC frequency between the placebo and treatment arms of

pDCs are mobilized into the blood prior to detection of viremia after ATI

RV397 (Supplemental Figure 2C). In contrast, ART-only treated participants in RV409 showed no increase in pDC frequency during the 10 weeks prior to ATI (Figure 1D), suggesting the transient increase seen after ATI is an early response to viral replication that follows ART interruption. Importantly, this increase was specific to pDCs, as a similar increase was not found within the frequency of CD1c⁺ mDC, CD141⁺ mDC, CD14⁺CD16⁻ classical monocyte, CD14⁺CD16⁺ intermediate monocyte, or CD14^{lo}CD16⁺ nonclassical monocyte subsets prior to detection of viremia (Supplemental Figure 3). pDCs are partially activated prior to detection of viremia after ATI To ascertain the activation state of pDCs entering the blood prior to detectable viremia, we analyzed expression of multiple markers by flow cytometry. Circulating pDCs showed evidence of activation, as measured by increased expression of the markers CD69, PD-L1, and CD40 (Figure 2A). Between the baseline time point of ATI and the last aviremic time point during ATI, significant increases were observed in the expression of CD69 (mean fluorescence intensity (MFI) of 256 vs 299, p < 0.01), PD-L1 (MFI of 113 vs 287, p < 0.01), and CD40 (190 vs 239, p < 0.01) (Figure 2B). Upon activation, pDCs can differentiate into more conventional dendritic cells, losing the capacity to produce large amounts of IFN α but gaining the capacity to stimulate T cells (42-44). This maturation is characterized by increased expression of the costimulatory marker CD86 and the maturation marker CD83, among others (42, 43, 45). There was no difference in the expression levels of CD86 or CD83 on pDCs prior to detectable viremia after ATI (Figure 2C). There were also no significant differences in expression of activation markers between arms in

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RV397 (Supplemental Figure 4). These data indicate that after ATI, pDCs entering the blood prior to detection of viremia are activated but not differentiated.

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pDCs are primed to traffic to sites of inflammation prior to detection of viremia To assess the destination of the pDCs entering the blood, we analyzed the expression of pDC surface proteins involved in cell trafficking. Due to sample availability, all remaining experiments were performed on samples from RV397 only. pDCs showed no changes in expression of the chemokine receptors CCR7 or CCR9 prior to detectable viremia, but did have decreased expression of integrin β 7, which mediates homing to mucosal sites (46), at the last aviremic time point compared to baseline ATI (392 vs 299, p < 0.05; Figure 3A). pDCs had increased expression of CD29 (integrin β 1)(1905 vs 2535, p<0.05), which is used for firm adhesion prior to extravasation from the blood vessel (47), and CXCR4 (1010 vs 1516, p < 0.01), which mediates chemotaxis to lymph nodes via high endothelial venules, particularly during inflammation (Figure 3A). No differences were observed between placebo and treatment arms for all of the measured markers. Further, plasma levels of stromal cell-derived factor 1α (SDF-1α), the primary ligand for CXCR4, were also elevated relative to baseline ATI prior to detectable viremia (126 vs 255, p < 0.01)(Figure 3B). These data suggest that the pDCs are primed for recruitment to secondary lymph organs and extravasation at sites of inflammation. Transient loss of interferon production occurs in pDCs prior to detection of viremia

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As these changes to pDCs occurred prior to detection of viral RNA in the blood, we assessed their functional capacity to respond to virus by measuring IFNα and TNFα production after stimulation with the TLR7 agonist imiquimod (Figure 4A). Prior to detectable viremia, there was

a transient decline in the percentage of pDCs which produced IFN α upon in vitro stimulation (Figure 4B). As this drop in IFNα production tended to correspond with peak pDC frequency, we compared the frequency of cells producing IFNa at the time of peak pDC frequency with that of the week prior to peak pDC frequency. There was a significant decline in the frequency of IFN α^+ pDCs (19.6% vs 14.1%, p < 0.05)(Figure 4C) and a trend towards a lower frequency of TNF α^+ pDCs (54.2% vs 45.3%, p=0.08) (Figure 4D, Supplemental Figure 5A) at the time of peak pDC frequency. It is important to note that the four participants with increased frequencies of IFN α^+ pDCs at the time of peak pDC frequency had the lowest frequencies of IFN α^+ pDCs at the visit prior. For two of these participants, the decreased capacity of pDCs to produce IFNα after stimulation occurred the week prior to peak pDC frequency, when the frequencies of pDCs in the blood had increased but not yet reached maximum. For the other two participants, rebound occurred very early, within just 2 weeks of treatment interruption, and the limited sampling prior to rebound may not have been sufficient to capture the transient decline. Moreover, there was a trend towards decreased IFN\alpha MFI at the time of peak pDC frequency among all participants (7144 vs 6442, p=0.15) (Supplemental Figure 5B), suggesting that not only were fewer cells able to produce IFN α , but also that less IFN α could be produced on a per cell basis after in vitro stimulation.

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pDC activation in participants with extended aviremia

The two participants in RV397 with the longest time off ART before detection of viremia (≥ 20 copies/mL) both had an increased frequency of pDCs in the blood after ATI, with transient increases in expression of activation markers (Figure 5A). Participant 7242 had a delayed rebound of 65 days, but showed an increased frequency of pDCs in the blood with a decreased

capacity to produce IFNα one week after ATI. pDC function in Participant 7242 recovered three weeks after ATI but was lost again prior to detection of viremia. While Participant 3499 experienced the longest time to rebound (42 weeks), there was a viral blip identified by a high sensitivity single-copy assay and an increased frequency of pDCs in the blood was observed immediately before this viral blip. A transient impaired capacity to produce IFNα after in vitro stimulation was also seen in these two participants (Figure 5B). Despite a viral blip of less than 2 copies/mL six weeks after ATI, pDCs from Participant 3499 still showed decreased capacity to produce IFNα prior to the blip. Participant 7242 did not have a detectable viral blip by singlecopy assay, but six of the other participants in RV397 had measurable virus of 2.1 copies/mL or less at the last time point the participant was considered aviremic (VL<20 copies/mL). One of these participants had a viral load of just 21 copies/mL at the first visit viral load was detectable by commercial assay, but already had a viral load of 1.5 copies/mL one week prior as measured by the single copy assay. These data indicate that viral replication may begin over a week before it is detectable by commercial assays, and suggest that the increased frequency and decreased function of pDCs after ATI may be a marker for low level viral replication, even when detectable viremia does not occur until weeks or months later.

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pDCs undergo a refractory state prior to detection of viremia

Previous studies reported a decline in IFNα production by pDCs after peak viremia during acute SIV and HIV-1 infections or after detectable viremia during treatment interruption (14, 16-18, 22, 23, 48). In SIV infection, this decline was attributed to an egress of immature pDCs from the bone marrow into the blood after peak viremia (25). To determine whether the increased frequency of pDCs in the blood prior to detection of viremia was due to bone marrow egress, we

measured the frequency of cells expressing Ki-67, a marker of immature pDCs (26). There was no difference in the frequency of Ki-67⁺ pDCs between the week of peak pDC frequency compared to the week prior (Supplemental Figure 6A), suggesting that the decreased frequency of IFN α ⁺ pDCs after in vitro stimulation was not due to an influx of immature cells in blood. An inability to produce IFN α has also been attributed to exhaustion of pDCs over time during HIV-1 infection (22). To determine if the decreased ability to produce IFN α at the time of peak pDC frequency was due to an influx of exhausted cells, characterized by Tim-3 expression, we measured the frequency of Tim-3⁺ pDCs. At the time of peak pDC frequency, there was a decline in the frequency of pDCs expressing Tim-3 compared to the week prior (7.3% vs 5.7%, p<0.01)(Supplemental Figure 6B). Thus, the decreased IFN α production seen prior to detectable viremia was not a result of an influx of exhausted pDCs into the blood.

During in vitro activation of pDCs with HIV-1, there is a delayed production of IFN α that has been associated with increased phosphorylation of the signaling molecule SYK as a result of interactions between gp-120 and CD303 (19). To assess if the decreased IFN α production by pDCs was due to exposure to gp-120 in vivo, we measured the basal percentage of SYK phosphorylation in eight RV397 participants at a visit prior to ATI (pre-ATI visit) and a visit with the nadir IFN α response or the only available visit remaining after ATI (ATI visit). There was a significant increase in the percentage of SYK phosphorylation at the ATI visit compared to the pre-ATI visit (median 68.1% vs 58.7%, p<0.01)(Supplemental Figure 7A). However, there was no correlation between the basal levels of SYK phosphorylation and the capacity to produce IFN α . Thus, while these data suggest that a portion of the pDCs may have come in contact with

HIV-1 in vivo resulting in higher SYK phosphorylation, this phosphorylation did not appear to be the cause of the decreased IFN α production in vitro.

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The decreased IFN α production in vitro could also be a result of the pDCs being in a refractory state after in vivo activation by HIV-1 (18, 49). We measured the capability of pDCs from eight participants in RV397 to phosphorylate IRF7 (pIRF7) and NF-κB (pNF-κB), key transcription factors in the production of IFN α and TNF α , respectively. We assessed whether the capacity to activate IRF7 or NF-κB correlated with IFNα production. There were negative correlations between the levels of pIRF7 or pNF-κB after stimulation and the loss of IFNα capacity, as measured by the fold decrease in IFN α^+ pDCs after in vitro stimulation at the ATI visit compared to the week prior (r = -0.76, p < 0.05, r = 0.71, p = 0.06, respectively)(Figure 6A, Supplemental Figure 7B-C). These data indicate that pDCs at the ATI visit were capable of producing IFNα when the upstream transcription factors were activated, but lost IFNα capacity when signaling was hampered. We further hypothesized that if the pDCs were in a refractory state due to activation by HIV-1, then earlier production of IFNα in vivo would be seen by higher plasma IFN α 2 levels. Along these lines, we found that there was a negative correlation between plasma IFNα2 levels at the ATI visit and the levels of pIRF7 (r = -0.78, p < 0.05) and pNF-κB (r = -0.64, p=0.10) in pDCs after imiquimod stimulation (Figure 6B, Supplemental Figure 7D). In contrast, the levels of pIRF7 and pNF-κB after imiquimod stimulation of a sample from before ATI correlated with the plasma IFN α 2 levels at the ATI visit (r=0.79, p<0.05, r=0.92, p<0.01, respectively)(Figure 6C, Supplemental Figure 7E), indicating that the pDCs had the capacity to phosphorylate IRF7 before ATI. Cumulatively, these data suggest that prior to detectable viremia after ATI pDCs are activated and produce IFN α before entering into a refractory state.

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IFNα production.

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It has been reported that IRF7 mRNA levels are lower in pDCs from people living with HIV-1 during both chronic untreated infection and after ART, resulting in lower IFNα production (50, 51). To determine if the decreased IFNα production measured after ATI was related to decreased expression of key signaling molecules, we measured the mRNA levels of interferons, multiple cytokines and chemokines, surface receptors, and signaling genes in sorted pDCs from pre-ATI and ATI samples from eight separate RV397 participants (Figure 7A). Several genes showed lower expression in pDCs from the ATI visit compared to the pre-ATI visit. In particular, genes related to type I IFN signaling were lower at the ATI visit, including: IRF7 (median 1.44-fold, p < 0.05); TLR7 (median 1.29-fold, p < 0.01); STAT1 (median 1.57-fold, p < 0.01); and two interferon-stimulated genes (MX1, median 1.77-fold, p < 0.01; MX2, median 1.41-fold, p < 0.01), with the latter four genes maintaining significance after correction for multiple comparisons (Figure 7A). To determine if the changes in interferon signaling genes were intrinsic to pDCs or were common to other innate cells, we also measured gene expression in sorted CD1c⁺ mDCs (Supplemental Figure 7F). mDCs had very little change in gene expression between the pre-ATI and ATI time points, only showing a decrease in MX2 expression (1.34-fold, p < 0.05) and an increase in IRF1 expression (1.40-fold, p < 0.01) neither of which remain significant when corrected for multiple comparisons. These data suggest that there is a pDC-specific loss in expression of genes related to type I IFN signaling that may contribute to the loss of in vitro

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Consistent with the decreased in vitro IFNα production, pDCs had lower expression of type I IFN genes. *IFNA5* and *IFNB*1 transcript levels were 2.14- and 1.87-fold lower in the pDCs from

the ATI visit, respectively (p<0.05)(Figure 7A). Though the 2.53-fold decrease in *IFNA2* expression did not remain significant after correcting for multiple comparisons, there was a significant positive correlation between the expression levels of *IFNA2* at the ATI visit and the subsequent plasma IFNα2 levels measured after rebound (r=0.9048, p<0.01)(Figure 7B). A similar correlation between *IFNA2* expression and rebound plasma IFNα2 levels was not found in the mDCs (Supplemental Figure 7G), suggesting that pDC function after ATI drives early IFNα production after rebound. Interestingly, there were negative correlations between the levels of expression of type I IFN genes and the time from the ATI visit to viral detection (VL≥20 copies/mL)(Figure 7C). *IFNA1*, *IFNA2*, *IFNA7*, and *IFNA8* levels were all significantly negatively correlated with time to viral detection after correction for multiple comparisons (r=0.9132, p<0.01 for all), and *IFNA21/1* and *IFNB1* levels only reached significance without correction (r=-0.8498 and -0.7864, respectively, p<0.05). These data suggest that the loss of type I IFN gene expression by pDCs after ATI may be indicative of a more effective early innate immune response, correlating with a longer time to viral detection.

Discussion

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Using longitudinal samples from participants who interrupted ART as part of ATI studies, we show that there is an increased frequency of activated pDCs in the blood prior to detection of plasma viremia. These data from clinical trials are in agreement with prior observations of increased frequency of pDCs preceding peak plasma viremia during acute SIV infection in macaques (26, 28). As pDCs are one of the first innate cells recruited to the site of infection (27), we posit that the increased frequency, and decreased function, of pDCs measured here is an indicator of low-level viral replication in the tissues (Figure 8). After ATI, viral replication in CD4 T cells occurs in a mono- or oligoclonal manner (Step 1)(52). The location of these initial reactivation events is yet to be determined but they likely occur in lymphoid tissues, where CD4⁺ T cells harboring HIV-1 DNA reside (53-55). Successful viral replication initiates a secondary immune response, presumably starting with the recruitment and activation of pDCs at the site of replication, similar to that seen during the primary infection (Step 2)(27). Production of CCL4, and other cytokines, by pDCs at the site of replication can then recruit other immune cells, including CD4⁺ T cells that can propagate virus through new infection or by cellular activation and recrudescence of quiescent virus (Step 3). With increased viral replication and dissemination to distal sites, we hypothesized that mobilization of innate immune cells should be evident in the blood. Our data demonstrate the presence of an increased frequency of activated pDCs in the blood prior to detectable viremia after ATI (Step 4). Peripheral blood pDCs were activated, as measured by expression of CD69, PD-L1, and CD40, but had not differentiated into antigenpresenting cells expressing CD83 and CD86. These cells also expressed increased CXCR4 and CD29, suggesting they are being trafficked to sites of inflammation (56, 57). Consistent with the immature phenotype of the pDCs there was no increase of CCR7 expression prior to detectable

viremia, and the pDCs will be responsive to the higher levels of SDF-1 α in the plasma at this time (58). A partially activated phenotype, similar to that seen here, has previously been associated with an ability to continuously produce IFN α by pDCs exposed to HIV-1 in vitro (44). However, pDCs in the blood of ATI participants showed a transient decline in the ability to produce IFN α after in vitro stimulation (Step 5), similar to the refractory state that has been reported in pDCs from after peak viremia during AHI, after rebound in ATI, and in SIV-infected AGMs (14, 16-18, 59).

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Identification of a refractory state instead of a state of persistent IFNα production may depend on the timing of HIV-1 exposure and IFN α measurement. pDCs are refractory to IFN α production after multiple in vitro stimulations with influenza virus, R848, or CpGB, indicating a mechanism to control persistent activation and IFNα production (44, 60). However, IFNα production is delayed in pDCs stimulated with HIV-1 compared to these other ligands, with peak production not occurring until 24-48 hours after activation compared to just 4 hours with other viruses (19). Thus, induction of the refractory state after exposure to HIV-1 is also likely delayed until after peak IFN α production. The refractory state of pDCs in the blood prior to detectable viremia would then imply that these pDCs had already reached peak IFNα production in vivo. We found that there were increases in plasma IFNa2 levels in some participants at peak pDC frequency, an observation that may have been restricted by the limitations of weekly sampling to identify what would be expected to be a very low, transient increase of IFN α produced prior to viremia (29). However, the added observation that plasma IFN α 2 levels negatively correlated with the ability of pDCs to phosphorylate IRF7, is consistent with a refractory state of the pDCs at this stage (Step 5).

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The observations made here were limited by the inclusion of participants enrolled in ATI studies performed in Thailand alone. While it may be presumed that a similar increase in pDC frequency and decrease in function would be seen after ATI in other cohorts, further studies are needed to rule out any effects of genetic background or HIV-1 clade on these observations. We did include participants from RV397 who received VRC01 treatment during ATI in this study after showing that there was no difference in changes of pDC frequency or phenotype compared to placebo treated participants, but it is possible that VRC01 had an indirect effect on pDCs that we did not identify in our analysis. It has been shown that VRC01 did not alter IFNa production by pDCs exposed to HIV-1 in vitro, suggesting that VRC01 does not affect pDC sensing of HIV-1 (61, 62). However, pDCs can produce IFNα after sensing immune complexes (63), and, although we did not see differences in pDC increase and activation between placebo and VRCO1 treated participants, we cannot rule out the potential that VRC01 formed immune complexes in vivo which could have enhanced viral sensing by pDCs in VRC01 treated participants. Though we combined participants from multiple ATI studies for this analysis, the number of participants who received no therapeutic intervention was limited and analysis of pDC function and viral sensing in future ATI studies would help further these observations. Sampling frequency was also a limitation of the ATI studies, particularly for the participants who had detectable viral load within two weeks of ATI and thus only had one aviremic time point. Unfortunately, the current sampling regimen is already burdensome for participants and nonhuman primate studies may be necessary for more frequent sampling to better characterize the innate response after ATI. The unavailability of tissue samples in this analysis prevented us from confirming that low level viral replication in tissues is indeed the source of pDC activation. However, the identification of

changes in blood pDCs as a potential indicator of HIV replication provides critical information to time the sampling of future studies in order to confirm when viral replication initiates in the tissue, investigate where early replication begins, and study the earliest immune responses to viral replication after ATI.

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It has been estimated that viral replication is initiated once every 5-8 days after ATI based on the time to detectable plasma viremia of greater than 20 copies/mL (64). However, that calculation does not take into account the potential of local control of replicating virus by the immune system. One participant in RV397 did not have plasma viral loads above 20 copies/mL until 42 weeks but showed an increased frequency of activated pDCs in the blood early after ATI just prior to a low-level peripheral blood viral load blip of 1.5 copies/mL. These data support our conjecture that pDC dynamics in the blood are an indicator of viral replication in the tissue and may be used to develop a more sensitive estimate of viral reactivation after ATI. Due to the longer time intervals between sampling points during prolonged ATI, we were unable to determine whether multiple reactivation events occurred in the participants with the longest times of aviremia. It has previously been reported that the frequencies of CD30⁺ T cells increased months prior to viral detection in two participants who had extended aviremia (65). Unfortunately, the levels of HLA-DR and CD38, which we used to measure T cell activation in the three ATI studies, did not change on the T cells prior to viral rebound so we were not able to identify correlations between pDC activation and T cell activation in our analysis. Experiments in nonhuman primates may lead to further understanding of the timing and mechanisms of these early innate immune responses to viral replication, and to identify aspects of the immune response that contribute to control of replication in those with extended aviremia.

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While our data suggest that pDCs are sensing low level HIV-1 replication after ATI, other factors that could be causing pDC activation should also be considered. ART could have an offtarget effect on pDCs, resulting in pDC activation after antiretroviral (ARV) clearance. However, given that pDC frequency and function recover after ART initiation, this seems unlikely (13, 14). Further, participants who were on the same ART regimen at the time of ATI had varied times to pDC activation, from 1-3 weeks after ATI, suggesting that ARV clearance was not responsible for pDC activation. It is also conceivable that ARVs were having an indirect effect on the control of other viruses which were then reactivated after ARV clearance and caused pDC activation. However, a virome analysis to identify other viruses activated after ATI was outside the scope of this work. Finally, pDC activation could be a result of microbial translocation in the gut. As early as two weeks after SIV infection, damage to the intestinal epithelium results in microbial translocation that is associated with immune activation, including increased plasma IFN α levels and T cell activation (66, 67). While low levels of IFN α were detected prior to viral detection in participants of the ATI cohorts, there was no evidence of T cell activation before viral detection (40). This does not exclude the potential for microbial translocation, but gut biopsies were not available before viral detection to investigate whether pDCs were being activated and mobilized do to microbial translocation.

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Our data show that activated pDCs were mobilized to the blood prior to detectable viremia after ATI, and that they entered a refractory state after in vivo production of IFNa. Whereas the early activation of pDCs in response to viral infections is well documented, less information is available on the mechanisms that blunt the pDC response to prevent excessive IFNa production.

Decreased levels of pIRF7 after in vitro stimulation were associated with higher levels of plasma IFNα2, suggesting that the refractory state of the pDCs is a result of in vivo activation. Decreased expression of type I IFN genes prior to viral detection was associated with a longer time to reach a viral load over 20 copies/mL, indicating that the refractory state of the pDCs may be indicative of a favorable immune response during ATI. Indeed, *IRF7* levels decreased in pDCs over time after in vitro stimulated with the TLR7 agonist R848 (44). If the extent to which type I IFN gene expression is lost is a measure of the extent to which pDCs were activated in vivo, these data suggest that a robust and thorough activation of pDCs is associated with longer time to detection of viremia after ATI.

While the contribution of this pDC activation towards controlling viral replication is yet to be determined, it is known that the timing of IFN α production is important for viral clearance during acute viral infections. Early administration of IFN α protects against rectal SIV infection of rhesus macaques, but a delay in IFN α signaling results in more rapid disease progression (31). A delayed IFN α response to robust replication of LCMV CL-13 and SARS-CoV in mice is associated with a stronger cytokine storm and disease progression, but early introduction of IFN α is ameliorative (68, 69). Further, administration of pegylated-IFN α just prior to and during ATI resulted in extended viral control and decreased integrated HIV DNA in a portion of recipients (70). While many experiments suggest that pDCs are not the main producer of IFN α in later stages of acute and chronic HIV-1 and SIV infection (24, 59, 71, 72), an early role of pDC function prior to detection of viremia has not been extensively studied. Importantly, although pDCs are not the main producer of IFN α involved in the control of LCMV CL-13 viremia during the normal course of infection (68), knock out of the negative regulator *OASL1* in LCMV CL-13-

infected mice resulted in prolonged IFN α production by pDCs which was associated with maintenance of CD8 T cell function and eventual LCMV CL-13 clearance (60). These studies show that early and sustained IFN α production is important for an effective immune response and viral clearance. Thus, while the early induction of IFN α production after ATI may have the potential to be beneficial, a blunting of the pDC response may hinder the mobilization of a successful immune response to clear the virus. Therefore, new strategies to boost and sustain the initial interferon-mediated response to replicating virus are needed to potentially sustain HIV-1 remission after treatment interruption.

Methods

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ATI study design. Twenty-nine individuals with HIV-1 who initiated ART during acute infection as part of the RV254 cohort in Thailand (clinicaltrials.gov NCT00796146) and underwent ATI were included in this study (Table 1). Participants in the RV254 cohort initiated ART during the earliest stages of AHI, as staged according to a previously described sequential algorithm of pooled nucleic acid testing and immunoassays (73). Early initiation of treatment is associated with preserved immune function and decreased viral reservoir seeding, making individuals in the RV254 cohort ideal for enrollment in ATI studies to identify post-treatment controllers (74, 75). As such, participants included in this study were enrolled in one of three ATI studies: RV411, RV409, or RV397 (Supplemental Figure 1). Eight participants who initiated ART during Fiebig stage I of AHI were enrolled in the RV411 study (clinicaltrials.gov NCT02614950)(40). Participants in the RV411 study underwent ATI without additional interventions. Plasma viral load was monitored weekly, except weeks 2-6 after ATI when twice weekly monitoring occurred. PBMC samples for RV411 participants were limited to the following timepoints: baseline, last aviremic (VL<20 copies/mL), rebound, and 2 weeks after ART-reinitiation. The RV409 study (clinicaltrials.gov NCT02475915) included 15 participants who initiated ART during Fiebig stages III-IV of AHI, 14 of whom interrupted ART (Kroon, unpublished observations). During the 10 weeks prior to ATI, participants were treated with ART alone (n=5) or with vorinostat, hydroxychloroquine, and maraviroc (VHM) to induce production of latent virus while maintaining ART (n=10). Plasma viral load was monitored weekly for the first 12 weeks after ATI, and biweekly thereafter. PBMC samples were collected

at baseline ATI, weekly through week 4, and biweekly thereafter. During the ten weeks pre-ATI,

PBMCs were collected biweekly. The RV397 study (clinicaltrials.gov NCT02664415) included 18 participants who initiated ART during Fiebig stages I-III of AHI (41). Participants received intravenous injections of the broadly neutralizing antibody VRC01 (n=13) or placebo (n=5) every three weeks during ATI, starting on the day ART was ceased and continuing for 24 weeks or until ART resumption. Plasma viral load was monitored weekly, with twice weekly testing occurring during weeks 2-6 and 26-30. PBMC samples were collected from RV397 participants at baseline, weekly through week 3, and once every 3 weeks after that. All participants in these three studies experienced viral rebound, and ART was resumed after two consecutive viral loads >1,000 copies/mL. At the time of enrollment in the ATI studies, all participants had a CD4 count > 400 cells/μL and had maintained viral suppression (HIV-1 RNA <50 copies/mL) for at least 48 weeks.

RV409 participants receiving VHM and ART were not included in the current analysis due to detection of intervention induced pDC activation prior to ATI. Further, two participants in RV411 and one participant in the ART only arm of RV409 were not included in any analysis due to an extended amount of time between the last aviremic sample collected and the detection of viral RNA in the blood (>2 weeks).

Measurement of HIV-1 RNA. Plasma viral load was measured quantitatively with the COBAS TaqMan HIV-1 Test (Roche Diagnostics, Branchburg, NJ) with a lower limit of detection of 20 copies/mL. Single copy HIV-1 RNA levels were measured retrospectively in samples from RV397 using ultrasensitive hybrid real time/digital PCR with a limit of detection of 0.45 copies/mL, as previously described (76, 77).

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524	Antibodies and reagents for flow cytometry. BV421-labeled anti-CCR7 (clone G043H7), PE-
525	Cy7-labeled anti-CCR9 (clone L053E8), BV785-labeled anti-CD11c (clone 3.9), Pacific Blue-
526	labeled anti-CD11c (clone 3.9), BV510-labeled anti-CD14 (clone M5E2), BV785-labeled anti-
527	CD14 (clone M5E2), BV510-labeled anti-CD19 (clone SJ25C1), PE/Dazzle 594-labeled anti-
528	CD1c, APC-Cy7-labeled anti-CD29 (clone L161), BV510-labeled anti-CD3 (clone UCHT1),
529	AlexaFluor 700-labeled anti-CD303 (clone 201A), PE-labeled anti-CD303 (clone 201A),
530	AlexaFluor700-labeled anti-CD45 (clone 2D1), BV510-labeled anti-CD56 (clone 5.1H11),
531	BV605-labeled anti-CD86 (clone IT2.2), BV605-labeled anti-CXCR4 (clone 12G5), BV711-
532	labeled anti-HLA-DR (clone L243), APC-labeled anti-integrin β7 (clone 29E.2A3), PE-Cy7-
533	labeled anti-PDL1 (clone 29E.2A3), BV650-labeled anti-Tim-3 (clone F38-2E2), and PE-Cy7-
534	labeled anti-TNF α mAbs (clone MAb11) were obtained from BioLegend (San Diego, CA).
535	PerCP-Cy5.5-labeled anti-CD123 (clone 7G3), APC-labeled anti-CD141 (clone 1A4), BUV496-
536	labeled anti-CD16 (clone 3G8), BV650-labeled anti-CD3 (clone UCHT1), BUV395-labeled anti-
537	CD40 (clone 5C3), BUV737-labeled anti-CD69 (clone FN50), BUV737-labeled anti-CD83
538	(clone HB15e), BUV395-labeled anti-Ki-67 (clone B56), AF488-labeled anti-IRF7
539	(pS477/pS479) (clone K47-671), GolgiPlug (catalogue 555029), BD Perm/Wash Buffer
540	(catalogue 554723), and BD Phosflow Perm Buffer III (catalogue 558050) were obtained from
541	BD Biosciences (San Jose, CA). PE-labeled anti-phospho-NF-κB p65 (Ser536) (clone 93H1),
542	and AlexaFluor 647-labeled anti-phospho-SYK (Tyr352) (clone 65E4) antibodies were obtained
543	from Cell Signaling (Danvers, MA). APC-labeled anti-IFN α antibody (clone LT27:295) was
544	from Miltenyi Biotec (Auburn, CA). The Foxp3/Transcription Factor Staining Buffer Set
545	(catalogue 50-112-8857) was from Thermo Fisher Scientific (eBioscience, San Diego, CA).

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Flow cytometry analysis. Frozen PBMCs were thawed for phenotypic analysis by flow cytometry. Longitudinal samples from each participant were processed on the same day. Cells were stained with Live/Dead for 10 minutes at room temperature before staining for surface markers at 4°C for 20 minutes. Cells were washed twice with PBS containing 2% fetal bovine serum (wash buffer) before fixation with PBS containing 2% formaldehyde. For intracellular cytokine staining, cells were permeabilized in BD Perm/Wash Buffer at room temperature for 15 minutes after overnight fixation. The cells were stained with anti-IFNα and anti-TNFα mAbs at room temperature for 30 minutes followed by three washes with perm/wash buffer. All cells were resuspended in PBS containing 2% formaldehyde before being analyzed on a LSRII (BD Biosciences). All flow cytometry data was analyzed with FlowJo v10 (FlowJo, LLC, Ashland, OR). For measurement of SYK phosphorylation, PBMCs were stained immediately after thawing. For measurement of phosphorylated IRF7 and NF-κB, PBMCs were stimulated with 2.5ug/mL imiquimod as described below. Cells were stained with Live/Dead and surface markers as previously described. Cells were fixed in pre-warmed PBS containing 2% formaldehyde and incubated for 10 minutes at 37°C. Cells were then permeabilized in BD Phosflow Perm Buffer III and incubated for 20 minutes on ice. Cells were washed 3 times in PBS containing 10% fetal bovine serum, either immediately or after overnight incubation at -20°C. After incubation in wash buffer for 30 minutes on ice, cells were stained for 20 minutes at room temperature followed by two washes with wash buffer. For intracellular staining of Ki-67, cells were stained for surface markers as described in the methods followed by fixation and permeabilization with the Foxp3/Transcription Factor Staining Buffer Set. The cells were stained 568 with anti-Ki-67 mAbs for 30 minutes at room temperature followed by washes with Foxp3 569 permeabilization buffer and wash buffer, respectively 570 571 Plasma SDF-1α levels. Plasma SDF-1α levels were measured using Luminex technology with a 572 ProcartaPlex multiplex immunoassay (Assay MXH49YW)(Life Technologies Corporation, 573 Carlsbad, CA). Samples were run according to the manufacturer's instructions and cytokine 574 standards were provided by the manufacturer. A Bioplex-200 system was used to acquire 575 samples, and the data was analyzed with the BioPlex Manager Software (Bio-Rad Laboratories, 576 Hercules, CA). 577 **pDC stimulation.** Thawed PBMCs were reconstituted at 2x10⁶ cells/mL in RPMI 1640 578 579 supplemented with 10% fetal bovine serum, penicillin/streptomycin, and 20ng/mL of IL-3 580 (PeproTech, Rock Hill, NJ), and allowed to rest for 5 hours. Cells were stimulated with 581 2.5ug/mL imiquimod (Invivogen, San Diego, CA) or left in media alone for 6 hours, with 582 GolgiPlug added for the last 4 hours of stimulation. 583 584 Plasma IFNα2 levels. Plasma IFNα2 levels were measured using the SIMOA Human IFNα kit 585 (Quanterix, Lexington, MA) according to the manufacturer's instructions. The lower limit of 586 quantification of this assay is 4.7fg/mL. 587 588 **qRT-PCR.** Thawed PBMCs were stained for flow cytometric sorting on a BD FACSAria (BD 589 Biosciences). Replicates of 250 pDCs (Lin-HLA-DR+CD11c-CD123+CD303+) or 1000 mDCs 590 (Lin-HLA-DR+CD11c +CD303-CD1c+) were sorted into 10uL of SuperScript III-Platinum Taq

One-Step qRT-PCR master mix (Invitrogen, Carlsbad, CA) in 0.2mL PCR tubes for immediate lysis. Direct reverse transcription and PCR pre-amplification with gene specific primers was performed as previously described (78). Pre-amplification product was diluted 5-fold before use for multiplexed qPCR on a Fluidigm BioMark HD system (Fluidigm, South San Francisco, CA). Gene expression was measured using the 96.96 BioMark dynamic Array Chip for Gene Expression (Fluidigm) using TaqMan assays (Supplemental Table 1, Life Technologies Corporation) according to manufacturer's instructions, and as previously described (78). Data were analyzed with JMP Software (SAS, Cary, NC).

Statistics. All summary data provided in the text is in the form of the median value unless otherwise noted. Samples were excluded from analysis if taken more than two weeks from detection of viral RNA (last aviremic point) or the week of peak pDC frequency (week prior point). Statistical analyses were performed using the Wilcoxon matched-pairs singed rank test for comparisons between two visits, and the Mann-Whitney test for treatment group comparisons. Gene expression data were analyzed with the one sample Wilcoxon test to identify changes in expression that were significantly different than 0. Correlations were performed with the nonparametric Spearman test. When multiple comparisons were made for gene expression or correlations, the Benjamin-Hochberg Procedure was used to correct for a false discovery rate of 10%. All statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA) and significance was defined as p < 0.05 for two-sided testing.

- 612 **Study approval.** Informed consent was obtained from all participants prior to inclusion in the
- studies. All studies were approved by the Chulalongkorn University and Walter Reed Army
- 614 Institute of Research institutional review boards.

Author Contributions

J.L.M. designed and performed the experiments, analyzed the data, and wrote the manuscript. H.T. helped design and perform the experiments, and edited the manuscript. R.M. performed the Luminex assay. D.J.C., E.K., T.A.C., C.S., S.Pu., K.B., and R.T. managed participant recruitment and follow-up in the studies. D.J.C., E.K., and T.A.C. edited the manuscript. S.Pi. provided help in statistical analyses. R.L.T. provided supervision. L.F. provided supervision and edited the manuscript. V.R.P. and F.M. provided resources. D.L.B. provided resources and edited the manuscript. E.K.H. provided conceptual advice and edited the manuscript. S.R.L. provided conceptual advice and edited the manuscript. S.R.L. provided support for the clinical studies. J.A. designed the clinical studies, provided conceptual advice, and edited the manuscript. L.T. designed the experiments, analyzed the data, and wrote the manuscript.

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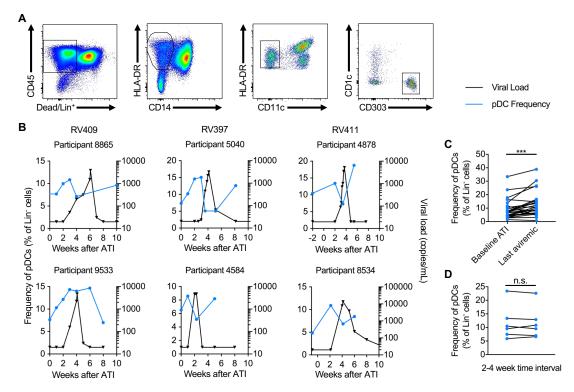


Figure 1. Frequency of pDCs in the blood increased prior to detectable viremia. (A) pDCs were identified as CD45⁺HLA-DR⁺CD303⁺ cells that were negative for CD14, CD11c, CD1c and the lineage markers CD3, CD14, CD19, and CD56 (Lin⁻). (B) The frequency of pDCs in the blood was measured as a percentage of the lineage negative cells by flow cytometry. Representative graphs show the pDC frequency (blue) relative to the viral load after ATI (black). (C) The pDC frequency at the time of ATI was compared to the frequency at the last aviremic time point to show increased pDC frequency before viral RNA was detected in the blood (*** p < 0.001, Wilcoxon test, n = 25, 10 of whom received VRC01). (D) The frequency of pDCs in the blood prior to ATI is shown for the 5 participants in the placebo arm of RV409. Changes in pDC frequency were compared when measurements were taken at a 2- or 4-week time interval.

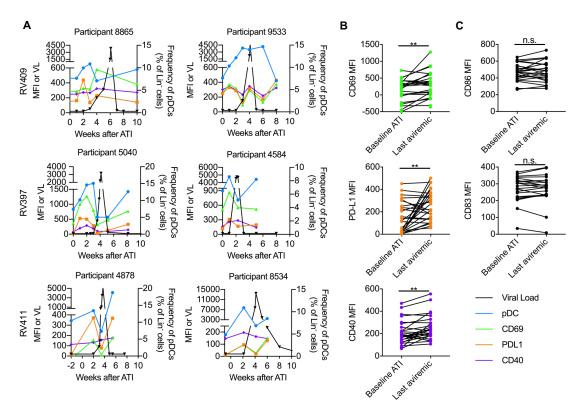


Figure 2. Expression of activation markers increased on pDCs prior to detectable viremia. (*A*) The mean fluorescence intensity of CD69, PD-L1, and CD40 on pDCs was measured by flow cytometry. Representative graphs show CD69 MFI (green), PD-L1 MFI (orange), and CD40 MFI (purple) relative to the changes in pDC frequency (blue) and viral load (black). (*B*) The MFI of CD69, PD-L1, or CD40 at ATI was compared to that at the last time point with undetectable viral RNA in the plasma (** p<0.01, Wilcoxon test). (*C*) Changes in CD86 and CD83 MFI between baseline ATI and the last aviremic time point are shown. n = 25, 10 of whom received VRC01.

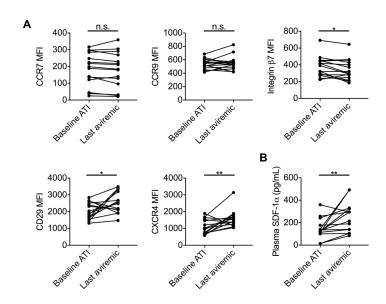


Figure 3. Expression of migration markers increased on pDCs prior to detectable viremia.

(A) Changes in CCR7, CCR9, integrin β 7, CXCR4 and CD29 MFI between baseline ATI and the last aviremic time point are shown. (B) SDF-1 α levels were measured in the plasma of participants in RV397, and changes between baseline ATI and the last aviremic time point are shown (* p<0.05, ** p<0.01, Wilcoxon test). n = 15 participants from RV397, 10 of whom received VRC01.

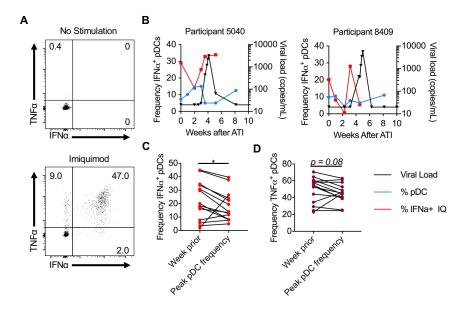


Figure 4. pDCs have a transient decrease in capacity to produce IFNα in vitro. (*A*) Total PBMCs were stimulated with imiquimod for 6 hours and IFNα production by pDCs was measured by flow cytometry. (*B*) The percentage of pDCs that produced IFNα in response to imiquimod stimulation is shown (red). pDC frequency (blue) and HIV-1 viral load (black) are included for reference. The percentage of pDCs producing IFNα (*C*) or TNFα (*D*) in response to imiquimod were compared between the time point before viremia at which the highest pDC frequency occurred and the time point immediately prior (* p<0.05, Wilcoxon test). n = 14 participants from RV397, 11 of whom received VRC01.

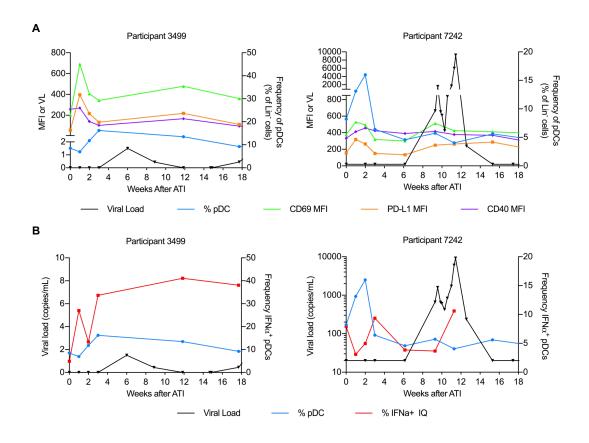


Figure 5. Increased frequency and activation of pDCs in participants with the longest aviremia. (A) The frequency of pDCs as a percentage of lineage negative cells is shown in blue for two participants in RV397 who had the longest period of aviremia (VL<20 copies/mL). The mean fluorescence intensity of CD69 (green), PD-L1 (orange), and CD40 (purple) on the pDCs is shown. Single copy viral load (black) is shown for participant 3499. (B) The frequency of pDCs that produced IFNα after in vitro imiquimod stimulation is shown (red) with pDC frequency and viral load for reference. Both participants received VRC01.

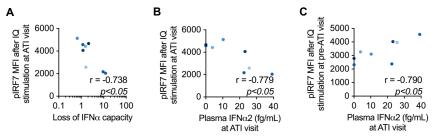


Figure 6. pDC signaling capacity negatively correlates with plasma IFNα2 levels. (*A*) The phosphorylation levels of IRF7 were measured in pDCs by flow cytometry after in vitro imiquimod stimulation of PBMCs from the visit with lowest IFNα response or the only remaining sample available after ATI (ATI visit). Spearman correlations were performed between the MFI of pIRF7 and the loss in IFNα producing capacity, as measured by the fold decrease in pDCs producing IFNα in vitro at the ATI visit compared to the visit prior. (*B-C*) Plasma IFNα2 levels were measured by SIMOA at the ATI visit in RV397 participants. Spearman correlations were performed between the plasma IFNα2 levels at the ATI visit and the MFI of pIRF7 in pDCs after in vitro imiquimod stimulation of PBMCs from the ATI visit (*B*) or a visit prior to ATI (pre-ATI visit) (*C*). n = 8 participants from RV397, all received VRC01.

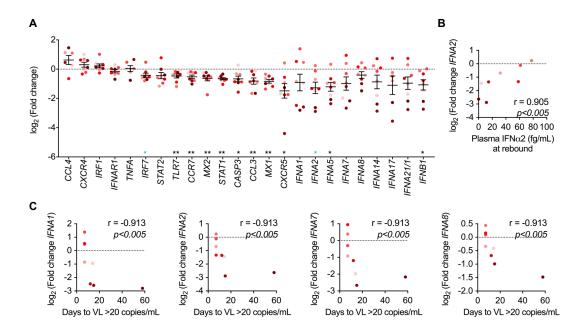


Figure 7. Type I IFN gene expression negatively correlates with time to rebound. (*A*) The mRNA levels of cytokines, chemokines, surface receptors, and interferon signaling molecules were measured by BioMark in pDCs sorted from the pre-ATI and ATI visits. Shown is the difference in CT values between the ATI time point and pre-ATI time point after normalization to GAPDH (mean \pm SEM, * p<0.05, ** p<0.01, One sample Wilcoxon test, blue indicates values that were no longer significant when corrected for a false discovery rate of 10% by the Benjamin-Hochberg Procedure). (*B*) Spearman correlation between the plasma IFN α 2 levels after viral rebound and the change in *IFNA2* levels at the ATI time point. (*C*) Spearman correlation between the change in expression of *IFN* genes at the ATI time point and the days from the ATI time point to viral detection. n = 8 participants from RV397, 4 of whom received VRC01.

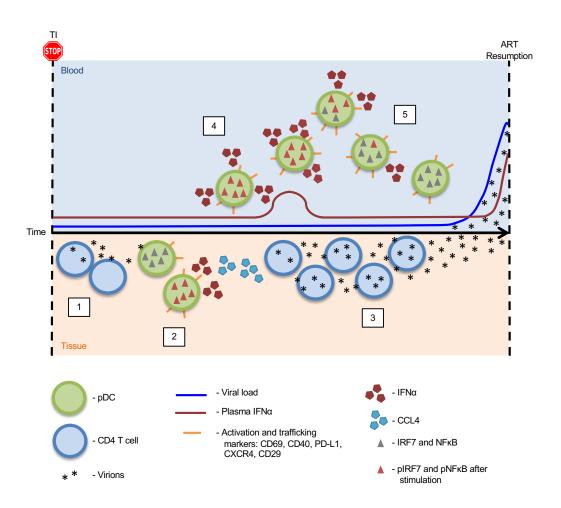


Figure 8. Schematic representation of pDC sensing of viral replication after ATI.

Table 1. Characteristics of participants included in this study.

At Treatment Interruption	n=29
Age, years Median (interquartile range[IQR])	27.5 (24.5-34.5)
Gender (Male:Female)	28:1
Duration from ART Initiation, years Median (IQR)	2.9 (2.5-3.9)
CD4 ⁺ T cell count, cells/mm ³ Median (IQR)	703 (550-839)
Time to viral detection (VL≥20 copies/mL), days Median (IQR)	22 (14-31)
Study Enrollment	
RV411	6
RV397 - placebo arm	5
RV397 - VRC01 arm	13
RV409 - ART only arm	5
At ART Initiation	
Fiebig stage at ART initiation, n (%)	
I (RNA+, p24–)	7 (24)
II (p24+, IgM-)	10 (34)
III (IgM+, WB-)	12 (41)
HIV RNA, log ₁₀ copies/mL Median (IQR)	5.6 (4.6-6.4)
HIV Subtype, n (%)	
CRFO1_AE	20 (69)
В	4 (14)
CRF01_AE and B co-infection	1 (3)
CRF01_AE/B recombinant	2 (7)
CRF01_AE/B/C recombinant	1 (3)
Nontypeable	1 (3)
CD4 ⁺ T cell count, cells/mm ³ Median (range)	377.5 (124-740)