Early Treatment with Reverse Transcriptase Inhibitors Significantly suppresses Peak Plasma IFN α *in vivo* During Acute Simian Immunodeficiency Virus Infection

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Abstract:

Innate interferons (IFN) are comprised of multiple Type I and III subtypes. The *in vivo* kinetics of subtype responses during human immunodeficiency virus (HIV) infection is not well defined. Using the acute simian immunodeficiency virus (SIV) infection model, we show that plasma IFN α levels peak at day 10 post-infection (pi) after which they rapidly declined. The mRNA expression of Type I and III IFN subtypes were significantly elevated in the lymph nodes (LN) at day 10 pi. Though the expression levels of all subtypes declined by day 14 – 31 pi, numerous subtypes remained elevated suggesting that ongoing viral replication in LN continues to drive induction of these subtypes. Interestingly, treatment with reverse transcriptase (RT) inhibitors at day 7 pi significantly suppressed plasma IFN α responses by day 10 pi that significantly correlated with cell-associated SIV DNA loads suggesting that RT byproducts such as viral DNA likely plays a role in driving IFN responses during acute SIV infection. Quantification of Type I and III subtype transcripts in sorted subsets of LN CD4⁺ and CD8⁺ T cells, CD14⁺/CD14⁻ monocytes/macrophages, and total CD11c/CD123⁺ dendritic cells (DC) at day 10 pi showed that DC expressed ~3 – 4 log more subtype transcripts as compared to the other subsets. Taken together, our results provide new insights into the kinetics of innate interferon responses during early stages of infection, and provide evidence that DC's are a major *in vivo* source of innate IFN during acute SIV infection.

Introduction

Human and simian immunodeficiency virus (HIV and SIV) infections are characterized by significant acute pathogenesis that is accompanied by massive viral replication, systemic loss of CD4 memory T cells, acute immune activation, and innate immune responses.

Innate interferons (IFN) are anti-viral cytokines that are released during viral infections in response to the stimulation of various pathogen recognition receptors (PRR) such as Toll-like receptors (TLR), RIG-I, MDA-5 etc. IFN exert their anti-viral action either by inducing the expression of interferon-stimulated genes (ISG) that directly suppresses viral replication or by modulating innate and adaptive immune responses[1; 2; 3; 4]. Numerous studies have shown that IFN α potently inhibits HIV by inhibiting reverse transcription and viral expression from integrated provirus, HIV replication in primary monocyte-derived macrophages, and virion release from infected cell lines[5; 6; 7; 8; 9; 10]. IFN- α treatment of HIV-1 infected cells suppressed viral replication[11; 12; 13; 14; 15; 16]. Others have shown that acute SIV infection is characterized by the release of IFN and upregulation of ISG[17; 18; 19; 20], whereas IFN- α treatment of HIV-1 infected cells suppressed viral replication during early stages of replication[11; 12; 13; 14; 15; 16]. Blocking of IFN signaling with antibodies to IFNR1 was shown to increase viremia whereas treatment with IFN- α 2 increased the expression of ISG[21] in SIV infected macaques suggesting a protective role for IFN responses in controlling viral infection.

Innate IFN include two multi-gene families that code for numerous Type I and III IFN subtypes. Type I IFN gene family includes IFN β , IFN ω and multiple subtypes of IFN- α 1,

- α 2, - α 4, - α 5, - α 6, - α 7, - α 8, - α 10, - α 13, - α 14, - α 16, - α 17 and - α 21 subtypes in humans[22], and IFN α -01/13, 02, 06, 08, 14, 16, 23, 24, 25, 26, 27, 28, 29 in rhesus macaques[23]. Both humans and macaques express the type III IFN gene family that encodes for IFN λ -1, IFN λ -2, IFN λ -3 subtypes[22; 23]. Recent studies have identified IFN- λ 4 as a new Type III subtype in humans[24] with antiviral activity[25].

All Type I IFN subtypes signal through a common cell-surface receptor that is composed of INFA-1 and INFA-2, whereas Type III IFN signals through a receptor complex consisting of the IL-28R and IL-10R β [26; 27; 28].

Interestingly, however, different subtypes have been shown to display different binding affinities for their receptors[29; 30; 31] that in turn was shown to influence their action and potency. Some subtypes such as IFN α -10 has been shown to bind the IFNAR1/2 receptor at 10 - 100 fold greater affinity than IFN α -01[32]. IFN α -10 was found to be highly effective against Semliki forest virus (SFV) and Vesicular stomatitis virus (VSV) whereas IFN α -02 was the least effective among the 9 different subtypes tested[33; 34]. On the other hand, IFN α -02 binding was found to induce chemotaxis genes and shown to be most effective against HIV-1[35] whereas IFN α -08 induced ISG's that protected against HCV replication[36]. Other studies have reported that there were significant differences in the *in vitro* anti-viral and anti-proliferative effects between subtypes[33; 37; 38; 39; 40], with variable effect on T cells and dendritic cells (DC), and B cell proliferation[41; 42].

These studies raise the prospect that various subtypes may play a differential role in acute HIV infection. Harper et al[43] examined the expression of different IFN α subtypes in HIV-1 exposed plasmacytoid dendritic cells (pDC), and determined the

potency of each IFN α subtype *ex vivo* using the Lamina propria aggregate culture model. They reported that IFN subtypes were highly expressed in pDC after exposure to HIV-1, and the relative potencies of subtypes was influenced by their binding affinities to the Type I IFNR with IFN α 8 and IFN α 14 being most potent at inhibiting HIV infectivity. Zaritsky et al[44] examined the expression of both total IFN α mRNA and the pattern of IFN α subtype mRNA expression in pigtailed macaques (PTM) between day 7 and 21 pi and reported that expression and pattern of subtypes expressed differed between the brain, lung and spleen. There is limited information regarding the *in vivo* expression of various Type I and III subtypes in the lymph nodes, jejunal mucosa and PBMC and the source of the various Type I and III subtypes during the early acute stages of HIV infection.

We sought to address this question using the SIV infected rhesus macaque model and examined the kinetics of IFN α levels in the plasma during the 1st two weeks of infection and compared them to animals that were treated with reverser transcriptase (RT) inhibitors very early during the course of infection. To determine if acute SIV infection was characterized by differences in the expression of various subtypes, we examined the mRNA expression profile of both Type I and Type III IFN subtypes in peripheral blood mononuclear cells (PBMC), jejunal mucosa and lymph nodes (LN) during early stages of SIV infection using a quantitative RT-PCR assay. Our results showed that plasma IFN α levels were significantly elevated at day 10- post infection (pi) in untreated animals but was significantly suppressed in treated animals when ART was initiated at day 7 pi. Plasma IFN α levels significantly correlated with CD4 T cell associated SIV DNA loads suggesting a role of viral DNA in the induction of IFN α . Both

Type I and III IFN subtypes were differentially expressed during acute SIV infection with most subtypes being significantly elevated in the LN as compared to the mucosa and peripheral blood. Analysis of various cellular sources in the LN at day 10 pi showed that dendritic cells were the primary *in vivo* source of all IFN subtypes as compared to other cellular subsets.

Materials and Methods

Animals, infection and samples

Archived cryopreserved cells from peripheral blood (PBMC; uninfected n = 10; day 10 pi n = 10; day 14 - 31 pi n = 8), lymph nodes (LN; uninfected n = 5; day 10 pi n = 8; day 14 - 31 pi n = 9) and jejunum (uninfected n = 5; day 10 PI n = 8; day 14 - 31 pi n = 5) that were collected from healthy and SIVmac251 infected rhesus macaques (*Macaca mulatta*) of Indian origin were used in this cross-sectional study. Additionally, archived cells and plasma from rhesus macaques that were treated with a combination of RT inhibitors PMPA (Tenofovir) and FTC (Emtricitabine) were used for analysis; PMPA and FTC were administered at 20-30 mg / Kg BW / day starting at day 7 pi. The animals were housed in accordance with the American Association for Accreditation of Laboratory Animal guidelines and were seronegative for SIV, simian retrovirus (SRV) and simian T-cell leukemia virus (STLV) type-1 prior to SIV challenge.

PBMC was isolated by density gradient centrifugation and cells from LN were isolated by mechanical disruption. Cells from the jejunal mucosa were isolated by enzymatic digestion and percoll gradient centrifugation as per procedures described previously[45; 46; 47; 48; 49; 50].

Plasma viral loads were determined by real-time PCR using reverse-transcribed

viral RNA as the template, as previously described [51]. CD4 T cell associated viral loads were determined in sorted memory CD4 T cell subsets using a highly quantitative PCR assay for SIV-gag as described previously[52]. The level of IFN α in plasma was determined using a human IFN α pan ELISA kit as per manufacturers instructions (limit of detection: 4 pg/ ml).

Antibodies and flow cytometry

Cryopreserved cells were labeled with a panel of CD3-Cy-7APC, CD4-APC, CD8-Alexa700, CD95-FITC and CD28-Cy-5PE (BD Biosciences) and memory CD4 T cells were sorted using a Becton Dickinson Aria sorter and used for determining CD4 T cell-associated viral loads. Memory CD4 T cells was discriminated based on the expression of CD28 and CD95 as described previously[52; 53]. All the antibodies were titrated using rhesus macaque PBMC.

To determine the expression of IFN subtypes in cell subsets, live LN cells at day 10 pi were stained with Vivid live dead marker and anti-CD3-Cy-7APC, CD4-BV605, CD8-Alexa700, CD14-FITC, CD11c-APC and CD123-PE, and CD3+CD4+ T cells (CD4), CD3+CD8+ T cells (CD8), total CD3-CD11c-CD123-CD14+/CD14- monocytes/macrophages (CD14+/CD14-) and CD3-CD14-CD11c+CD123+ dendritic cells (DC) were sorted using BD Aria sorter. Dead cells were excluded and live cell subsets that were sufficient to yield a minimum of ~500 ng of RNA were sorted for each subset (>98% purity) and used for RNA extraction. The number of cells required to yield ~500 ng of RNA was determined in a cell titration experiment.

Absolute quantification of *Macaca mulatta* IFN subtype mRNA levels by qRT-PCR

RNA was extracted from whole cell populations (total PBMC, LN and jejunum cells), and sorted cell subsets (CD4, CD8, monocytes, macrophages, and DC) using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and treated with RNase-free DNase (Qiagen). Total RNA was quantified using a Nanodrop spectrophotometer and 500 ng of RNA was reverse transcribed with the Verso cDNA Synthesis Kit (Thermo Scientific, Rockford, IL, USA) using a mixture of random hexamers and anchored oligo dT primers. The run conditions were as follows: 42°C for 30 min, 95°C for 2 min, 4°C for ∞ . Samples were subsequently treated with RNase H (New England Biolabs, Ipswich, MA, USA) following the manufacturer's instructions.

The copy numbers of type I and III IFN was determined by qRT-PCR as previously described[23; 54]. Briefly, *Macaca mulatta* type I and III interferon subtype (IFN α -01/13, 02, 06, 08, 14, 16, 23, 24, 25, 26, 27, 28, 29, IFN β , IFN ω , IFN λ -1 and IFN λ -3) specific primers and probes[23] were distributed into 384-well assay plates with the Solo automated multi-channel pipettor (Hudson Robotics, Inc., Springfield, NJ), dried, and stored in the dark at 4°C until use. TaqMan Fast Universal PCR Master Mix and the primer/probe sets for GAPDH and 18S were purchased from Applied Biosystems (Foster City, CA, USA). Primers for the IFN transcripts and Molecular Beacon probes were synthesized by the Facility for Biotechnology Resources at the Center for Biologics Evaluation and Research (Silver Spring, MD, USA). LNA probes were synthesized by Sigma-Aldrich (Saint Louis, MO, USA). All primer and probe stocks were purified by high-performance liquid chromatography. The master mix/water mixtures, sample cDNA, housekeeping gene primer/probe sets, and standards were added to each well using electronic multichannel pipettes (Thermo-Fisher Scientific,

Waltham, MA, USA). No template controls (NTC) and four point standard curves of linearized plasmids containing the IFN sequences as inserts were included on each assay plate. The total volume of each PCR reaction per well was 7.5 μ l (3.75 μ l PCR Master Mix, 2.25 μ l primer/probe sets and 1.5 μ l cDNA template). Sealed plates were centrifuged at 1500 rpm, mixed with a MixMate two-dimensional plate vortexer at 2600 rpm (Eppendorf, Westbury, NY, USA), and centrifuged again. After centrifugation, the qRT-PCR assay plates were run on the ViiA 7 Real-Time PCR System (Life Technologies, Grand Island, NY, USA) using the following run conditions: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 sec, 60°C for 1 min. Data was analyzed using the ViiA 7 RUO Software (Life Technologies) and exported into a Microsoft Excel Spreadsheet designed for in-house analysis. Each four point standard curve set was graphed and analyzed for linearity. Absolute copy numbers of each IFN transcript were calculated based on the four point standard curve and target gene transcripts were normalized to micrograms of RNA input per well.

Data analysis

Flow cytometric data was analyzed using FlowJo version 9.2 (Tree Star, Inc., Ashland, OR). Statistical analysis was performed using GraphPad Prism Version 5.0 software (GraphPad Prism Software, Inc. San Diego, CA). *Mann Whitney U* test was used to determine significance and *spearman's rank* was used to determine correlations. A $p < 0.05$ was considered significant. Error bars represent standard error.

Results

Kinetics of plasma IFN α levels correlate with viral infection

Previous studies[19; 55] have shown that acute SIV infection was characterized by an increase in plasma IFN α levels. To confirm these findings, we examined the levels of IFN α in plasma collected prior to infection, and at day 7, 10 and 14 pi and correlated them with plasma viral loads. Our results showed that plasma viremia increased after infection and peaked at day 10 pi and continued to remain high at day 14 pi (Fig. 1a). As reported previously[19; 55; 56; 57], plasma IFN α levels were found to peak at day 10 pi that was followed by a significant decline to near baseline levels by day 14 pi (Fig. 1b). Interestingly, plasma viral loads ($r = 0.4949$; $p = 0.0521$) at day 10 pi did not significantly correlate with plasma IFN α levels at day 10 pi (Fig. 1c).

Reverse transcriptase inhibitors administered at day 7 pi significantly suppresses plasma IFN α levels and CD4 T cell associated SIV-DNA loads at day 10 pi

Studies[58; 59] have shown that endosomal Toll-like-receptors (TLR) such as TLR-7, 8, and 9 play a key role in induction of IFN α . However, *in vivo* administration of TLR-7 and 9 antagonists had little or no effect on plasma IFN α levels[19] suggesting to a role for other mechanisms in this process. On the other hand, recent studies[60] have shown that a cytoplasmic DNA sensor, cGAS plays a role in induction of IFN α responses in macrophages raising the possibility that similar mechanisms may be driving early IFN α responses during SIV infection. To address this question we examined plasma IFN α levels in rhesus macaques that initiated anti-retroviral therapy with reverse transcriptase (RT) inhibitors at day 7 pi and compared them SIV infected untreated animals.

Our results show that initiation of therapy early during infection was associated with a significant decrease in peak plasma and cell-associated SIV DNA as compared

to untreated animals (Fig. 1a and 1d). Suppression of infection was accompanied by a significant decline in plasma IFN α levels to near baseline levels (Fig. 1b). Though plasma viremia remained high in treated animals plasma IFN α levels remained at near baseline levels in treated animals at day 10 and 14 pi. These findings along with the lack of a significant correlation between plasma viral loads and plasma IFN α levels at day 10 pi suggest that byproducts of reverse transcription such as viral DNA maybe playing a key role in driving IFN α responses very early during SIV infection. In line with this argument, we found a significantly high positive correlation between cell-associated SIV DNA loads and plasma IFN α levels ($r = 0.7762$, $p = 0.0022$) at day 10 pi (Fig. 1e).

IFN subtypes are differentially expressed in PBMC, LN and Jejunal mucosa

SIV has been shown to extensively replicate in the LN and mucosa during acute stages of infection[52; 61; 62; 63; 64]. We hypothesized that these tissues were a likely source for the significant increase in IFN levels during the acute SIV infection. As IFN harbors numerous subtypes, we quantified the copy numbers of both Type I and III IFN subtype mRNA in total cells isolated from the PBMC, LN and jejunal mucosa at day 10, 14 – 31 pi and compared them to pre-infection levels (Fig. 2).

Our results showed that the expression of IFN subtypes was highly restricted in PBMC with only IFN α -01/13 and IFN λ -1 being significantly upregulated at day 10 pi as compared to uninfected animals. Interestingly, IFN α -14 and 16 levels significantly declined by day 10 pi as compared to pre-infection levels. Like PBMC, cells from the jejunal mucosa at day 10 pi were found to selectively upregulate IFN α -01/13, 06, 08, 23, IFN ω and IFN λ -1 subtypes as compared to preinfection values. Expression levels in both PBMC and jejunal mucosa declined significantly by day 14 – 31 pi with the loss

being more pronounced in the jejunal mucosa that likely coincided with the massive loss of cells reported to occur at these sites during acute stages of infection.

Unlike PBMC and jejunal mucosa, however, the expression levels of all the Type I and III subtypes except for IFN α -26 were significantly upregulated at day 10 pi in the LN as compared to uninfected animals. The levels of IFN expression in LN were significantly higher in magnitude than both PBMC and jejunal mucosa suggesting that LN were a significant source of early IFN response. Though peak expression levels declined by day 14 – 31 as compared to day 10 pi, the expression levels of IFN α -01/13, 08, 23, 24, 25, 28, 29, IFN β and IFN λ -1 remained significantly higher than preinfection levels suggesting that ongoing viral replication continues to drive innate IFN responses in organized lymphoid tissues after viral replication had peaked. IFN λ -3 was undetectable in all the three tissues we examined.

Dendritic cells are the primary producers of both Type I and III IFN during acute SIV infection

Our results showed that LN expressed ~ 3 – 4 logs more Type I and III IFN subtype mRNA transcripts at day 10 pi as compared other tissues. To determine the cellular source of the different subtypes, we sorted highly purified subsets of CD4 and CD8 T cells, total monocytes/macrophages (CD14+/CD14-) and total DC's (CD11c+ and CD123+) from the LN at day 10 pi and quantified the expression of IFN subtypes in these subsets (Fig. 3). We were unable to obtain sufficient LN samples prior to infection for sorting cell subsets, hence restricted our analysis to samples collected at day 10 pi. Additionally, given the limited amount of samples we had and the low frequency of both CD11c+ myeloid DC and CD123+ plasmacytoid DC, we decided to sort total DC instead

of individual subsets to extract sufficient RNA needed for qRT-PCR analysis of various IFN subtypes. A similar rationale was used to sort total monocyte/macrophage subsets. Our results showed that except for IFN α -26, at peak of infection DC subsets expressed significantly higher numbers of all IFN subtypes *in vivo* as compared to the other cell subsets. There was no significant difference between the other four subsets we examined.

Discussion

HIV-1 and SIV infections are characterized by a significant innate immune response early in infection[65]. Others have reported upregulated expression of interferon stimulating genes (ISG) that have been associated with control of infection[65]. The mechanisms that drive early IFN response have been a matter of intense investigation over a number of years though the exact cause for induction of Type I, and III IFN response *in vivo* remains unclear. Our results suggest that RT byproducts such as viral DNA may play a key role in this process; initiation of anti-retroviral therapy with RT inhibitors at day 7 pi significantly suppressed plasma IFN α levels to that of baseline by day 10 pi as compared to ~3000 pg/ ml of IFN α at day 10 pi in SIV infected untreated animals. Interestingly, the plasma viral loads remained high (~6 logs) in treated animals at day 10 pi suggesting that endosomal compartmentalization of virus may not be the key driver of early IFN responses. Otherwise, we should have seen higher levels of plasma IFN α as viral RNA levels remained significantly high in the plasma from treated animals. In line with this argument, studies[19] have shown that treatment of SIV infected rhesus macaques with

TLR7 and TLR9 antagonists had little or no effect on peak plasma IFN α levels during the first two weeks of SIV infection.

On the other hand, suppression of IFN responses by RT inhibitors suggests that RT byproducts such as the reverse transcribed viral DNA might be driving early IFN responses during acute SIV infection. Recent studies have demonstrated a key role of the cytoplasmic DNA sensor cGAS in macrophages infected with HIV and SIV[60]. Lahaye et al[66] showed that host sensing of HIV-2, a virus that is more similar to SIV, required viral cDNA synthesis rather than nuclear entry or genome integration, and this sensing in DC's were mediated by cGAS. Martin-Gayo et al[67] showed that conventional DC from HIV infected elite controllers produce high levels of innate IFN that was associated with an accumulation of viral reverse transcripts and blocking of cGAS or reverse transcription inhibited these responses. Herzner et al[68] showed that single stranded HIV-1 DNA activates cGAS and HIV-1 reverse transcripts were the predominant viral DNA species in the cytoplasm of macrophages during early infection. The above studies along with our results suggest that reverse transcribed viral DNA and cytoplasmic sensors likely play an important role in the induction of innate IFN responses *in vivo* during acute SIV infection.

That innate IFN response in plasma peaks very early during infection has been known for some time. Previous studies[19; 55] have shown that plasma IFN α levels peak between days 7 - 10 pi in SIV infected animals after which they rapidly decline to baseline levels. Our findings were in line with these earlier reports. What is, however, not clear is the kinetics of the various IFN subtype responses *in vivo* during acute stages of SIV infection, and if these responses differ between tissues. Our results

showed that various subtypes were differentially expressed in mucosal and peripheral tissues with nearly all of the detectable subtypes being expressed at significantly high levels in the LN as compared to PBMC and jejunum; at day 10 pi only IFN α -01/13 and IFN λ -1 transcripts were significantly upregulated in PBMC, whereas IFN α -01/13, 06, 08, 23, IFN ω and IFN λ -1 subtypes were significantly upregulated in the jejunal mucosa. IFN α -26 and IFN λ -3 were undetectable in all the tissues we examined. The expression levels in both PBMC and jejunal mucosa significantly declined by day 14 – 31 pi. Interestingly, there was a significant decline in IFN α -14 and 16 subtypes in PBMC by day 10 pi. Harper et al[43] using an *ex vivo* model of HIV infection showed that IFN α -06, 08 and 14 were the most potent of subtypes examined that showed significant HIV restriction in the mucosa. On the other hand, IFN α -01 and 02 had the weakest antiviral activity suggesting that decline of these highly restrictive subtypes may contribute to the increased pathogenesis and replication in the gut mucosa[43]. Likewise, Lavender et al[69] showed that IFN α -14 had significantly higher antiviral activity against HIV infection in humanized mouse models as compared to other subtypes.

In contrast to both PBMC and jejunal mucosa, the expression and magnitude of most subtypes were significantly upregulated in the LN at day 10 pi suggesting that LN was a significant source of early IFN response during acute SIV infection. Unlike the PBMC and jejunum, however, the expression levels of IFN α -01/13, 08, 23, 24, 25, 28, 29, IFN β and IFN λ -1 transcripts remained significantly higher at day 14 – 31 pi as compared to pre-infection levels suggesting that ongoing replication continues to drive specific IFN subtype responses in the LN. The exact reason why only a subset of IFN subtypes were expressed is difficult to determine at this point. Previous studies[44]

examined the relative expression of IFN α subtypes in SIV infected PTM during the 1st 3 weeks of infection and found tissue specific differences in the expression pattern of various IFN α subtypes; only subtypes 2, 6, and 13 were expressed in the brain of SIV infected PTM at day 7 pi, whereas only subtypes 6 and 13 were upregulated in the lung. In contrast to the brain and lung, all the 13 subtypes were expressed in the spleen with subtypes 2, 8, and 13 being the most abundantly expressed at day 7 pi whereas subtypes 4, 17 and 21 were least abundantly expressed.

It is unlikely that the lower levels of IFN subtypes expressed in the jejunum as compared to the LN was due to the lower frequency of IFN producing cells at these sites as fewer cells are likely to lower the magnitude of expression rather than the pattern of expression of IFN subtypes. It is, however, difficult to rule this out. The loss of DC subsets from peripheral blood may explain the decline in expression of subtypes in blood as numerous studies have documented that both DC and macrophages migrate to the gut mucosa during SIV infection[56; 70; 71; 72; 73; 74; 75]. The simultaneous decline in IFN expression in both mucosal and peripheral tissues, however, raises the possibility that DC are either lost or become refractive in both PBMC and the mucosa at the same time. Wonderlich et al[57] reported that macrophages and myeloid DC subsets lose stimulating function and was associated with decreased IFN α production during SIV infection.

Compared to PBMC and jejunum, LN is enriched for IFN producing cells such as DC that might explain the significantly higher levels of all IFN subtypes in the LN. In fact, we observed significantly higher levels of all detectable IFN subtypes in the DC's from the LN as compared to other cell subsets; DC's had ~100,000 fold more copies of IFN

subtype transcripts than either T cells or monocyte/macrophage subsets. Our results for the first time provide *in vivo* evidence for previously reported *in vitro* studies[76; 77; 78; 79; 80] showing that DC produce significantly higher amounts of IFN than other cell types.

Previous studies have reported that LN DC's were major producers of Type I IFN α during acute SIV infection[55; 81; 82] whereas others have shown that pDC's were actively recruited from circulation into the LN during SIV infection[71; 83]. Bruel et al[55] showed that pDC were the major producers of IFN α in the gut and lymphoid tissues during acute SIV infection. Interestingly, there was no difference in the IFN transcript levels between CD4⁺ T cells and other non-DC subsets we examined even though CD4 T cells are highly infected at day 10 pi (Fig. 1d). There is little or no evidence in the literature showing that CD4 T cells were capable of making innate IFN responses during viral infection though they have been shown to upregulate the expression of ISG during acute stages of SIV infection. Like CD4 T cells, it was somewhat surprising that we did not see any significant upregulation of IFN transcripts monocytes/macrophage subsets even though previous studies have shown that macrophages carry SIV DNA[84]. The exact reason why macrophages did not express IFN *in vivo* very early in infection has to be examined further and additional studies are needed to clarify these questions in detail. It is difficult to determine from our studies if cellular subsets other than DC's in the LN upregulated the expression of IFN subtypes at day 10 pi as we did not have sufficient cells from healthy LN to compare. However, there is little or no evidence of CD8 T cells producing IFN α during SIV infection and transcript levels in CD8 T cells did not differ from the other non-DC subsets suggesting that cell subsets other than DC's

likely did not upregulate the expression of innate IFN. Future studies will examine this question in greater detail.

In conclusion, our studies provide new insights into the kinetics of different Type I and III IFN subtypes during acute SIV infection and identifies DC's as a major *in vivo* cellular source of IFN subtypes. The significant suppression of plasma IFN α with RT inhibitors suggest that viral DNA and cytoplasmic DNA sensors likely play a role in driving Type I and III IFN responses during acute SIV infection.

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Figure Legends

Figure 1. Reverse transcriptase inhibitors significantly suppressed plasma IFN α during acute SIV infection. (a) Kinetics of plasma viral loads (Limit of detection is 30 copies /ml of plasma) and (b) plasma IFN α levels during the 1st 2 weeks of SIV infection in untreated (n = 8) and treated (n = 4) animals. Treated animals received reverse transcriptase inhibitors starting at day 7 post-infection. (c) Correlation between plasma viral loads and plasma IFN α levels at day 10 pi from untreated (n = 8) and treated (n = 4) animals. (d) Kinetics of SIV DNA loads in CD4 memory T cells in untreated and treated animals during the 1st 2 weeks of SIV infection. (e) Correlation between CD4 memory T cell-associated SIV DNA loads and plasma IFN α levels at day 10 pi from untreated (n = 8) and treated (n = 4) animals. Line of fit was determined using linear regression analysis and correlations were derived using Spearman's rank test. Error bars represent standard error and * denotes $p < 0.05$.

Figure 2. Most Type I and III IFN subtypes are significantly upregulated in the LN at day 10 post infection. Absolute copies of Type I and III IFN subtypes was determined in total cells isolated from peripheral blood (PBMC), jejunum, and lymph nodes (LN) at day 10 and 14 – 31 post infection using a qRT-PCR assay and compared to pre-infection values. Absolute copy numbers were determined using rhesus macaque IFN subtype specific standards. Statistical analysis was performed using Mann-Whitney U test and a $p < 0.05$ (*) was considered significant.

Figure 3. Dendritic cells are the primary producers of all IFN subtypes at 10 days post infection. Highly purified populations of T cells (CD4+ and CD8+), monocytes/macrophages (CD14+/CD14-) and dendritic cells (DC) were sorted using a

748 BD FACS Aria sorter and used for quantifying IFN subtype transcripts using a qRT-PCR
749 assay. Absolute copy numbers were determined using rhesus macaque IFN subtype
750 specific standards. Statistical analysis was performed using Mann-Whitney U test and a
751 $p < 0.05$ (*) was considered significant.

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Figure 1

Figure 2

Figure 3

Day 10 Post Infection