MAJOR ARTICLE

Bone Marrow–Imprinted Gut-Homing of Plasmacytoid Dendritic Cells (pDCs) in Acute Simian Immunodeficiency Virus Infection Results in Massive Accumulation of Hyperfunctional CD4$^+$ pDCs in the Mucosae

Haiying Li,[1] Tristan I. Evans,[2] Jacqueline Gillis,[2] Michelle Connole,[2] and R. Keith Reeves[1,2]

[1]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, and [2]Division of Immunology, New England Primate Research Center, Harvard Medical School, Southborough, Massachusetts

Plasmacytoid dendritic cells (pDCs), a primary source of interferon α (IFN-α), provide a first line of innate immune defense against human immunodeficiency virus infection. However, their kinetics and functions during acute infection are poorly understood. In mucosal tissues of normal rhesus macaques, we found CD4$^+$ pDCs to be the subset responsible for most IFN-α and tumor necrosis factor α (TNF-α) production in response to Toll-like receptor (TLR) 7/8 stimulation, compared with relatively anergic CD4$^-$ pDCs. During acute simian immunodeficiency virus (SIV) infection, gut homing was imprinted on pDCs in the bone marrow, resulting in a decline in pDCs from circulation and secondary lymphoid tissues. Although the accumulated pDCs in the gut mucosae had robust cytokine responses to TLR7/8 stimulation in vitro, pDC gut migration occurred after infection and detection of SIV in plasma. Our data suggest that innate pDC responses do not control initial SIV seeding and dissemination but instead may contribute to ongoing immune activation in the gut.

Keywords. plasmacytoid dendritic cell; mucosal immunology; SIV pathogenesis.

Plasmacytoid dendritic cells (pDCs) are potent type I interferon (IFN)–producing cells mobilized in response to viral infections [1, 2]. pDCs sense viral nucleic acids and CpG oligodeoxynucleotides through Toll-like receptor 7 (TLR7) and TLR9, respectively, both of which could induce secretion of type I IFN, through the MyD88-IRF7 pathway, and production of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin 6, through NF-κB signaling [3–5]. These cytokines subsequently activate natural killer cells and other DCs, and they also regulate adaptive immunity by enhancing T-lymphocyte cytotoxicity, promoting T-helper cell type 1 polarization and B-cell differentiation into plasma cells [3, 6].

Numerous reports from human and nonhuman primate (NHP) studies have shown that pDCs are dramatically lost from peripheral blood during primary and chronic human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infection [7–16], and the loss of pDCs is correlated with increasing plasma virus load [8, 11]. After initiation of antiviral therapy, pDC numbers increase as viral load decreases [9, 10]. Correspondingly, elite controllers have greater pDC frequencies and increased IFN-α production, compared with viremic patients [17]. Furthermore, it has been demonstrated that pDC-derived IFN-α can limit HIV replication in vitro [18]. A study of sequential plasma samples collected from acutely HIV-1–infected individuals reveals a systemic cytokine cascade prior to peak viremia, and IFN-α is one of the first cytokines to have elevated levels after infection [19]. Collectively,

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Correspondence: R. Keith Reeves, Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, 3 Blackfan Cir, Boston, MA 02215 (rreeves@bidmc.harvard.edu).

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these data suggest a role for pDCs in the acute control of HIV replication, likely through production of type I IFN.

However, recent evidence indicates that pDCs may also induce chronic immune activation and immunopathogenesis in persistent viral infections, such as HIV and SIV infection [13, 20–23] and lymphocytic choriomeningitis virus (LCMV) infection [24], and in autoimmune diseases [25–27]. A murine study has shown that blockade of type I IFN during LCMV infection enhances viral clearance, suggesting that type I IFN signaling is linked to immune activation and viral persistence [24]. Genomic analyses of rhesus macaques and sooty mangabeys, a natural host of SIV infection, reveal that sooty mangabeys rapidly control IFN-stimulated gene expression and immune activation, whereas rhesus macaques have prolonged production of IFN-α and expression of IFN-stimulated genes [22]. However, other studies have shown that blocking IFN-α production by pDCs during early SIV infection did not diminish immune activation [25], and treatment with high levels of IFN-α during acute SIV infection did not induce African green monkeys to exhibit chronic immune activation [28]. This evidence suggests that IFN-α may not be the main factor involved in the immunopathogenesis of HIV and SIV infection. Hence, it is still unclear whether the contribution of pDCs in HIV infection is advantageous or detrimental to the host [29].

The gastrointestinal (GI) tract is a major site of HIV and SIV replication and $CD4^+$ T-cell depletion, particularly during the acute phase of disease [30, 31]. Limited data shows that an elevated IFN-α level is observed in the gut mucosa of HIV-1–infected patients and correlates with chronic immune activation [32]. Our laboratory and others have reported that pDCs traffic from circulation and nonlymphoid organs to the gut mucosa [32–35] and that this trafficking is coupled with the production of multiple cytokines by pDCs during chronic SIV infection [33]. However, although it is well established that acute infection induces an efflux of pDCs out of the bone marrow and increased apoptosis in lymph nodes [16], potential gut homing by pDCs during the acute stage of infection and the role of pDCs in the GI tract during this stage are less well characterized. The underlying mechanisms causing the dynamic and functional alteration of pDCs in tissues during the acute stage of infection is unclear. To address these deficits, we investigated pDC functions in specific anatomic tissue microenvironments during acute SIV infection.

METHODS

Animals and SIV Infections

Twelve Indian rhesus macaques were analyzed in this study, including 6 SIV-naive animals and 6 infected intravenously with SIVmac239, and euthanized at day 14 following infection. Infection was verified by plasma virus quantification using reverse transcription polymerase chain reaction (RT-PCR) analysis. All animals were housed at the New England Primate Research Center, cared for in accordance with American Association for Accreditation of Laboratory Animal Care standards, and were free of simian retrovirus type D, simian T-lymphotropic virus type 1, and simian herpes B virus. Animal procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee of Harvard Medical School.

Collection and Processing of Peripheral Blood and Tissues

Peripheral blood specimens were collected before infection and at days 1, 3, 6, 7 or 8, 10, and 14 following infection, and peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation over lymphocyte separation media (MP Biomedicals, Solon, Ohio). Tissue specimens from bone marrow, liver, spleen, colon, jejunum, peripheral lymph node, mesenteric lymph node, and pararectal/paracolonic lymph node were collected from naive and acutely infected animals at day 14 after infection. Before infection (on day −14), lymph node and colorectal biopsy specimens were also collected. Lymphocytes were isolated from mucosal tissues by mechanical and enzymatic disruption as described previously [34].

Antibodies and Flow Cytometry

Antibodies to the following antigens were included in this study, and except where noted, all were obtained from BD Biosciences: α4β7-APC (clone A4B7; NHP reagent resource), CD3-APC-Cy7 (clone SP34.2), CD4-FITC (clone L-200), CD11c-PE and APC (clone S-HCL3), CD14-Alexa 700 and Pacific blue (clone M5E2; BioLegend), CD20-PerCP-Cy5.5 (clone L27), Pacific blue (clone 2H7; BioLegend), CD45-FITC (clone D058-1283), PerCp-Cy5.5 (clone Tu116), HLA-DR-PE-Texas Red (clone Immu-357; Beckman-Coulter), CD123-PE-Cy7 (clone 7G3), and Ki-67-FITC (clone B56). Ki-67-FITC monoclonal antibody was used for intracellular stains. Levels of spontaneous apoptosis were examined by annexin V staining with a commercially available kit (BioVision, California). Flow cytometry acquisitions were performed on an LSR II (BD Biosciences, La Jolla, California), and FlowJo software (version 9.6.4; Tree Star, Ashland, Oregon) was used for all analyses. pDCs were gated as $CD3^-CD14^-CD20^-HLA\text{-}DR^+CD123^+CD11c^-$ cells among live $CD45^+$ mononuclear cells. Pestle (version 1.6.2) and SPICE (version 5.1) were used for multiparametric analyses.

In Vitro Stimulation and Intracellular IFN-α Staining

Mononuclear cells were stimulated with TLR7/8 ligand (R848; imidazoquinoline 5 μg/mL; InvivoGen) or cultured in medium (Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum) alone, as adapted from a previous report [36]. Golgiplug (brefeldin A) was added at a final concentration of 10 μg/mL. After culture for 5 hours at 37°C in 5% CO_2, cells were fixed and permeabilized (Cytofix/Cytoperm; BD

Bioscience), followed by staining with surface markers and intracellular anti-IFN-α (FITC conjugate; clone MMHA-2; PBL Biomedical Laboratories), and anti-TNF-α (Alexa700 conjugate; clone Mab11) for 30 minutes.

Luminex Cytokine Assays

Concentrations of plasma IFN-α and TNF-α were measured using a custom Luminex assay as previously described [37]. Each sample was tested in duplicate wells, and cytokine standards supplied by the manufacturer were included in each plate.

Plasma Virus Load Quantification

Plasma SIV RNA copy numbers were determined using a standard quantitative real-time RT-PCR assay based on amplification of conserved gag sequences, as described previously [38].

Statistical Analysis

All statistical and graphic analyses were performed using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, California). Statistical significance between groups was analyzed with the nonparametric Mann–Whitney *U* test. The Wilcoxon matched pairs test was used to compare matched samples from before infection and after infection. A *P* value of <.05 was considered statistically significant.

RESULTS

Early Redistribution of pDCs to GI Tract During Acute SIV Infection

To explore the impact of acute SIV infection on pDC distribution in tissues, we evaluated 6 rhesus macaques that were intravenously infected with SIVmac239 and euthanized at day 14 after infection and compared them with 6 naive animals in a planned study of euthanized animals. There was significant variability in the percentages of pDCs among mononuclear cells in circulation following infection, but all 6 acutely infected animals exhibited significant depletion of pDCs by day 10 coincident with peak viremia (Figure 1*A* and Supplementary Figure 1). Consistent with a previous report [30], the incidence of pDCs in the liver of uninfected rhesus macaques ranged from 0.33% to 1.16% (median, 0.68%) among mononuclear cells, significantly higher than in peripheral blood (median, 0.14%; range, 0.01%–0.27%; $P < .001$) or spleen (median, 0.17%; range, 0.07%–0.37%; $P < .01$), whereas pDCs were relatively rare in the colon and jejunum (median, 0.02% and 0.03%, respectively; Figure 1*B*). Interestingly, compared with naive animals, pDC frequencies declined not only from the peripheral blood of acutely infected animals with up to a 9-fold decrease, but were also dramatically reduced in the spleen and liver, with 7-fold and 6-fold decreases,

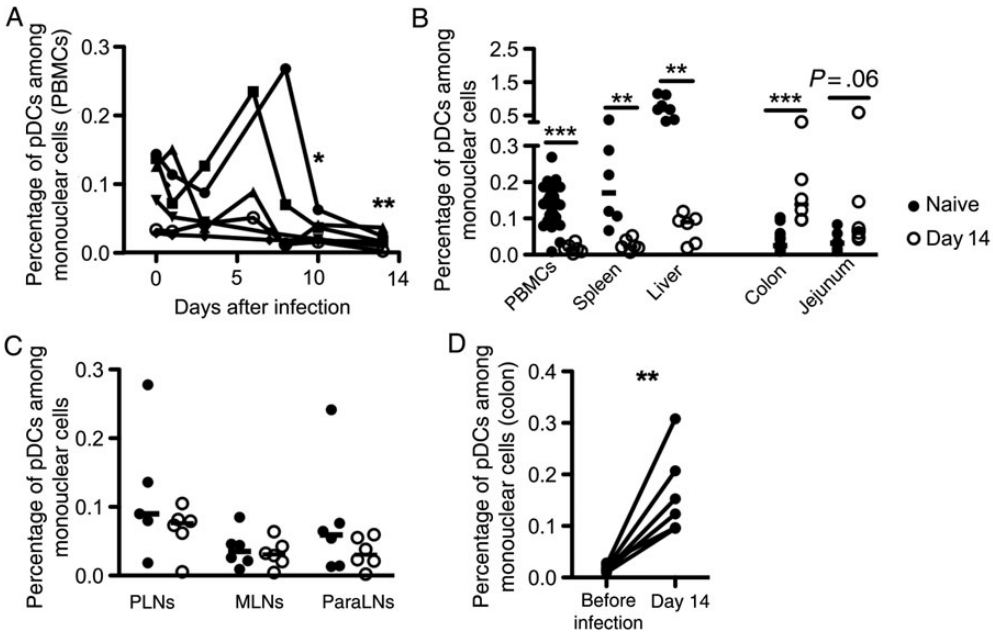

Figure 1. Redistribution of plasmacytoid dendritic cells (pDCs) to the intestinal mucosae during acute simian immunodeficiency virus (SIV) infection. *A*, Longitudinal frequency of peripheral blood pDCs among peripheral blood mononuclear cells (PBMCs) in acutely infected rhesus macaques. Frequencies of pDCs in PBMCs, spleen, liver, colon, and jejunum (*B*) and peripheral lymph nodes (PLNs), mesenteric lymph nodes (MLNs), and pararectal/paracolonic lymph nodes (ParaLNs; *C*) were compared between SIV-naive animals and acutely infected animals at day 14 after infection. Black horizontal lines indicate medians. *D*, The frequency of pDCs in the colon was compared between biopsy specimens obtained from animals before infection and on day 14 after infection, after animals were euthanized. The nonparametric Mann–Whitney *U* test was used to compare the statistical significance of differences between the naive and acutely infected groups. The Wilcoxon matched pairs test was used to compare matched samples obtained before infection to those obtained after infection. Each line represents an individual animal. *$P < .05$, **$P < .01$, and ***$P < .001$.

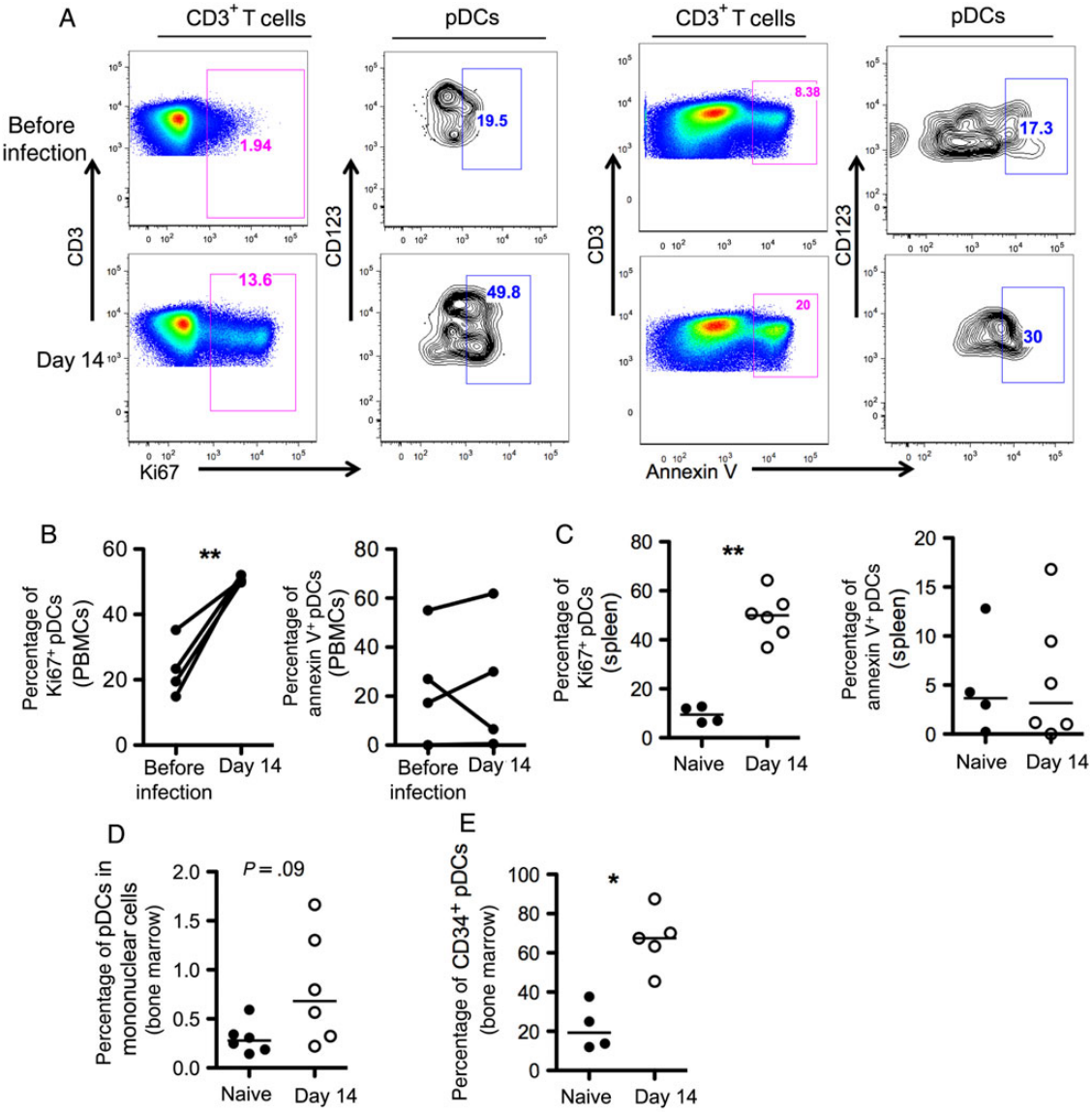

Figure 2. Increased proliferation and mobilization, but not apoptosis, of plasmacytoid dendritic cells (pDCs) during acute simian immunodeficiency virus infection. *A*, Representative gating strategies for Ki-67 intracellular staining and annexin V expression on circulating $CD3^+$ T cells and pDCs obtained before infection and at day 14 after infection. *B*, Percentages of Ki-67$^+$ and annexin V$^+$ pDCs within the total population of peripheral blood pDCs were compared between before infection and day 14 after infection (day 14). *C*, Percentages of Ki-67$^+$ and annexin V$^+$ pDCs among the total pDC population in the spleen were compared between naive and acutely infected animals. *D*, The frequency of pDCs among mononuclear cells in the bone marrow was compared between naive and acutely infected macaques. *E*, The percentage of $CD34^+$ pDC precursors within the total population of pDCs in bone marrow was compared between naive and acutely infected macaques. The Wilcoxon matched pairs test was used in the analysis summarized in panel *B*. The nonparametric Mann–Whitney *U* test was used in analyses summarized in panels *C*–*E*. **P* < .05 and ***P* < .01. Abbreviation: PBMC, peripheral blood mononuclear cell.

respectively. In contrast pDC frequencies increased by 5-fold in the colon and 2-fold in the jejunum (Figure 1*B*). No change was observed in the pDC frequency in the peripheral, mesenteric, and paracolonic lymph nodes (Figure 1*C*). Consistently, all 6 acutely infected macaques exhibited a significant increase of pDCs in the colon at day 14 after infection, compared with colon biopsy samples obtained before infection (Figure 1*D*). These data suggest that SIV rapidly induces pDCs redistribution to the intestinal mucosae. Furthermore, this redistribution was highly specific to pDCs, and we find that, by comparison, $CD11c^+$ myeloid DCs do not change in frequency in the gut mucosae (Supplementary Figure 2)

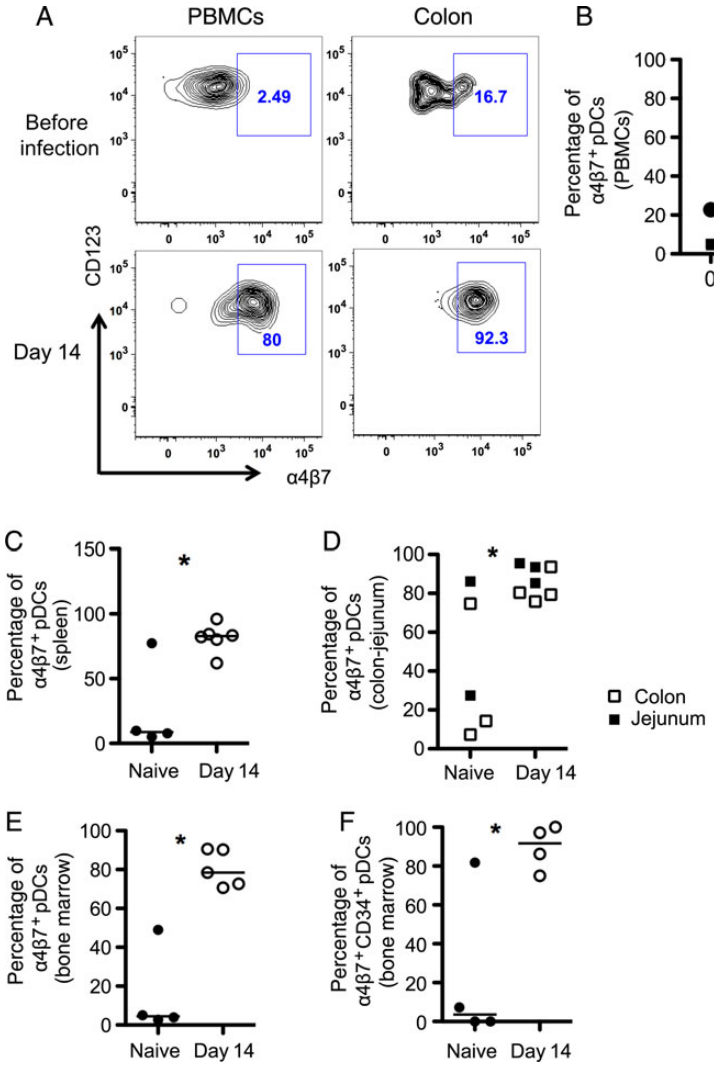

Figure 3. Increased expression of α4β7 on plasmacytoid dendritic cells (pDCs) in tissues during acute simian immunodeficiency virus (SIV) infection. *A*, Representative gating strategies of α4β7 on pDCs from peripheral blood mononuclear cells (PBMCs) and colon before infection and at day 14 after infection. *B*, Longitudinal frequency of $\alpha4\beta7^+$ pDCs among the total population of circulating pDCs in3 rhesus macaques during acute SIV infection. Percentages of $\alpha4\beta7^+$ cells among pDCs in the spleen (*C*), colon and jejunum (*D*), and bone marrow (*E*) were compared between naive and acutely infected macaques. *F*, The percentage of $\alpha4\beta7^+$ cells among $CD34^+$ pDCs in bone marrow was compared between naive and acutely infected macaques. The nonparametric Mann–Whitney *U* test was used in analyses summarized in panels *C–F*. $^*P < .05$.

Heightened Proliferation and Mobilization of pDCs During Acute SIV Infection

We speculated that mechanisms involved in the loss of pDC could be due to increased pDC apoptosis and/or decreased proliferation based on previous observations [39]. We first analyzed expression of the apoptotic marker annexin V and the proliferation marker Ki-67 (Figure 2*A*). Longitudinal data clearly showed dramatically increased expression of Ki-67 in circulating pDCs at day 14 following SIV infection, but no significant change in annexin V expression, compared with preinfection samples (Figure 2*B*). Splenic pDCs also exhibited a >5-fold increase in Ki-67 expression, when comparing naive and acutely infected animals (median, 9.49% and 49.95%, respectively), but no change in annexin V expression (Figure 2*C*). These data indicated that acute SIV infection rapidly induces heightened proliferation of pDCs in the peripheral blood and spleen but does not appear to increase apoptosis in these tissues. However, it is possible that the increased numbers of pDCs may eventually undergo apoptosis in other tissues, as has been described for chronic SIV infection [39], and, alternatively, pDCs could be undergoing apoptosis not identified by annexin V expression.

To further understand the mechanisms of pDC redistribution during acute SIV infection, we next analyzed the mobilization of pDCs from bone marrow. Compared with naive animals,

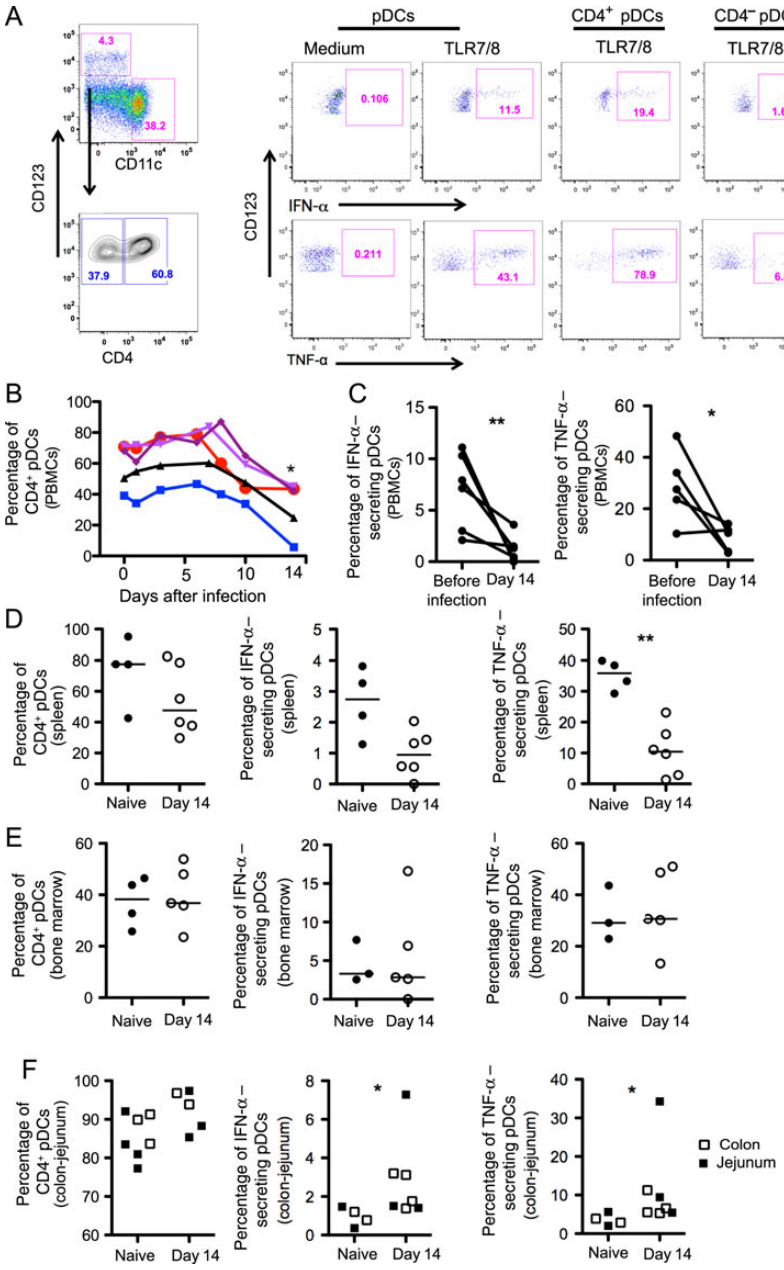

Figure 4. Cytokine secreting plasmacytoid dendritic cells (pDCs) in tissue specimens obtained during acute infection. *A*, Representative gating strategies for interferon α (IFN-α) and tumor necrosis factor α (TNF-α) within the total population of pDCs, $CD4^+$ pDCs, and $CD4^-$ pDCs after stimulation with Toll-like receptor 7/8 (TLR7/8) or medium only. *B*, Longitudinal frequencies of $CD4^+$ pDCs among the total population of circulating pDCs in rhesus macaques during acute simian immunodeficiency virus infection. *C*, The percentage of IFN-α or TNF-α-producing cells after gating on circulating pDCs with TLR7/8 stimulation was compared between before infection and 14 days after infection. The percentages of $CD4^+$ pDCs and IFN-α– or TNF-α–secreting cells among the total population of pDCs in spleen (*D*), bone marrow (*E*), and colon and jejunum (*F*) with TLR7/8 stimulation were compared between naive and acutely infected macaques. The Wilcoxon matched pairs test was used in analysis summarized in panels *B* and *C*. The nonparametric Mann–Whitney *U* test was used in analyses summarized in panels *D* and *E*. $^*P < .05$ and $^{**}P < .01$. Abbreviation: PBMC, peripheral blood mononuclear cell.

acutely infected animals had significantly higher frequencies of pDCs (Figure 2*D*) and $CD34^+$ pDC precursors (Figure 2*E*) in bone marrow, which suggested that despite the overall decline of pDCs in blood and spleen, SIV induces expansion of pDCs in bone marrow during acute infection.

Imprinting of pDC Gut Homing Occurs in the Bone Marrow

We previously reported that circulating pDCs from chronically infected animals had increased expression of the trafficking marker α4β7, which mediates migration and retention of pDCs into the gut mucosa [33, 34]. Therefore, we speculated

that gut migration of pDCs might also occur during the acute stage of SIV infection. Longitudinal data from acutely infected animals showed that circulating pDC α4β7 expression increased by day 6 after infection peaking at day 10, coinciding with peak viral load, and remained significantly elevated even at day 14 (Figure 3*A* and 3*B*). Compared with naive animals, acutely infected animals had significantly increased expression of α4β7 on pDCs in the spleen (Figure 3*C*) and colon and jejunum (Figure 3*D*). Consistently, longitudinal data also showed that pDCs in the colon at day 14 after infection expressed significantly higher levels of α4β7, compared with preinfection colon biopsy specimens ($P < .05$). Interestingly, acutely infected animals also had dramatically increased expression of α4β7 on pDCs (Figure 3*E*) and $CD34^+$ pDC precursors (Figure 3*F*) in bone marrow. These findings suggested that pDC trafficking is a primary mechanism of early redistribution of pDCs to the intestinal mucosae and likely occurs because of early imprinting of migration in the bone marrow.

Altered Cytokine Production by pDCs in Tissues

To address the impact of acute SIV infection on the functions of pDCs in tissues, we first evaluated cytokine production by pDCs from uninfected animals in response to TLR7/8 stimulation. As shown in Figure 4*A*, pDCs were divided into $CD4^+$ and $CD4^-$ subgroups. Interestingly, $CD4^+$ pDCs were consistently identified as the major producers of IFN-α and TNF-α, compared with $CD4^-$ pDCs, in peripheral blood, spleen, bone marrow, colon, and jejunum specimens (Supplementary Figure 3*A*). pDCs from the intestinal mucosae produced less IFN-α and TNF-α in response to TLR7/8 stimulation than did pDCs from the peripheral blood and bone marrow (Supplementary Figure 3*A*). To our surprise, acute SIV infection rapidly decreased the frequency of $CD4^+$ pDCs among total circulating pDCs at day 14 after SIV infection (Figure 4*B*). Furthermore, we found that the cytokine-secreting function of circulating pDCs was profoundly impaired at day 14 after infection, compared with before infection (Figure 4*C*). In contrast, the percentage of $CD4^+$ pDCs among splenic pDCs decreased only slightly in acutely infected animals, concomitant with a trend toward decreased IFN-α production and significantly impaired production of TNF-α in response to TLR7/8 stimulation, compared with splenic pDCs from naive animals (Figure 4*D*). This evidence suggested that SIV profoundly impairs the cytokine-secreting function of pDCs by rapidly depleting $CD4^+$ pDCs from peripheral blood and spleen. Interestingly, compared with cells from naive animals, pDCs from the bone marrow had an unimpaired ability to produce IFN-α and TNF-α in response to TLR7/8 stimulation, consistent with the unchanged expression of CD4 on pDCs (Figure 4*E*). To exclude the possibility that TLR7/8 stimulation could alter CD4 expression on pDCs and thus compromise our results, we compared CD4 expression on pDCs after stimulation with TLR7/8 and medium-only controls but found no evidence of modulation (Supplementary Figure 3*B*).

We speculated that the accumulated pDCs in gut mucosae might have impaired cytokine production during acute infection, as they mostly came from the peripheral blood and spleen. Interestingly, >80% of pDCs in the intestinal mucosae of acutely infected animals were $CD4^+$, which we show are the primary pDCs responsible for cytokine production (Figure 4*F*). Consistently, these cells had significantly higher production of IFN-α and TNF-α in response to TLR7/8 stimulation, compared with cells from naive animals (Figure 4*F*). Furthermore, in comparison with naive macaques, multiparametric analysis revealed that more pDCs from the jejunum of acutely SIV-infected macaques are dual-functional cells that produce both IFN- α and TNF-α (Supplementary Figure 4).

DISCUSSION

In this study, we describe several findings on the response of pDCs in peripheral blood and multiple tissues during acute SIV infection. First, we identified that $CD4^+$ pDCs are the main producers of IFN-α and TNF-α in response to TLR7/8 stimulation by analyzing multiple tissues from SIV-naive rhesus macaques. Considering that HIV gp120 specifically binds with CD4, it is theoretically reasonable that HIV and SIV could have important impacts on pDC function. Correspondingly, it has been reported that CD4 binding to gp120 is required for virion engulfment and activation of pDCs via TLR7 engagement [40, 41]. The question is whether HIV could impair pDC secretion of cytokines by downregulating CD4 expression or depleting $CD4^+$ pDCs. Here we reported that pDC frequencies rapidly declined in peripheral blood, spleen, and liver and that the remaining pDCs in circulation were $CD4^-$ pDCs, whereas $CD4^+$ pDCs expressing high levels of α4β7 mainly trafficked to and accumulated in the intestinal mucosae. These data suggest that gut-homing pDCs are primarily of the $CD4^+$ subset and that SIV rapidly impairs the function of circulating pDCs through dramatic depletion of this functional subset from peripheral blood. Our data are consistent with a recent report that demonstrated that the rapid decline in acute IFN-α plasma concentration was associated with the loss of IFN-α production ability by circulating pDCs [28]. The authors found that Ki-67^+ pDCs in peripheral blood have no ability to produce IFN-α, and hence the activation/death-driven pDC renewal rapidly blunts IFN-α production in vivo [28]. Here we proposed an alternative mechanism for the decline in IFN-α plasma concentration during acute infection. We suggest that the combined effects of the lower frequency of pDCs in circulation and the functional impairment of remaining cells caused by the depletion of $CD4^+$ pDCs together cause the quick elimination of plasma IFN-α during acute infection.

Our second main finding was that migration of pDCs to the gut mucosae during acute infection may be imprinted in the

bone marrow. Our data show that both bulk pDCs and $CD34^+$ immature pDCs from the bone marrow significantly upregulate α4β7 in acutely SIV-infected animals. This suggests that pDCs in infected macaques begin expressing α4β7 and likely gain the ability to home to the gut even before they have emigrated from the bone marrow. While the mechanisms are not entirely clear and why this might deviate from the normal homing of pDCs in the absence of infection is not known, these findings are in line with the overall mobilization of pDCs to traffic to the gut. Interestingly, the expression of α4β7 on circulating pDCs did not increase until 1 week after SIV infection, which could suggest that pDCs do not migrate to and accumulate in the intestinal mucosae until after viremia is detectable in blood. By this time, virus has likely already disseminated and replicated in local tissues, and the lymphatic tissue reservoir is established [42–44]. Thus, even though many pDCs migrated to the gut mucosae in acute disease, they are unlikely to play a pivotal role in controlling viral dissemination or hampering reservoir establishment in GI tissues. On the other hand, we observed that the IFN-α level was generally low or undetectable in plasma until day 10 after infection. These data suggest that the secretion of IFN-α by pDCs may also occur too late to play a critical role in the earliest events controlling viral replication. Although the level of IFN-α in plasma significantly increased when the virus load reached peak value at day 10 after infection, the plasma IFN-α level quickly declined by day 14, at which time viral load was still high. This could be associated with the trafficking and accumulation of IFN-α–producing pDCs in the gut by this time point. It is important to note, however, that all these infections were performed intravenously and that it is possible that the kinetics of both pDC trafficking and associated IFN-α might differ after a mucosal challenge. Additional studies will be required to address these points. We could also speculate that gut homing of pDCs during the acute stage could block SIV replication in the intestinal mucosae by secreting IFN-α. Although attempts to measure IFN-α in mucosal sites found it undetectable by Luminex or enzyme-linked immunosorbent assay (data not shown), IFN-stimulated genes are upregulated during SIV infection [22]. Regardless, the presence of infection indicates these responses are insufficient and/or likely too late to impact virus acquisition. Interestingly though pDCs from the GI mucosae of uninfected animals produced much less IFN-α and TNF-α than circulating pDCs in response to TLR7/8 stimulation. In contrast, during acute infection, pDCs from the gut produced robust IFN-α and TNF-α, likely because of the influx of more-potent $CD4^+$ pDCs. Accumulated pDCs in the mucosae during acute infection were also multifunctional cells, producing IFN-α and high levels of TNF-α, a proinflammatory cytokine that has been demonstrated to contribute to mucosal immunopathogenesis in inflammatory bowel disease [45, 46]. Therefore, pDC responses may be unlikely to control SIV dissemination and replication but, rather, contribute to ongoing immune activation in the gut.

To conclude, we are the first to demonstrate that $CD4^+$ pDCs from multiple tissues are major producers of IFN-α and TNF-α. After imprinting in the bone marrow, $CD4^+$ pDCs migrated from the peripheral blood, spleen, and liver to the GI tissues. Even though functional pDCs accumulate in the GI tract during acute SIV infection, this accumulation is likely too late to control local viral expansion and dissemination but instead might play a role in ongoing immunopathogenesis.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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