

# Altered dynamics and differential infection profiles of lymphoid and myeloid cell subsets during acute and chronic HIV-1 infection

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## ABSTRACT

The dynamics of immune cell populations during acute HIV-1 infection are not fully deciphered, especially for non-T cells. In this study, we tested whether specific cellular subsets of the innate arm of the immune response are affected early after HIV-1 infection. Using a cohort of HIV-1-infected individuals, we have monitored the relative frequency of blood T lymphocytes, monocytes, and DCs at various infection stages and measured their respective intracellular HIV-1 DNA loads. The HIV-1 DNA load in naïve CD4<sup>+</sup> T lymphocytes, which are lost very early during acute infection, was ten- to 100-fold lower than in CD57<sup>+</sup> and CD57<sup>+</sup> memory CD4<sup>+</sup> T lymphocytes. We observed that despite rapid, persistent loss after HIV-1 infection, pDCs represented a non-negligible HIV-1 DNA reservoir. CD16<sup>+</sup> proinflammatory cDCs and monocytes accumulated gradually, and HIV-infected CD16<sup>+</sup> monocytes contained higher HIV-1 DNA loads than their CD16<sup>−</sup> counterpart during acute infection. During chronic infection, CD16<sup>+</sup> cDCs exhibited higher HIV-1 DNA loads than the CD16<sup>−</sup> population. Overall, our results demonstrate that non-T cell compartments are a major HIV-1 DNA reservoir, and CD16<sup>+</sup> monocytes and CD16<sup>+</sup> cDCs potentially play an important role in HIV-1 dissemination. *J. Leukoc. Biol.* 89: 785–795; 2011.

## Introduction

The earliest phases of HIV-1 infection, which include early innate and adaptive immune responses, have a profound impact

on the long-term disease outcome [1, 2]. Acute infection with HIV-1 is characterized by a rapid and high burst in viremia, invariably between 10<sup>5</sup> and 10<sup>7</sup> RNA copies/mL, which declines over the ensuing weeks, coinciding with the resolution of symptoms [3]. The viral set-point reached following the induction of HIV-1-specific immune responses is associated with disease progression and outcome [4, 5]. This short window from time of transmission to resolution of peak viremia enables HIV-1 to infect a large number of leukocytes, causing damage to the immune system that becomes apparent later in infection.

Pools of memory/activated CD4<sup>+</sup> T cells are preferentially infected and destroyed during the early stages of acute infection with an associated reduction in cell numbers [6, 7]. The viruses transmitted and found early in HIV-1 infection predominantly use the CCR5 coreceptor, a CC-chemokine receptor expressed at higher density on CD4<sup>+</sup> T cells from infected individuals in comparison with healthy, control individuals [8]. As the GALT contains a high proportion of activated CD4<sup>+</sup>CCR5<sup>+</sup> T cells, it is believed that the majority of early viral replication occurs in the GALT in HIV-1 infection of humans and SIV infection of nonhuman primates [9, 10], although LNs and bronchus-associated lymphoid tissue may also be a major location of viral replication [11]. Later, during chronic infection, an array of blood lymphocytes is infected with HIV-1, including CD4<sup>+</sup> T cells of the naïve and memory pools and to a lesser extent, CD8<sup>+</sup> T cells [12, 13].

In addition to T lymphocytes, HIV-1 interacts with a large array of other cell types of the innate immune system. cDCs have been postulated to play a role in viral transmission at mu-

Abbreviations: APC=allophycocyanin, cDC=conventional DC, DC-SIGN=DC-specific ICAM-3-grabbing nonintegrin, pDC=plasmacytoid DC, WB=Western blot

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cosal surfaces, where HIV-1 can be captured and transferred to CD4<sup>+</sup> T cells locally or after migration to the LNs (reviewed in ref. [14]) through a *trans*-infection mechanism [15] or through *cis*-infection, where the cDCs are themselves infected and producing virus (reviewed in ref. [14]). pDCs, which express CD4, CXCR4, and CCR5, are susceptible to HIV-1 infection [16–18] and produce IFN- $\alpha$  upon encountering HIV-1, which inhibits viral replication and stimulates other immune cells [19]. An inverse correlation between blood pDC counts and the HIV-1 viral load during the chronic stage of infection has been reported previously [20]. Similar to cDCs, pDCs can transmit HIV-1 to CD4<sup>+</sup> T cells with a preference for antigen-stimulated CD4<sup>+</sup> T cells, which include HIV-1-specific T cells [21]. Last, monocytes and macrophages can also be productively infected with HIV-1, despite their intrinsic antiviral activity (reviewed in ref. [22]), and their susceptibility to HIV-1 infection has been shown to correlate with CCR5 cell surface expression [23, 24]. Within the pool of CD11c<sup>+</sup>CD14<sup>+</sup> monocytes, a CD16<sup>+</sup> subset with proinflammatory capacity has been shown to be more permissive to HIV-1 infection than their CD16<sup>−</sup> counterpart, to preferentially harbor HIV-1 in vivo, and to transfer virus to resting CD4<sup>+</sup> T cells [25–27].

In this study, we hypothesized that the earliest events occurring during the acute phase of HIV-1 infection not only affect the adaptive arm but also components of the innate arm of the immune system. Using samples from a cohort of HIV-1-infected patients, we report that the pDCs are depleted from the blood from the earliest stage of HIV-1 infection as naive CD4<sup>+</sup> T cells and that CD16<sup>+</sup> monocytes and cDCs preferentially accumulate during the course of the infection. Overall, CD57<sup>−</sup> memory CD4<sup>+</sup> T cells represent the major cell subset contributing to the HIV-1 DNA load in blood during the acute stage, but non-T cell targets of HIV-1 infection become more important HIV-1 DNA reservoirs as infection progresses, and pDCs, CD16<sup>+</sup> cDCs, and CD16<sup>+</sup> monocytes are particularly effective carriers of HIV-1 DNA load.

## MATERIALS AND METHODS

### Study subjects

Twenty-four subjects (men and women,  $\geq 18$  years old) diagnosed with clinical symptoms of acute HIV-1 infection were recruited for this study (Supplemental Table 1). Women with adequate contraception were included, whereas pregnant and breastfeeding women were excluded. All of the subjects gave informed consent in compliance with the Academic Medical Center of the University of Amsterdam Medical Ethical Committee (Amsterdam, The Netherlands). No patients received antiretroviral therapy at the time of study entry.

Patients were enrolled based on detectable plasma HIV-1 RNA load, as measured with the bDNA 3.0 assay (Bayer Diagnostics, Berkeley, CA, USA) or the NucliSens ultrasensitive assay (BioMerieux, Boxtel, Netherlands). The samples were analyzed further using two assays: the HIV antigen/antibody Combo assay (Abbot AxSYM System, Wiesbaden, Germany), which detects the presence of p24 in the sample and/or anti-HIV antibody reactivity (referred to as ELISA), and the HIV Blot 2.2 assay (MP Diagnostics, Illkirch, France), which detects antibody reactivity against HIV proteins *env* (gp41, gp120, gp160), *gag* (p17, p24, p51, p55), and *pol* (p31, p39, p66) by WB. The patients were subsequently grouped according to the Fiebig score nomenclature [28] as follows: (Fiebig II/III) ELISA<sup>+</sup> WB<sup>−</sup> ( $n=4$ ); (Fiebig IV) ELISA<sup>+</sup> WB<sup>+/−</sup> (lacking reactivity against one or several HIV antigens, including p31;  $n=9$ ); and (Fiebig V/VI) ELISA<sup>+</sup> WB<sup>+</sup> with a documented negative HIV screening test in the preceding 180 days ( $n=11$ ). A group of chronically infected individuals, who were therapy-naïve or had not received therapy during the previous 3 years, was also included ( $n=10$ ; Supplemental Table 2). Last, blood cell populations were also analyzed in a group of HIV-1-negative subjects ( $n=11$ ).

### Blood sample collection

Approximately 65 mL blood was collected in BD Vacutainer™ CPT™ cell preparation tubes with sodium citrate (BD Biosciences, Franklin Lakes, NJ, USA). At the time of sample collection, plasma HIV-1 RNA was measured, and blood CD4<sup>+</sup> T cell, CD8<sup>+</sup> T cell, B cell, and monocyte counts were determined. After cell isolation, PBMCs were aliquoted, frozen in RPMI medium, supplemented with 40% DMSO and 15% FCS, and eventually stored at  $-150^{\circ}\text{C}$  until further use. Plasma samples were aliquoted and stored at  $-80^{\circ}\text{C}$ .

### FACS and flow cytometry analysis

Freshly thawed PBMCs were analyzed and sorted using mAb against human cell surface markers. All washings and reagent dilutions were performed using PBS containing 2% FCS and 0.02% sodium azide. The stained cells were fixed with 1% PFA for at least 30 min prior to cell sorting. All cell subsets were isolated with a FACS-ARIA cell sorter (BD Biosciences), following sorting strategies depicted in Supplemental Fig. 1. For T cell subsets (Table 1), anti-human mAb, targeting the following cell surface markers, were used: CD57 (FITC-labeled, clone HNK-1), CD45RO (PE, clone UCHL1), CD4 (PerCP-Cy5.5, clone SK3), CD3 (PE-Cy7, clone SK7), and CD8 (APC-Cy7, clone SK1; BD Biosciences) and CD27 (APC, clone 0323; eBioscience, San Diego, CA, USA). For monocytes and cDC and pDC subsets (Table 2), we used mAb against blood DC antigen 2 (FITC, clone AC144; Miltenyi, Bergisch Gladbach, Germany) and CD16 (PE, clone 3G8), CD33 (PerCP-Cy5.5, clone P67.6), CD14 (PE-Cy7, clone M5E2), CD11c (APC, clone S-HCL-3), CD3, and CD19 (APC-Cy7, clones SK7 and SJ25C1; BD Biosciences). Post-sort analyses were performed to check for purity of the sorted cell populations, which were typically above 95%. The main contaminating cells were found to be CD8<sup>+</sup> T lymphocytes and B cells, which do not impact the HIV-1 DNA load measurements [12]. We compared the degree of purity with the HIV-1 DNA loads for all fractions, and no correlations were found (Supplemental Fig. 2). When the number of harvested PBMCs was limited, we only performed the T cell sort. Consequently, samples from one Fiebig II/III, two Fiebig IV, one Fiebig V/VI, and one chronic patient were excluded from the monocytes and cDC and pDC

TABLE 1. Cell Surface Markers and T Cell Phenotype

T cell subset	Phenotype					
	CD3	CD4	CD8	CD45RO	CD27	CD57
Naive CD4 <sup>+</sup> T cells	+	+	−	−	+	−
CD57 <sup>−</sup> memory CD4 <sup>+</sup> T cells	+	+	−	+	+/−	−
CD57 <sup>+</sup> memory CD4 <sup>+</sup> T cells	+	+	−	+/−	−	+

**TABLE 2. DC and Monocyte Phenotype**

DC and monocyte subset	Phenotype				
	CD33	CD14	CD11c	BDCA2	CD16
pDC	low/–	–	–	+	–
CD16 <sup>–</sup> monocytes	+	++	+	–	–
CD16 <sup>+</sup> monocytes	+	+	+	–	+
CD16 <sup>–</sup> cDC	+	–	+	–	–
CD16 <sup>+</sup> cDC	low	–	+	–	+

quantitative PCR analysis. The frequencies obtained from the flow cytometry analysis were used to calculate the number of cells per fraction based on the clinical leukocyte counts/ $\mu$ L blood.

### Real-time PCR HIV-1 DNA quantification

The intracellular HIV DNA viral load was quantified in each sorted cell fraction as published previously [29]. In brief, cells were resuspended in Tris-EDTA (10 mM, pH 8.3) containing 0.5 unit/ $\mu$ L proteinase K (Roche Applied Science, Switzerland) and used directly for PCR amplification after incubation (1 h at 56°C and 10 min at 95°C). The HIV-1 DNA was detected with a semi-nested real-time PCR assay. A 15-cycle preamplification step only amplified fully transcribed viral DNA, using 5' and 3' primers located in the 5' LTR U3 region and the *gag* gene, respectively. The pre-amplified product was quantified subsequently by real-time PCR amplification that was performed in duplicate, as described previously [29]. The standard used was a linear plasmid DNA containing the viral genome region targeted by the assay and was also subjected to the pre-amplification step. The detection limit for the assay was set at five DNA copies/input, although the assay could detect as few as one copy/input. Cells ( $5 \times 10^4$ ) of each cellular fraction were subjected to this semi-nested real-time PCR assay. For some fractions (pDC, CD57<sup>+</sup> memory CD4<sup>+</sup> T cells, and cDC fractions), the number of cells recovered after cell sorting was low, and less than  $5 \times 10^4$  cells were used for the semi-nested real-time PCR assay. All values were normalized for  $10^5$  cells. The cut of values shown in figures was set to be equal for all fractions to visualize between the negative and positive fractions for HIV-1 detection. Distinction between fractions, susceptible or not to HIV-1, was clear. The positive fractions were always higher than 50 copies/ $10^5$  cells, and adjustment of the cut-off, as a result of varied cell numbers' isolated values, will not induce positive fractions to become negative.

### Statistical analyses

Data were subjected to the Mann-Whitney U test analysis for comparisons of two groups, as implemented by Graphpad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). For multiple comparisons, the Kruskal-Wallis analysis was applied as described by Conover [30], including a "Bonferroni"-like correction for multiple testing. The statistical analysis comparing HIV-1 DNA loads in cell subsets was performed on the whole study population (i.e., negative and positive fractions), and the median values of only the positive fractions are depicted in the figures to allow for easier comparisons to be made between specific cellular subsets once found HIV-1-infected. The obtained *P* values were considered significant with \* when  $P < 0.05$ , \*\* when  $P < 0.01$ , and \*\*\* when  $P < 0.001$  (see figures).

## RESULTS

### Differential dynamics of T cell subsets during HIV-1 infection

We identified 24 HIV-1 individuals in the acute stage of HIV-1 infection, which were divided into three subgroups based on

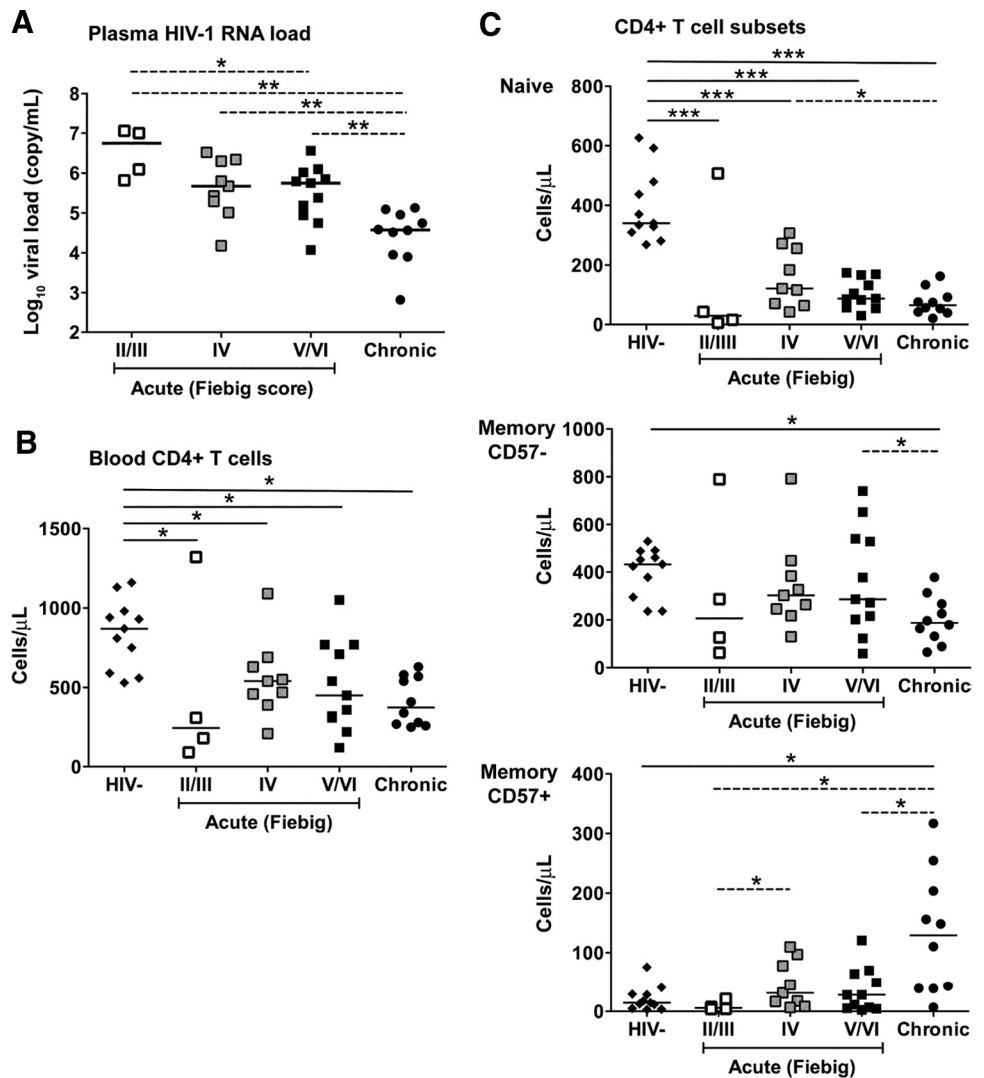
their HIV-1 antigen and antibody status, according to the Fiebig score nomenclature [28]. These patients were compared with HIV-negative and chronically infected individuals. All acutely infected individuals exhibited a significantly higher HIV-1 RNA plasma load when compared with the chronically infected group, with a gradual decrease between the different acute infection stages (Fig. 1A), indicating that the patients were categorized correctly.

We first validated our technical approach by analyzing the CD4<sup>+</sup> T cell population at various stages of HIV-1 infection. As expected, the absolute count and frequency of CD4<sup>+</sup> T cells in the blood dropped in the HIV-1-infected patients as compared with HIV-negative individuals (Fig. 1B and Supplemental Fig. 3A). This decrease occurred early after infection, as it was already apparent in the Fiebig II/III group, and was maintained during the complete course of the infection, confirming that the loss of CD4<sup>+</sup> T lymphocytes is fast and persistent. The percentage and number of CD8<sup>+</sup> T lymphocytes increased to reach 60–80% of blood T cells in the HIV-1-infected subjects (Supplemental Fig. 3A and B), and the blood CD4/CD8 T cell ratio inverted at all stages of infection (Supplemental Fig. 3C).

Although a global CD4<sup>+</sup> T cell drop was observed immediately following onset of HIV-1 infection, we questioned whether discrete subpopulations might be affected differentially over time. The CD4<sup>+</sup> T lymphocytes were subdivided into naïve and memory fractions based on surface expression of CD45RO, CD27, and CD57 (Table 1), as described previously [12]. The absolute count and frequency of naïve CD4<sup>+</sup> T cells decreased significantly by half in the infected individuals as compared with the non-infected group (Fig. 1C and Supplemental Fig. 4A). The memory CD4<sup>+</sup> T cell compartment was divided according to the surface expression of CD57, which marks terminally differentiated effector T cells [12]. The number of blood CD57<sup>–</sup> memory CD4<sup>+</sup> T cells was relatively stable at all stages of acute infection but dropped by half during chronic infection, as compared with the non-infected individuals (Fig. 1C and Supplemental Fig. 4B). In contrast, the number of CD57<sup>+</sup> effector memory CD4<sup>+</sup> T lymphocytes increased gradually during HIV-1 infection, culminating in chronically infected patients (Fig. 1C and Supplemental Fig. 4C), which is in line with previous findings [31, 32].

### Levels of HIV-1 DNA load in T cell subsets during HIV-1 infection

Having confirmed that the dynamics of the various T cell subsets are differentially affected during the course of HIV-1 infection, we next assessed whether these differences correlated with differential susceptibility to HIV-1 infection. We sorted naïve, CD57<sup>–</sup> memory and CD57<sup>+</sup> memory CD4<sup>+</sup> T cell fractions from the blood of acute and chronic patients (Table 1 and Supplemental Fig. 1A) and measured their respective intracellular HIV-1 DNA load using a quantitative PCR assay. A large majority (>90%) of sorted CD57<sup>–</sup> and CD57<sup>+</sup> memory CD4<sup>+</sup> T cell fractions was HIV<sup>+</sup>, and most of the patients also had detectable levels of HIV-1 DNA in the naïve CD4<sup>+</sup> T cell fraction at all stages of infection. Naïve CD4<sup>+</sup> T cells isolated from acute and chronic patients exhibited similar HIV-1 DNA



**Figure 1. Plasma HIV-1 RNA load and blood T cell counts during HIV-1 infection.** (A) Plasma RNA viral load (copy/mL) is shown for the acute infection (grouped according to Fiebig nomenclature) and chronic infection patients. (B) The blood counts of total CD4<sup>+</sup> T lymphocytes are shown for HIV-negative and -positive individuals. (C) Similarly, the blood counts of naïve (top), CD57<sup>-</sup> memory (middle), and CD57<sup>+</sup> memory CD4<sup>+</sup> T cells (bottom) were determined separately.

loads, and ~0.01% of the cells was infected (Fig. 2A). The plasma HIV-1 RNA viral loads and the naïve CD4<sup>+</sup> T cell HIV-1 DNA loads did not correlate during acute infection but showed a significant correlation in chronic patients (Fig. 2A). The infection levels in the CD57<sup>-</sup> memory CD4<sup>+</sup> T cell fraction were also similar at all stages of infection but at a higher rate than naïve CD4<sup>+</sup> T cells, and ~1.0% of the cells was infected (Fig. 2B). A positive correlation between HIV-1 DNA loads and plasma RNA viral loads was only observed during the chronic phase of infection, as shown previously [12]. In contrast, the HIV-1 DNA load in the CD57<sup>+</sup> memory CD4<sup>+</sup> T cell fraction decreased gradually during infection, and the highest levels were observed in the Fiebig II/III group (~10% infected cells). A positive correlation with plasma RNA viral loads was observed during acute infection (Fig. 2C). Of note, similar HIV-1 DNA load measurements in the CD4<sup>+</sup> T cell fractions during the chronic phase of the infection were reported previously [12].

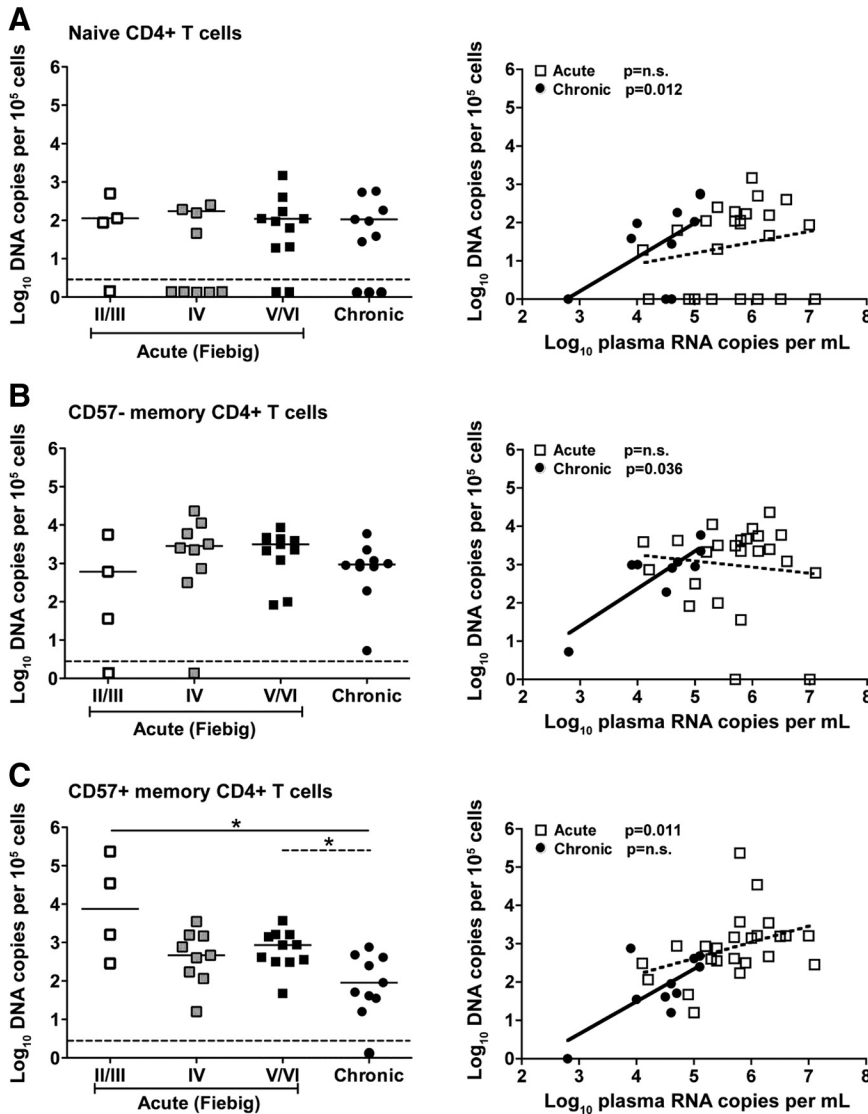
Overall, our results confirm that T cell dynamics are quickly altered after HIV-1 infection, and CD57<sup>+</sup> memory CD4<sup>+</sup> T

cells are infected at the highest rate early (Fiebig II/III) during the acute phase of infection.

### Cellular dynamics of monocytes and DC subsets during HIV-1 infection

As the impact of acute HIV-1 infection on cell subsets of the innate arm of the immune response has not yet been fully elucidated, we focused on the analysis of monocyte, cDC, and pDC subsets (Table 2) during the different stages of HIV-1 infection. As compared with non-infected individuals, the number of monocytes was moderately enhanced during the acute phase of infection only (Fig. 3A and Supplemental Fig. 5A). In contrast, the cDC subset exhibited an opposite profile, and the absolute counts and percentages of cDCs decreased during all stages of acute infection and eventually came back to control levels during the chronic stage of infection (Fig. 3A and Supplemental Fig. 5B). The numbers as well as percentages of pDCs dropped early after infection (Fiebig II/III group) and persisted at a low level





**Figure 2. HIV-1 DNA load in T cell subsets.** The HIV-1 DNA load was measured in the sorted CD4<sup>+</sup> T cell subsets by quantitative PCR. (A) The naïve CD4<sup>+</sup> T cell HIV-1 DNA loads were determined for each stage of infection (left). All symbols located under the dotted line correspond to HIV-negative fractions. The HIV-1 DNA load was correlated with the plasma RNA viral load for the fractions isolated during acute (pooled groups; dotted line) and chronic (solid line) infection (right). The median value (horizontal bars) is calculated from the HIV-1<sup>+</sup> fractions only. A similar analysis was performed for (B) CD57<sup>-</sup> and (C) CD57<sup>+</sup> memory CD4<sup>+</sup> T lymphocytes.

during the entire course of HIV-1 infection (Fig. 3A and Supplemental Fig. 5C) [33].

The monocyte and cDC subsets were separated further according to the expression of CD16, which has been shown previously for monocytes to correlate with a proinflammatory state and higher susceptibility to HIV-1 infection [26]. Only CD16<sup>+</sup> monocytes significantly accumulated in the blood of infected individuals (Fig. 3B), eventually representing ~15% of all monocytes at the chronic stage, as compared with ~5% in noninfected individuals ( $P < 0.05$ ; Fig. 3C). Similarly, although CD16<sup>-</sup> and CD16<sup>+</sup> cDC counts were reduced severely early after infection (Fiebig II/III group), only CD16<sup>+</sup> cDCs accumulated gradually back to control values at the chronic stage (Fig. 3D). The CD16<sup>+</sup> cDC population eventually represented ~75% of all cDCs at the chronic stage, as compared with ~60% in non-infected individuals (Fig. 3E).

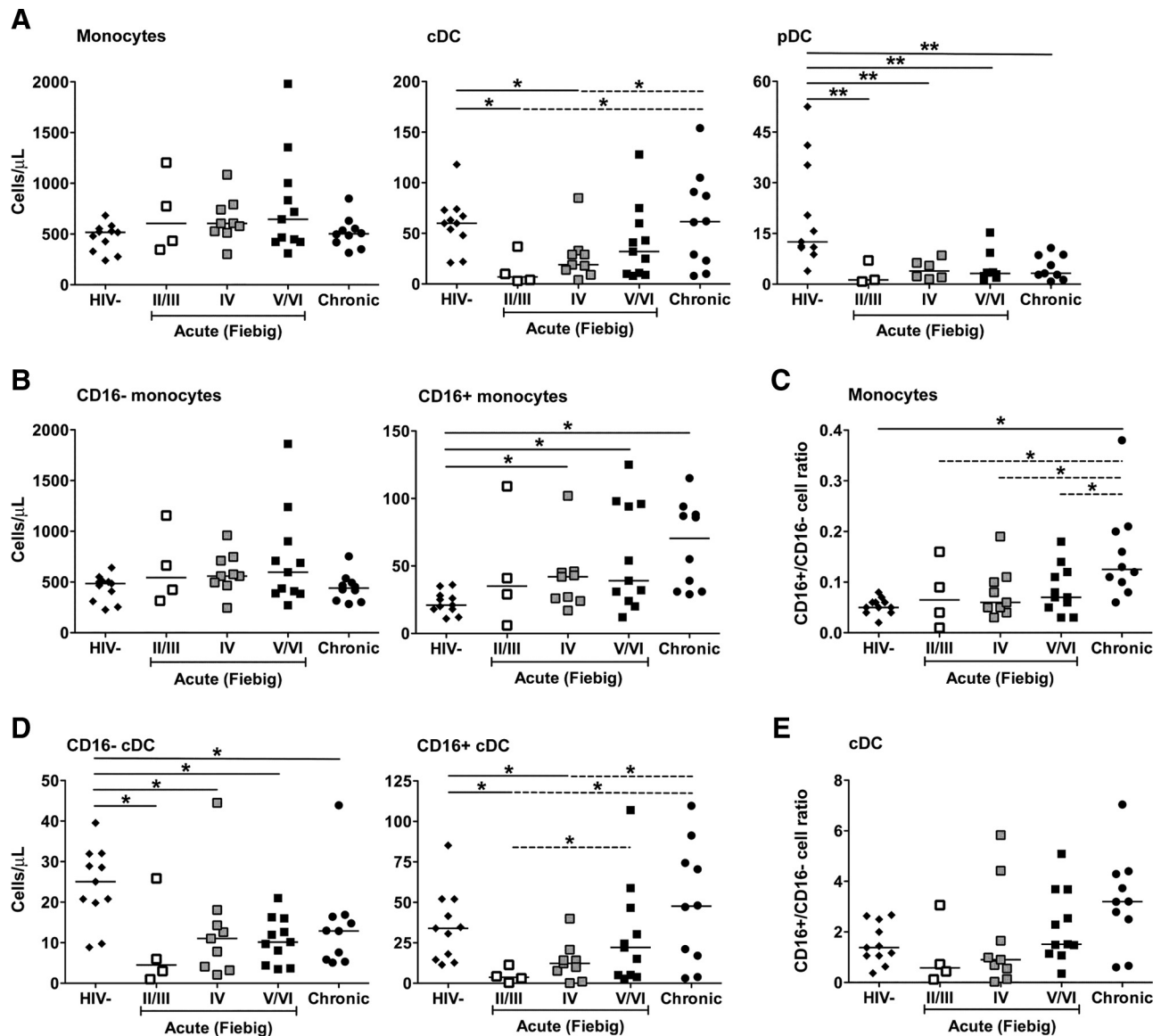
Overall, our results show that monocytes and DC blood subsets are significantly affected during HIV-1 infection, with a rapid, persistent disappearance of the pDC subset and a long-

term accumulation of proinflammatory CD16<sup>+</sup> monocytes and to a lesser extent, CD16<sup>+</sup> cDCs.

### HIV-1 infection levels in the monocytes and DC subsets

As pDC, monocyte, and cDC subsets were differentially affected during HIV-1 infection, we hypothesized that their respective level of infection may differ. After having sorted pDC and monocyte (CD16<sup>-</sup> and CD16<sup>+</sup>) and cDC (CD16<sup>-</sup> and CD16<sup>+</sup>) fractions from the blood of acute and chronic patients (Table 2 and Supplemental Fig. 1B), we measured their relative HIV-1 DNA content.

The proportion of individuals harboring detectable HIV-1 DNA loads in the CD16<sup>-</sup> and CD16<sup>+</sup> monocyte fractions was higher during acute infection (50–61%) as compared with the chronic stage (33–44%; Fig. 4A). Monocyte fractions with detectable HIV-1 DNA loads contained a number of DNA copies, reaching levels comparable with the CD57<sup>-</sup> memory CD4<sup>+</sup> T cell population (0.5–5% infected cells; Fig. 4A). Of note, for the patients with a detectable HIV-1 DNA load, the HIV-1<sup>+</sup>

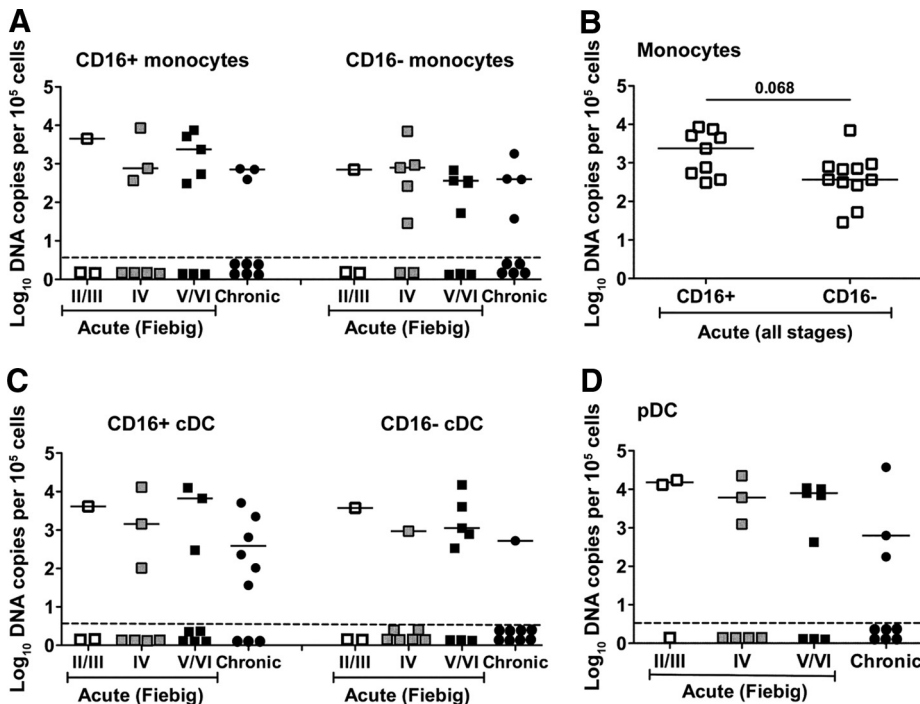


**Figure 3. Dynamics of monocytes and DC subsets during infection.** (A) Absolute counts of peripheral blood monocytes (left), cDCs (middle), and pDCs (right) were determined for acute and chronic infection patients, as compared with HIV-negative individuals. (B) Absolute counts of CD16<sup>-</sup> (left) and CD16<sup>+</sup> (right) monocytes in the blood of noninfected and acute- and chronic-infected individuals are shown. (C) The CD16<sup>+</sup>/CD16<sup>-</sup> monocyte ratio is given at every stage of infection, as compared with HIV-negative individuals. (D and E) A similar analysis was performed for the cDC population.

CD16<sup>+</sup> monocyte fractions showed higher HIV-1 DNA loads during acute infection than those measured in the CD16<sup>-</sup> fractions (Fig. 4B), and no differences were observed at the chronic stage of infection. In contrast, whereas a similar frequency of HIV-1<sup>+</sup> CD16<sup>-</sup> and CD16<sup>+</sup> cDC fractions was observed during acute infection (39%), a clear dichotomy was obtained with samples from chronic patients, with a higher proportion of HIV-1<sup>+</sup> CD16<sup>+</sup> cDCs (67%) than HIV-1<sup>+</sup> CD16<sup>-</sup> cDCs (11%; Fig. 4C). The HIV-1<sup>+</sup> cDC fractions also harbored HIV-1 DNA loads comparable with those of CD57<sup>+</sup> memory CD4<sup>+</sup> T cells, with no striking differences between the various stages of infection (Fig. 4C). The frequency of HIV-1<sup>+</sup> pDC

fractions was similar to what we observed for the monocyte fractions (Fig. 4D). The HIV-1 DNA load of the pDC fractions was comparable with the relatively high levels obtained from the CD57<sup>+</sup> memory CD4<sup>+</sup> T cells, with 1–10% infected cells and a trend toward higher HIV-1 DNA loads at the earliest stages of infection (Fig. 4D).

Collectively, our results indicate that monocytes, cDCs, and pDCs are not systematically found to harbor HIV-1 DNA. Still, when it is HIV-1<sup>+</sup>, the HIV-1 DNA load in these subsets is equivalent to what is observed with memory CD4<sup>+</sup> T cell subsets, and the highest level of infection is observed in pDCs at the Fiebig II/III stage of acute infection.



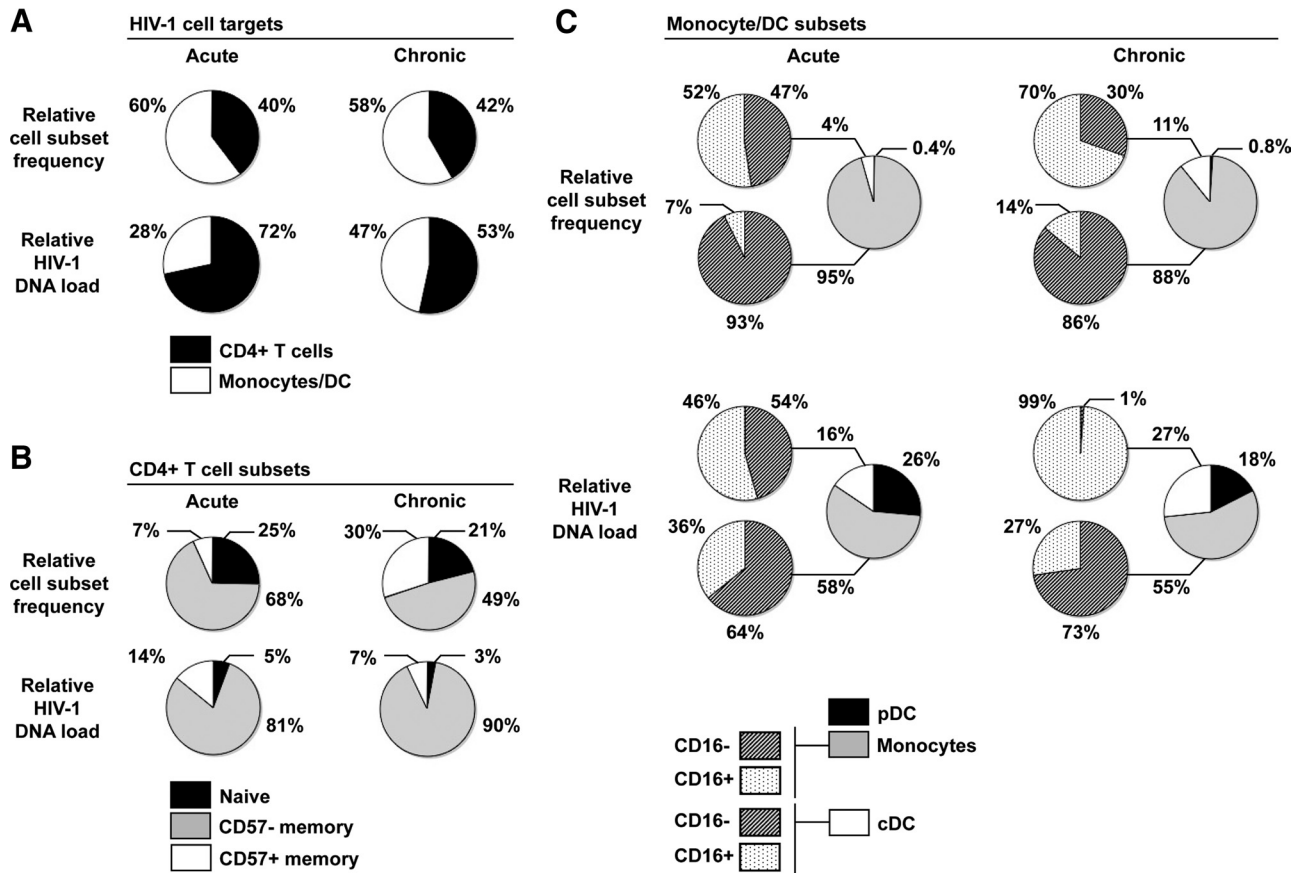
**Figure 4. HIV-1 DNA load in monocytes and DC subsets.** The HIV-1 DNA load was measured in the sorted monocyte, cDC, and pDC subsets by quantitative PCR. (A) The HIV-1 DNA loads of the CD16<sup>+</sup> and CD16<sup>-</sup> monocyte fractions were determined at all infection stages, as in Fig. 2B. (B) A comparison of the HIV-1 DNA loads of pooled HIV-1<sup>+</sup> CD16<sup>+</sup> and pooled HIV-1<sup>+</sup> CD16<sup>-</sup> monocyte fractions during acute infection is also shown for the patients with detectable HIV-1 DNA loads in these fractions. Similarly, the HIV-1 DNA loads measured from (C) cDC and (D) pDC fractions at all stages of the infection are shown.

## DISCUSSION

In this study, we provide an extensive temporal analysis of the alterations in specific cellular subsets from the innate and adaptive arms of the immune system during acute and chronic HIV-1 infection. As compared with other human studies, our results provide a global picture of cell dynamics during HIV-1 infection. Among the pool of potential cellular targets of HIV-1 infection found in the blood, CD4<sup>+</sup> T cells represent the largest pool of HIV-1 DNA reservoir during acute (72%) and chronic (53%) infection, although they only account for ~40% of these cellular targets (Fig. 5A). Among CD4<sup>+</sup> T cells, the CD57<sup>-</sup> memory subset is the major contributor to the total HIV-1 DNA load (81% at the acute stage; 90% at the chronic stage), despite the fact that it represents half of the CD4<sup>+</sup> T cells found in chronic infection (Fig. 5B). In other words, naive CD4<sup>+</sup> T cells and CD57<sup>+</sup> memory CD4<sup>+</sup> T cells, also described as terminally differentiated effector cells, only provide a minor contribution to the global blood HIV-1 DNA load.

It has been reported that massive infection of the mucosal CD4<sup>+</sup> T lymphocytes, which are mainly effector-memory CD4<sup>+</sup> T cells, occurs in the first weeks following infection and parallels the decrease of plasma RNA viral load [7, 9, 34]. Accordingly, we report here a positive correlation between the CD57<sup>+</sup> memory CD4<sup>+</sup> T cell HIV-1 DNA load and the plasma viral load during acute infection (Fig. 2C). Effector-memory CD4<sup>+</sup> T cells are an essential component of the mucosal, bronchial, and vaginal immune response and must be replenished to maintain immune function [35]. The effector-memory CD4<sup>+</sup> T cells intrinsically have a limited regenerative capacity and have to be generated continuously via proliferation, differentiation, and/or emigration of naive and memory CD4<sup>+</sup> T cell pools [36]. As a result of increased proliferation and im-

pairment of their own regenerative capacity [1], the naive and memory CD4<sup>+</sup> T cell compartments diminish during HIV-1 infection, as demonstrated here (Fig. 1C). Of note, naive and CD57<sup>-</sup> memory CD4<sup>+</sup> T lymphocytes are targets of HIV-1 infection, suggesting that these subsets can be a major source of virus production during chronic infection. Consistent with this idea, we observed during chronic HIV-1 infection a positive correlation between the HIV-1 DNA loads and the plasma RNA viral loads for naive and CD57<sup>-</sup> memory CD4<sup>+</sup> T lymphocytes (Fig. 2A and B). Moreover, it should be noted that similar HIV DNA load measurements in the CD4<sup>+</sup> T cell fractions during the chronic phase of the infection were reported previously [12]. Early during acute HIV-1 infection, the naive CD4<sup>+</sup> T cell population declines from blood circulation (Fig. 1C) and is infected with HIV-1 (Fig. 2A) at a stage where CCR5-tropic viruses are known to dominate, and virus genotypes are extremely homogenous, with limited quasi-species circulating [37]. In agreement with this observation, we previously showed a lack of divergence between viral envelope sequences, amplified from naive and memory CD4<sup>+</sup> T cell subsets, isolated from chronic patients infected with an array of HIV-1 subtypes, as well as infection of naive CD4<sup>+</sup> T cells with R5 signature viruses [29]. Overall, our results strongly support the notion that naive CD4<sup>+</sup> T cells can be infected with CCR5-tropic viruses during the acute stage of HIV-1 infection. This could reflect low expression of CCR5 on a proportion of naive CD4<sup>+</sup> T cells, including those stimulated recently with HIV-1 antigen, which should be abundant during this period [38]. However, HIV-1 enters naive CD4<sup>+</sup> T cells less efficiently than memory CD4<sup>+</sup> T cells [39], which may explain their lower HIV-1 DNA load and the lack of infected, naive CD4<sup>+</sup> T cells in some patients.



**Figure 5. Schematic view of temporal dynamics of HIV-1 cellular reservoir in blood.** (A) The relative frequency of CD4<sup>+</sup> T cells (black areas) and other HIV-1 target cells (monocytes, cDCs, pDCs, white areas) in blood is shown for acute (left) and chronic (right) infection (upper pie charts). The relative HIV-1 DNA load of these two populations is given (lower pie charts). (B) A similar analysis was performed within the CD4<sup>+</sup> T cell subset to distinguish among naive (black), CD57<sup>-</sup> memory (gray), and CD57<sup>+</sup> memory (white) CD4<sup>+</sup> T cells. (C) The same detailed analysis was performed for pDCs (black), monocytes (gray), and cDCs (white). Monocytes and cDCs were subdivided further between CD16<sup>-</sup> (hatched areas) and CD16<sup>+</sup> (dotted areas) populations. The relative cell population frequencies were derived from the number of cells/ $\mu$ L blood shown in Figs. 1 and 3. The relative HIV-1 DNA load between the various cell populations was calculated using the normalized values shown in Figs. 2 and 4 and correcting for the relative cell population frequencies.

CD4<sup>+</sup> T lymphocytes have been the major focus in HIV-1 pathogenesis studies, but cells from the innate immune system have also revealed their potential importance as carriers and transmitters of infection [40]. Our results illustrate the fact that monocytes, cDCs, and pDCs are also important contributors to the peripheral blood HIV-1 DNA load (Fig. 5C), especially at the chronic stage of infection, where they harbor approximately half of the total values (Fig. 5A). Interestingly, the relative contribution of cDCs and pDCs was relatively high, considering their rarity among blood leukocytes (Fig. 5C) and the persistent depletion of pDCs from blood (Fig. 3A). Last, the CD16<sup>+</sup> fraction of both monocytes and cDCs, was also infected more effectively by HIV-1, and >98% of all cDC HIV-1 DNA loads was detected in the CD16<sup>+</sup> cDC fraction at the chronic stage of infection (Fig. 5C). Monocytes/macrophages and cDCs play a dual role during HIV-1 infection, as they provide immune functions required to mount a beneficial response against HIV-1 but serve at the same time as an infected reservoir that can disseminate the virus. Here, we observed a

trend toward a moderate increase in monocyte numbers in the blood during acute infection, whereas cDCs were found to be depleted from the blood. At the chronic stage, both populations eventually returned to levels similar to those observed in HIV-negative individuals (Fig. 3A). In the SIV-macaque model, a similar increase in the blood monocyte counts was observed during acute infection, which was attributed to enhanced production and emigration from the bone marrow [41]. This increase in the blood monocyte counts during acute infection may also be a consequence of an increased resistance to apoptosis of monocytes induced by the virus [42]. The depletion of cDCs from blood circulation could be a result of homing to mucosal or lymphoid tissues. During acute infection, DC-SIGN<sup>+</sup> and CD40<sup>+</sup> cDCs have been shown to accumulate in peripheral LNs [43], whereas the proportion of cDCs in the spleen was similar in chronic patients and non-infected individuals [44], as reported here in the periphery (Fig. 3A). These observations suggest that in contrast to acute infection, cDCs are not majorly recruited to lymphoid tissues during



chronic infection. Alternatively, cDCs may be infected and depleted during the early stages of infection.

Although monocytes and cDCs exhibit opposite dynamics in blood, both populations demonstrate a marked increase of their respective CD16<sup>+</sup> subset (Fig. 3B–E), in agreement with previous studies in macaques and humans [25, 45]. CD16<sup>+</sup> monocyte and cDC subsets are described as proinflammatory cells producing TNF- $\alpha$  in response to bacterial endotoxin LPS [25, 46]. Bacterial translocation has been proposed to occur soon after HIV-1 infection as a consequence of damaged mucosa, resulting in LPS exposure beyond the mucosal barrier [47]. Such a mechanism, in combination with an altered cytokine environment observed during acute infection, could explain for the recrudescence of the CD16<sup>+</sup> subsets in the course of infection. Although it was suggested previously that the CD16<sup>+</sup> subsets are more susceptible to HIV-1 infection in HIV-positive patients during chronic infection [26, 48], we observed that the CD16<sup>+</sup> and CD16<sup>−</sup> cell fractions in monocytes and cDCs were infected to similar HIV-1 DNA load values at this stage of infection (Fig. 4). In contrast, when HIV-1 was detected, the CD16<sup>+</sup> monocytes isolated during acute infection were found to harbor higher HIV-1 DNA loads than their CD16<sup>−</sup> counterparts, suggesting that this cell population may play a key role in virus dissemination during the very early stages of infection. Of note, we observed that monocyte fractions, once found infected, exhibited similar HIV-1 DNA load than CD57<sup>−</sup> memory CD4<sup>+</sup> T cells. These results may differ from the data from Zhu et al. [40], as the type of cells analyzed is different (i.e., resting and activated CD4<sup>+</sup> T cells vs. naïve and memory CD4<sup>+</sup> T cells). However, there are clearly similarities between our two studies, as the monocyte fractions do not necessarily have detectable HIV-1 DNA loads as compared with the CD4<sup>+</sup> T cell fractions. In contrast, CD16<sup>+</sup> cDCs were infected in a higher proportion of patients during chronic infection as compared with the CD16<sup>−</sup> cDC fraction. The CD16<sup>+</sup> cDC subset partially overlaps with blood DC-SIGN<sup>+</sup> cDCs and may thus play a pivotal role in promoting HIV infection by transfer to CD4<sup>+</sup> T lymphocytes [49]. In conclusion, the CD16<sup>+</sup> subset differs in HIV-1 infectivity profile between monocytes and cDCs, as well as during the course of HIV-1 infection, possibly as a result of factors, such as changes in cellular activation through the different TLRs [50] or a differential response to the cytokine environment in which the cells reside.

The persistent loss of pDCs from blood circulation observed in our cohort of patients from the earliest stage of acute infection (Fig. 3A) is in agreement with previous studies in humans and macaques [51]. Similar to cDCs, the loss of pDCs from the blood may be a result of homing to lymphoid tissues after HIV-induced activation [52], but it may also reflect direct pDC destruction by HIV-1 cytopathic effects or by the induction of apoptosis through overstimulation. In the SIV-macaque model, pDCs are lost during acute infection from the peripheral blood and accumulate in the LNs, where they produce high levels of IFN- $\alpha$  [53], but this massive recruitment has been shown to correlate rapidly with induction of pDC apoptosis and necrosis [54]. We also observed that a large proportion of acute infection patients contained HIV<sup>+</sup> pDCs with a relatively

high HIV-1 DNA load (Fig. 4D), which suggests that pDCs may play a critical role early in infection.

Our results demonstrate which lymphoid and myeloid cell types are infected with HIV-1 early and late in acute infection; however, given the DNA assay used, we cannot distinguish between unintegrated and integrated DNA forms. From the literature, it is apparent that cells of the myeloid lineage likely possess less-integrated DNA than cells of lymphoid origin [55]. However, whereas monocytes from uninfected subjects are largely refractory to HIV-1 infection, *ex vivo* until macrophage differentiation, the presence of productive infection in circulating monocytes from infected persons has already been demonstrated, and infectious, proviral DNA has been isolated [40, 56–58]. Although integration has been shown to lead to higher levels of viral production, suggesting that the lymphoid cells contribute greater to plasma viral loads, there are implications to harboring high levels of HIV-1-infected myeloid cell populations. Circular HIV-1 DNA forms have been shown to reside in cells for long periods of time, potentially contributing to the overall viral reservoir [59, 60]. Gene transcription and translation from unintegrated DNA have also been shown to provide for Tat and Nef expression, which are both molecules with major physiological relevance concerning immune activation or immune recognition and cell destruction [61, 62]. The differential infection of myeloid cell populations, with limited integration, can still therefore hold consequences for HIV-1 infection and course of disease progression. Further analysis regarding which specific viral forms reside within each of the subsets will be able to address such questions. Additionally, it has been shown for resting T cells that reverse transcription and integration can be inhibited following infection, and again, it will be interesting to identify which replication forms are present within each lymphocyte subset [63, 64]. These HIV-1-infected but resting T cells with unintegrated forms may well progress to integrate when activated by specific cytokines, as has been shown *in vitro* [65], or conversely, the abortive HIV-1 DNA form may induce cell death and contribute to the perpetuation of the inflammation in tissue [66].

Overall, we show that HIV-1 infection strongly affects the cellular subsets from the innate arm of the immune system (monocytes, cDCs, and pDCs) from the earliest stages of acute infection. Relative to their respective cellular subset size, CD57<sup>−</sup> memory CD4<sup>+</sup> T cells, cDCs, and pDCs are the most effective contributors to the HIV-1 DNA load in the blood at all stages of the infection. Furthermore, the selective accumulation of CD16<sup>+</sup> monocytes and cDCs correlates with an increased capacity to support HIV-1 infection. It should be noted that the HIV-1 DNA PCR assay used in this study does not distinguish between productive and nonproductive infection with HIV-1, and it remains to be elucidated to what extent HIV-1<sup>+</sup> cells contribute to the overall viral production from these infected reservoirs. Furthermore, it is still to be determined whether early therapeutic interventions aiming at preserving innate immunity cell populations from the earliest stages of acute HIV-1 infection could have a long-term, beneficial outcome on disease.

## AUTHORSHIP

M.C. and N.L. designed and performed research, collected and analyzed data, and wrote the manuscript. R.S. and M.L.G. collected and analyzed the clinical data of the study subjects. R.v.d.S. performed research and collected data. M.B. and S.J. provided patient database information. B.B. analyzed data and wrote the manuscript. W.A.P. designed research, analyzed data, and wrote the manuscript. J.M.P. recruited the study subjects and designed research. G.P. initiated, designed, and performed research, collected and analyzed data, and wrote the manuscript.

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## KEY WORDS:

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