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# Defects in blood dendritic cell subsets in HIV-1 subtype C infected Indians

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***Background & objective*: DCs trigger both innate and adaptive immune responses to control HIV infection and represent a viral reservoir acting as target and HIV carriers for infection of permissive CD4$^+$ T-cells. DCs thus form a very attractive study subject to further our existing knowledge of HIV induced immunopathogenesis due to its diverse and crucial role in HIV infection establishment, viral dissemination, immune evasion, viral persistence, *etc*. We aimed to characterize the effect of HIV infection on myeloid and plasmacytoid dendritic cell subsets in a group of HIV-1 subtype C infected treated or untreated Indian individuals.**

***Methods*: Blood DC subset numbers and immunophenotype were studied for 79 HIV infected subjects at various stages of disease and compared with 13 HIV-uninfected controls. Comparisons were also made between groups of subjects based on their CD4$^+$ T cell counts and also experience of antiretrovirals.**

***Results*: Significant decreases were observed in blood DC counts and the two DC subsets in HIV infected individuals. Subjects with lowest CD4$^+$ T cell counts also had a drastically reduced DC subset pool which correlated positively with plasma viraemia and negatively with CD4$^+$ T cell counts. DC subsets from HIV infected subjects showed higher expression of co-stimulatory molecules CD40 and CD86, and HIV-1 co-receptors CXCR4 and CCR5 which correlated positively with HIV-1 plasma viraemia. The alterations in blood DCs were partly resolved in ART receiving study subjects.**

***Interpretation & conclusions*: Correlation between DC subset activation state and viraemia supports the role of DC activation on viral replication and CD4$^+$ T cell depletion.**

**Key words** Blood dendritic cell subsets - HIV-1 subtype C - immune activation - India - plasma viraemia

Dendritic cells (DCs), specialized antigen-presenting cells (APCs) in lymphoid and most nonlymphoid tissues, are critical for host immunity as these induce specific immune responses against a variety of pathogens[1]. Immature DCs in blood and tissues undergo highly regulated activation and maturation upon encountering invading pathogens or in the presence of pro-inflammatory cytokines[2]. The DC maturation results in increased surface expression of MHCs and co-stimulatory molecules CD40 and CD80/86 [3], and production of cytokines (IL-12, IFN-α)[4], thereby allowing efficient T cell activation. These mature DCs migrate to secondary lymphoid organs[5], where they present the pathogen derived antigens to antigen specific T cell and cause their activation and proliferation.

The human immunodeficiency virus-1 (HIV-1) infection is characterized by a progressive loss of $CD4^+$ T cells, chronic immune activation, and increased T cell exhaustion and apoptotic death, eventually rendering the patient susceptible to opportunistic viral and bacterial infections[6]. Several lines of evidence suggest the involvement of DCs in HIV dissemination and immunopathogenesis[7,8]. The DCs are amongst the first potential targets for HIV-1 during transmission, owing to their distinctive localization at mucosal surfaces like vagina and the gastrointestinal tract (GIT), coupled with their known proficiency in capturing antigens. DCs also express the receptors required for HIV-1 infection; CD4, and the most commonly used coreceptors CCR5 and CXCR4 [9], making them susceptible to infection by the virus.

The two distinct subsets of blood DCs, the CD11c expressing myeloid dendritic cells (mDCs), and the CD11c lacking plasmacytoid dendritic cells (pDCs) have distinct morphology and functions. On stimulation with invading pathogen, these show distinguishing cytokine secretion profile, and migration properties. Thus, the DC activation state and cytokine secretion profile can in turn determine the T-cell activation and immune response to invading virus[10,11]. The DCs, however, are themselves affected by the replicating virus, and studies have reported numerical as well as functional loss of blood DCs in HIV infected persons[12,13]. The DCs in HIV infected individuals also have a higher expression of activation markers CD40 and CD86, thereby contributing to the existent state of heightened immune activation.

We have previously reported the importance of immune activation in $CD4^+$ T cell depletion and disease progression during untreated HIV-1 infection, and have also documented the relation of immune activation with HIV-1 plasma viral load in a group of HIV-1 subtype C infected Indian individuals[14,15]. In this study, we aimed at delineating the influence of HIV infection on the number and activation state of blood dendritic cell subsets in HIV-1 subtype C infected Indian individuals. In addition, the effect of increasing HIV-1 plasma viraemia on the activation state of the DC subsets was examined. Also an attempt was made to determine restoration, if any, of the blood DC numbers and immunophenotype in presence of highly active antiretroviral therapy.

## Material & Methods

*Study population*: Seventy nine HIV infected individuals (62 males and 17 females, 58 therapy naïve, 21 on highly active antiretroviral therapy; HAART) at different stages of infection[16] were recruited from AIDS clinic of the Department of Microbiology at All India Institute of Medical Sciences (AIIMS), New Delhi from January 2007 to January 2009. Study population comprised all consecutive subjects who came to the clinic during the specified period and gave written informed consent for inclusion into the study. This clinic provides, HIV diagnostic facilities, pre- and post- test counselling, treatment and monitoring options to all patients referred to the clinic through various Out Patient Departments (OPDs), wards, and speciality clinics of AIIMS, and also to patients referred through any hospital or treatments centres across India. Based on clinical evaluation, 24 subjects were asymptomatic and thus in CDC stage A infection, 26 had CDC stage B infection, and 29 subjects had clinical AIDS[17]. The study was approved by the Institute Ethics Committee and all patients gave informed written consent prior to inclusion in the study. We also included 13 individuals (3 females and 10 males from the department staff and screened for acute illness and any other infection) negative for HIV-1/2 and for hepatitis B and C as healthy controls, which were screened for acute illness and any infection requiring medication.

*Sample collection*: Five ml of whole blood was collected from all study subjects by venipuncture in $K_3$EDTA vacutainer tubes (Becton Dickinson, USA). Plasma samples were stored at -70° C.

*Complete blood counts and CD4+ T cell enumeration*: Complete blood counts including total lymphocyte counts, and differential leucocyte counts were obtained from patient's records. For $CD4^+$ T cell enumeration, EDTA blood was processed according to manufacturer's instructions (Becton Dickinson) and the processed samples were analysed on FACSCount.

*Flowcytometry*: Four colour flowcytometric analysis was performed on whole blood samples obtained from study and control subjects using standard methodology in accordance with manufacturers' instructions (Becton Dickinson). The following monoclonal antibody panel was used for staining: fluorescein isothiocyanate (FITC)-labelled anti-Lineage ($Lin^-$) panel (CD3/CD14/CD16/CD19/ CD20/CD56) (BD, USA), peridinin chlorophyll protein (PerCP)-labelled anti-HLA-DR (BD), phycoerythrin (PE)-labeled anti-CD11c (BD PharMingen, USA), and either allophycocyanin (APC)-labelled CD40 (PharMingen, USA), -CD86

(PharMingen, USA), -CXCR4 (BD PharMingen, USA), or -CCR5 (BD PharMingen, USA). Appropriately labelled isotype controls were used in each experiment. A total of 50,000 to 300,000 events were acquired for each tube on a FACSCalibur (Becton Dickinson, San Jose, USA) flowcytometer within 2 h of processing the blood samples. Lymphocyte and monocyte population was gated, and dendritic cells were identified by gating on HLA-DR PerCP events negative for FITC labelled lineage markers. The DCs were differentiated into myeloid and plasmacytoid DCs on the basis of their expression of CD11c (Fig. 1). Absolute DC count was calculated using the percentage of these cells in relation to the mononuclear fraction determined by the automated differential blood count[18,19]. Further, the expression of HIV-1 co-receptor molecules: CXCR4 and CCR5, and co-stimulatory molecules: CD40 and CD86 were analysed on the two DC subsets. Isotype control values were subtracted from the values obtained from tubes with antibody staining, for calculation of mean fluorescence intensity (MFI) of all gated cells.

*HIV-1 viral RNA quantification*: HIV-1 viral RNA was extracted from plasma samples of study subjects and quantified using Amplicor HIV-1 Monitor Assay version 1.5 (Roche Diagnostics, Meylan, France) with a lower limit of detection of 400 copies/ml.

*Statistical analysis*: The Mann-Whitney *U* test was used for comparisons between HIV-1-infected and uninfected control subjects and also to analyse the differences between HAART naïve and HAART receiving study subjects. $CD4^+$ T cell counts of the study population were used to form three groups, among which the DC subsets were compared using Kruskal-Wallis test for equality of populations. Association of $CD4^+$ T cell counts and HIV-1 viral load with dendritic cell subset numbers and expression of co-receptor and co-stimulatory molecules on the subsets was examined using Spearman's $\rho$. Comparisons were made between HAART receiving subjects with adequate viral suppression and those with still detectable plasma viraemia. $P<0.05$ was considered significant. All the statistical analyses were performed using SPSS software version I6 (Chicago, IL).

## Results

Seventy nine HIV-1 infected individuals, with a median age of 34 yr, and 13 seronegative controls (median age, 27 yr) were included in the study. The

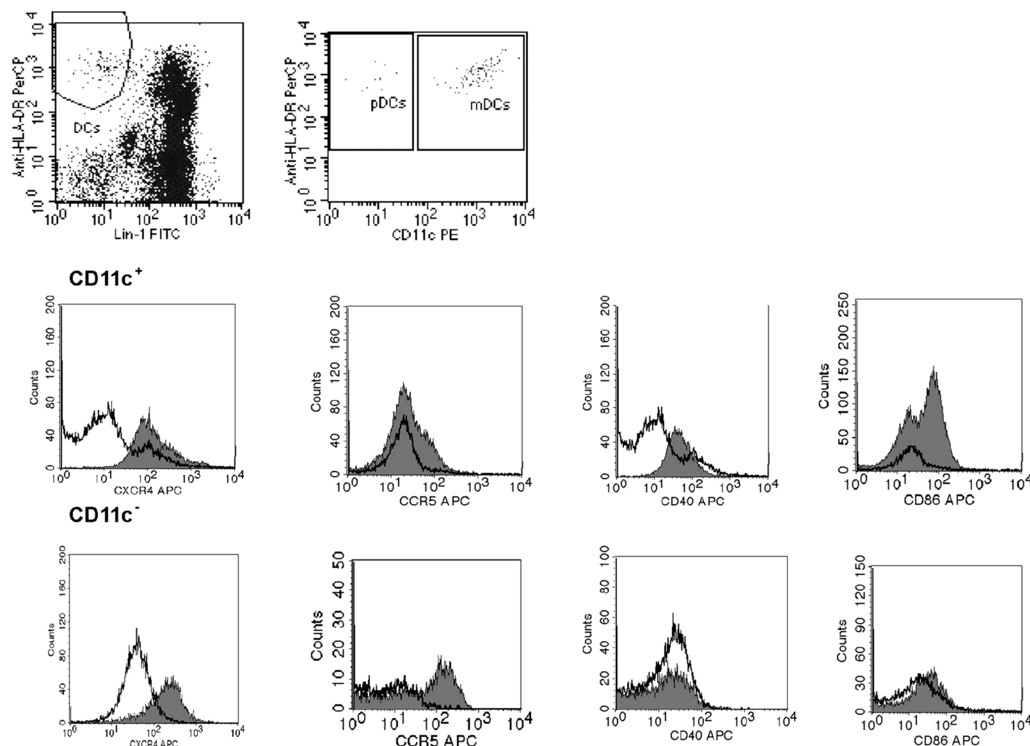


**Fig. 1.** Identification of blood dendritic cell (DC) subsets. DCs were identified by FACS analysis on the basis of their lack of labelling for the mononuclear cell markers CD3, CD14, CD16, and CD19 but positive staining for HLA-DR. The mDCs were differentiated from the pDCs by their expression of CD11c. The Fig. shows whole blood gating strategy for mDCs and pDCs. Filled histograms: specific staining, Hollow histograms: isotype.

median CD4$^{+}$ T cell counts were 294/µl for HIV infected subjects and 884/µl for control subjects ($P$<0.001). White blood cell counts and peripheral blood mononuclear cell population were not seen to be significantly altered in the HIV infected subjects as opposed to uninfected controls.

*Blood dendritic cell subsets in study and control subjects*: In ART naïve HIV infected study subjects, there was a marked decrease in the percentage of total DCs (median % of total DCs: control subjects, 1.07%; HIV-1-infected subjects, 0.41%; $P$<0.001) and absolute counts of blood DCs (median DC count: control subject, 27316 cells/ml; HIV-1-infected subjects, 7403 cells/ml; $P$<0.001) when compared to seronegative controls (Table I). On segregating the subjects on basis of clinical stage of HIV infection, a statistically significant decrease in DC per cent and absolute counts were observed with increasing disease severity (Table II).

**Table I.** Summary of comparison between uninfected control subjects and HIV-1 infected study subjects

| Characteristic | Control group (n=13) | HIV + (n=79) | | $P$ value |
|---|---|---|---|---|
| | | ART naïve (n=58) | On ART (n=21) | |
| Age (yr) | 27 (25 - 40) | 30 (18 - 53) | 37 (26 - 46) | 0.056 |
| CD4+ T (cells/µl) | 841 (547 - 1124) | 251 (21 - 1712) | 384 (64 - 1200) | <0.001 |
| % DCs in total PBMC | 1.07 (0.89 - 1.35) | 0.41 (0.05 - 0.98) | 0.46 (0.17 - 0.83) | <0.001 |
| No. of DCs/ml | 27316 (21417 - 37651) | 7403 (1601 - 25760) | 11555 (3848 - 30096) | <0.001 |
| % mDCs in total PBMC | 0.68 (0.50 - 1.00) | 0.20 (0.03 - 0.65) | 0.30 (0.11 - 0.56) | <0.001 |
| No. of mDCs/ml | 18900 (12443 - 24620) | 3920 (431 - 18158) | 6640 (3086 - 22277) | <0.001 |
| % pDCs in total PBMC | 0.34 (0.17 - 0.66) | 0.11 (0.01 - 0.39) | 0.17 (0.03 - 0.36) | <0.001 |
| No. of pDCs/ml | 8036 (4554 - 18411) | 2495 (61 - 12149) | 4178 (648 - 10010) | <0.001 |

Data are median values with ranges in parentheses. DCs, dendritic cells; mDCs, myeloid dendritic cells; pDCs, plasmacytoid dendritic cells; PBMC, peripheral blood mononuclear cells

**Table II.** Blood DC subsets in HIV-1 infected subjects segregated on basis clinical stage of HIV infection

| Characteristic | HIV disease stage | | | $P$ value |
|---|---|---|---|---|
| | CDC A (n=19) | CDC B (n=21) | CDC C (n=18) | |
| Age (yr) | 28 (19 - 53) | 30 (18 - 45) | 35 (22 - 50) | 0.032 |
| CD4+ T (cells/µl) | 462 (174 - 929) | 257 (25 - 1712) | 129 (21 - 885) | <0.001 |
| % DCs in total PBMC | 0.45 (0.05 - 0.98) | 0.44 (0.08 - 0.97) | 0.20 (0.12 - 0.69) | 0.04 |
| No. of DCs/ml | 11534 (1601 - 25760) | 8623 (1877 - 21445) | 4374 (1848 - 17105) | 0.021 |
| % mDCs in total PBMC | 0.23 (0.03 - 0.65) | 0.20 (0.02 - 0.34) | 0.12 (0.06 - 0.31) | 0.041 |
| No. of mDCs/ml | 7096 (986 - 18158) | 3764 (431 - 8818) | 2665 (1232 - 7730) | 0.008 |
| % pDCs in total PBMC | 0.14 (0.02 - 0.35) | 0.14 (0.01 - 0.39) | 0.07 (0.03 - 0.37) | 0.128 |
| No. of pDCs/ml | 4211 (496 - 10129) | 3540 (61 - 12149) | 1517 (449 - 9095) | 0.066 |

Data are median values with ranges in parentheses. DCs, dendritic cells; mDCs, myeloid dendritic cells; pDCs, plasmacytoid dendritic cells; PBMC, peripheral blood mononuclear cells

The percentage of DCs decreased with disease progression as was evidenced by marked reduction in DC per cent in subjects in CDC stage III with $CD4^+$ T cell counts less than 200 cells/ml (median, 0.23%) as opposed to DC percentages in CDC I with $CD4^+$ T cell counts more than 500 cells/ml (median, 0.43%) and CDC II *i.e.*, $CD4^+$ T cell counts 200-499 cells/ml subjects (median, 0.42%). The differences were statistically significant ($P$<0.001). Similar decreases were observed for DC absolute counts among the three stages, the differences being statistically significant ($P$<0.001 Table III).

HIV infected ART naive subjects showed decreases in blood DC subsets: mDCs and pDCs, relative to the uninfected control subjects. This decrease was observed when DCs were measured as a percentage of total PBMCs (median mDCs: control subjects, 0.68%; HIV-1-infected subjects, 0.20%; $P$<0.001; median pDCs: control subjects, 0.34%; HIV-1-infected subjects, 0.11%; $P$<0.001), and when measured as absolute numbers in the blood (median mDC count: control subjects, 18900 cells/ml; HIV-1-infected subjects, 3920 cells/ml; $P$<0.001; and median pDC count: control subjects, 8036 cells/ ml; HIV-1-infected subjects, 2495 cells/ml; $P$<0.001) (Table I). Similar differences in mDC and pDC percentages and absolute counts were observed when subjects were segregated on basis of $CD4^+$ T cell counts and disease stage (Tables II, III).

*Association between $CD4^+$ T cell counts, plasma viremia, and blood DC subsets*: A positive correlations was observed between blood DC count and peripheral $CD4^+$ T cell count (Spearman's $\rho$ 0.294, $P$=0.009). Similarly, mDCs (Spearman's $\rho$ 0.44, $P$<0.001) and pDCs ($\rho$ = 0.453, $P$<0.001) also displayed significant positive correlations with $CD4^+$ T cell counts (Fig. 2, A-C).

A progressive decline was observed in blood DC numbers with increasing plasma viraemia ($\rho$ = -0.31, $P$=0.012). Statistically significant negative correlations were detected between blood DC subset absolute counts and plasma viraemia (mDC, $\rho$ = -0.311, $P$=0.015; pDCs, $\rho$ = -0.334, $P$=0.008) (Fig. 2, D-F).

*Activation state of DC subsets in settings of HIV-1 infection*: Expression of co-stimulatory molecules differed in study and control subjects. The DC subsets had higher expression levels of co-receptors CXC;R4 and CCR5 in HIV infected subjects as compared to HIV-uninfected controls, with this difference being statistically significant ($P$<0.001) for CXCR4 expression on mDCs and CCR5 expression on both mDCs and pDCs. Similarly differences were observed in co-stimulatory molecules CD40 and CD86 expression on the two DC subsets in study and control groups, wherein the CD86 levels on both mDCs and pDCs being significantly higher in HIV infected subjects (Table IV).

*Association between HIV-1 RNA, CD4 counts and surface molecules on DC subsets*: A significant positive correlation was observed between CD86 expression on the two DC subsets and plasma viraemia (mDCs, $\rho$ = 0.283, $P$ = 0.022; pDCs, $\rho$ = 0.36, $P$=0.003). The CD40 expression on mDCs correlated significantly with plasma viraemia ($\rho$ = 0.322, $P$=0.009).

**Table III.** Blood DC subsets in HIV-1 infected subjects segregated on basis of $CD4^+$ T cell counts

| Characteristic | Control group (n=13) | CD4 > 500/µl (n=12) | CD4 200-500/µl (n=24) | CD4 < 200/µl (n=22) | $P$ value |
|---|---|---|---|---|---|
| % DCs in total PBMC | 1.07 (0.89 - 1.35) | 0.43 (0.05 - 0.98) | 0.42 (0.14 - 0.94) | 0.23 (0.12 - 0.97) | <0.001 |
| No. of DCs/ml | 27316 (21417 - 37651) | 14268 (1601 - 25760) | 9143 (2584 - 18970) | 4567 (1848 - 17105) | <0.001 |
| % mDCs in total PBMC | 0.68 (0.50 - 1.00) | 0.23 (0.02 - 0.65) | 0.22 (0.06 - 0.40) | 0.12 (0.07 - 0.31) | <0.001 |
| No. of mDCs/ml | 18900 (12443 - 24620) | 7893 (431 - 18158) | 4614 (1436 - 14505) | 2736 (1232 - 7730) | <0.001 |
| % pDCs in total PBMC | 0.34 (0.17 - 0.66) | 0.16 (0.02 - 0.39) | 0.145 (0.01 - 0.33) | 0.067 (0.03 - 0.37) | <0.001 |
| No. of pDCs/ml | 8036 (4554 - 18411) | 4949 (496 - 12149) | 3900 (61 - 7656) | 1517 (449 - 9095) | <0.001 |

Data are median values with ranges in parentheses. DCs, dendritic cells; mDCs, myeloid dendritic cells; pDCs, plasmacytoid dendritic cells; PBMC, peripheral blood mononuclear cells

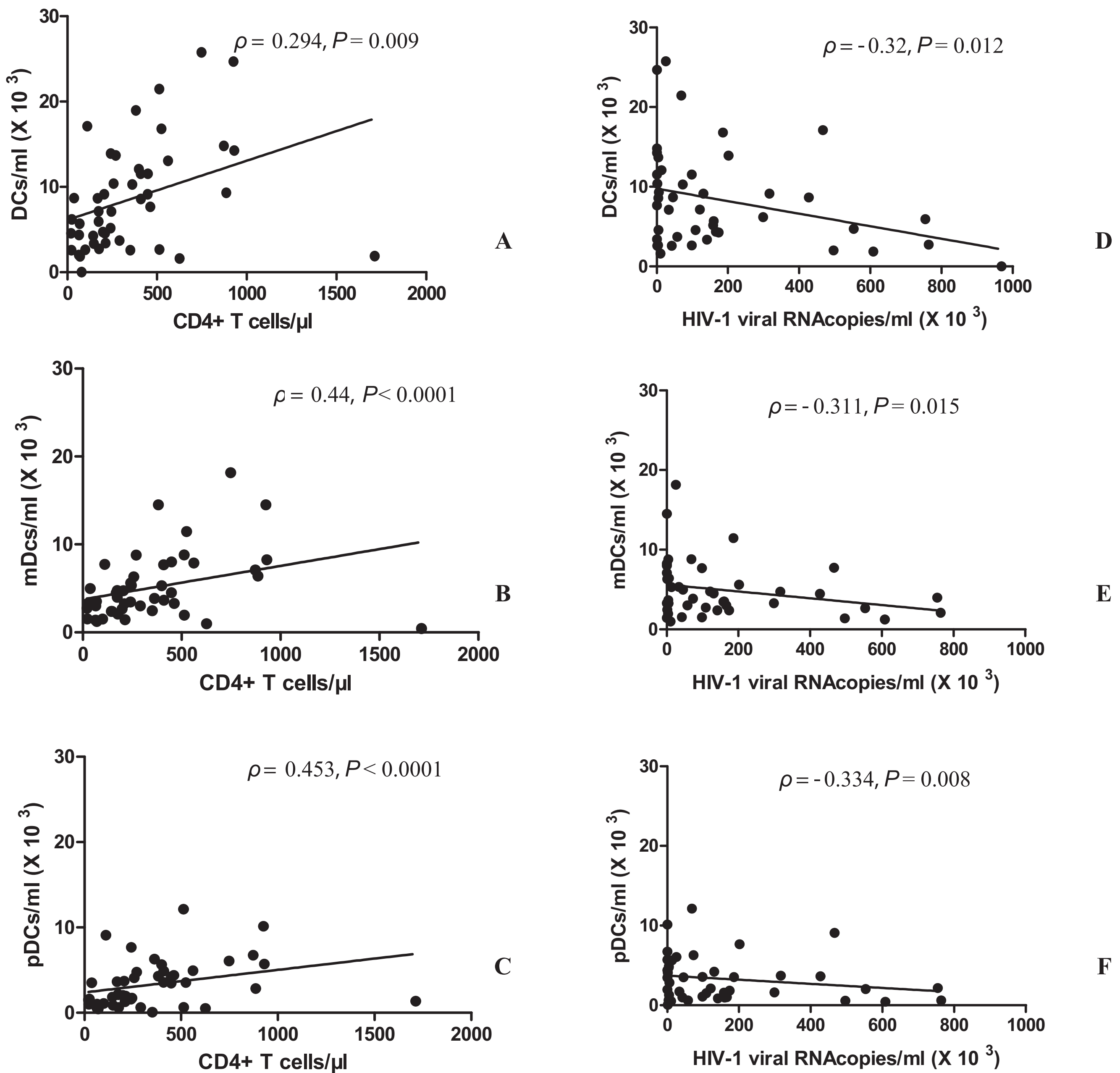


**Fig. 2.** Association of blood dendritic cells with markers of disease progression (n = 58). A-C: Association of blood DC subsets with peripheral blood $CD4^+$ T cell counts. D-F: Association of blood DC subsets with HIV-1 RNA load in plasma.

The CD86 expression on mDCs ($\rho$ = -0.126) and pDCs ($\rho$ = -0.26) were negatively correlated with peripheral $CD4^+$ T cells, but these approached statistical significance only in case of CD86 expression on pDCs ($P$=0.021). CD40 expression on pDCs did not show any association with $CD4^+$ T cell counts (data not shown).

Expression of co-receptors CXCR4 and CCR5 were positively correlated with plasma viraemia, with this correlation reaching significance only in case of CCR5 expression on pDCs ($\rho$ = 0.456, $P$<0.001). Weak and insignificant negative correlations were observed between percentage of DC subsets expressing either coreceptors molecule and $CD4^+$ T cell counts (data not shown).

*Blood DC subset number and immunophenotype in subjects on antiretroviral therapy*: Details of HAART regimen and treatment duration are summarised

in Table Va. To better define the effect to plasma viral replication on DC numbers the ART receiving subjects were divided into three groups based on their virologic status after treatment and further on duration of treatment. For duration of treatment, the median duration of ART was calculated for all subjects and the patients were segregated into groups with less than median duration and more than median duration of treatment. Thus, there were three groups based on these criteria: first group had subjects with undetectable plasma viraemia and less than 26 months of ART; second group had undetectable plasma viraemia with more than 26 months of ART, and the third group had detectable plasma viraemia with at least a year of ART experience (suspected HAART failure). Higher DC counts were observed in subjects with complete viral suppression when compared to suspect HAART failure cases irrespective of treatment duration. On comparing

**Table IV.** Difference in expression of surface molecules amongst study and control subjects

| Surface molecule expression | Controls (n=13) | HIV infected subjects (n=79) | | *P* value |
|---|---|---|---|---|
| | | ART naïve (n=58) | On ART (n=21) | |
| CD40 MFI on mDCs | 313 (145 - 567) | 596 (256 - 698) | 441 (225 - 703) | 0.167 |
| CD40 MFI on pDCs | 467 (141 - 688) | 574 (48 - 714) | 254 (39 - 531) | 0.179 |
| CD86 MFI on mDCs | 284 (119 - 454) | 1010 (789 - 1398) | 578 (351 - 733) | 0.023 |
| CD86 MFI on pDCs | 208 (97.5 - 431) | 507 (373 - 678) | 441 (289 - 637) | 0.018 |
| CXCR4 MFI on mDCs | 191 (66.5 - 286) | 642 (323 - 815) | 601 (259 - 887) | <0.001 |
| CXCR4 MFI on pDCs | 267 (42 - 539) | 300 (98 - 768) | 217 (69 - 562) | 0.228 |
| CCR5 MFI on mDCs | 279 (119 - 497) | 642 (275 - 856) | 601 (390 - 973) | 0.035 |
| CCR5 MFI on pDCs | 468 (176 - 672) | 568 (243 - 791) | 289 (68 - 553) | 0.027 |

Values are median with range in parentheses

**Table Va.** Details of subjects on highly active antiretroviral therapy (HAART)

| Patient | Age (yr) | Sex | CDC Stage | Months after HAART | Treatment | CD4 T cell count (cells /µl) | Plasma viral load (copies /ml) |
|---|---|---|---|---|---|---|---|
| 1 | 26 | M | A | 20 | LAM ZDV NVP | 512 | <400 |
| 2 | 40 | M | A | 30 | LAM ZDV NVP | 182 | <400 |
| 3 | 40 | M | B | 6 | LAM STA EFV | 373 | <400 |
| 4 | 27 | M | B | 22 | LAM ZDV NVP | 517 | <400 |
| 5 | 42 | M | B | 40 | LAM ZDV NVP | 384 | <400 |
| 6 | 40 | M | B | 24 | LAM ZDV NVP | 122 | <400 |
| 7 | 42 | M | C | 26 | LAM STA EFV | 350 | <400 |
| 8 | 38 | M | C | 48 | LAM ZDV NVP | 975 | <400 |
| 9 | 46 | M | C | 26 | STA LAM NVP | 553 | <400 |
| 10 | 35 | M | C | 37 | LAM STA EFV | 373 | <400 |
| 11 | 36 | M | C | 35 | STA LAM NVP | 503 | <400 |
| 12 | 40 | M | C | 37 | STA LAM NVP | 724 | <400 |
| 13 | 35 | F | A | 6 | LAM STA EFV | 367 | <400 |
| 14 | 32 | M | C | 12 | LAM ZDV NVP | 484 | 9194 |
| 15 | 35 | M | C | 16 | LAM ZDV NVP | 1181 | 3471 |
| 16 | 33 | M | C | 12 | LAM ZDV NVP | 425 | 2942 |
| 17 | 32 | M | C | 39 | STA LAM NVP | 96 | 6142 |
| 18 | 37 | M | A | 12 | LAM ZDV NVP | 1200 | 18182 |
| 19* | 40 | M | A | 31 | LAM ZDV NVP | 70 | 362469 |
| 20* | 38 | M | B | 60 | STA LAM NVP | 155 | 650000 |
| 21* | 35 | M | C | 30 | LAM ZDV NVP | 64 | 200041 |

LAM, lamivudine; ZDV, zidovudine; NVP, nevirapine; STA, stavudine; EFV, effavirenz

*Subjects with high plasma viraemia and low CD4+ T cell counts despite HAART, possible HAART failure

**Table Vb.** Effect of HAART duration on dendritic cell absolute counts and percentages

| Characteristic | Median (Range) | | |
|---|---|---|---|
| | ≤26 months on HAART (n=6) | >26 months on HAART (n=7) | Insufficient viral suppression (n=8) |
| CD4+ T cell/µl | 442 (350 - 553) | 466 (122 - 975) | 264 (64 - 1181) |
| Plasma viral load (copies/ml) | <499 | <499 | 7668 (2942 - 650000) |
| TLC (x $10^3$/$mm^3$) | 6400 (4500 - 13700) | 7528 (3900 - 10900) | 8225 (4100 - 11900) |
| % DCs in total PBMC | 0.57 (0.32 - 0.83) | 0.51 (0.19 - 0.81) | 0.42 (0.17 - 0.76) |
| No. of DCs/ml | 10587 (7142 - 17197) | 14976 (3847 - 28252) | 13152 (6473 - 30096) |
| % mDCs in total PBMC | 0.3 (0.15 - 0.54) | 0.31 (0.15 - 0.4) | 0.31 (0.11 - 0.56) |
| No. of mDCs/ml | 5526 (3312 - 11144) | 8245 (3085 - 11529) | 8078 (4316 - 22277) |
| % pDCs in total PBMC | 0.21 (0.14 - 0.33) | 0.17 (0.32 - 0.36) | 0.13 (0.05 - 0.19) |
| No. of pDCs/ml | 4259 (3038 - 5287) | 5320 (647 - 10010) | 3506 (1071 - 6717) |

the subjects with undetectable plasma viraemia subjects with less than median HAART duration with subjects with higher HAART duration, higher DC and subset absolute counts were observed in subjects who had been on therapy for a longer duration. Further, subjects with successful viral suppression and higher HAART duration also had higher median $CD4^+$ T cell counts (466 cells/µl, range: 122 - 975) as opposed to subjects with lower HAART duration (442 cells/µl, range: 350 - 553); while the subjects suspected to be experiencing HAART failure had still lower CD4+ T cell counts (264 cells/µl, range: 64 - 1181) (Table Vb).

Expression of co-stimulatory molecules CD86 and CD40 on DC subsets was lowered in ART receiving individuals as compared to ART naïve (Table IV). Similarly, co-receptors expression on the two DC subsets was also lower in ART receiving subjects.

## Discussion

HIV infection and disease progression have been associated with decrease in frequency of DC subsets in the peripheral blood[12,20]. In our study population of HIV-1 infected Indian individuals, we found that the absolute numbers and percentages of total blood DCs were markedly reduced in comparison to the HIV-1 uninfected controls. Also, the reduction was most apparent in CDC III subjects as compared to CDC I and II suggesting more severe DC pool depletion with progressing disease. In our study subjects, the peripheral mononuclear population was not significantly reduced when compared to the uninfected controls, thereby highlighting a selective loss of DCs from periphery as opposed to general impairment in homoeostasis. The loss of DCs was not restricted to either cell population, the number of both mDCs and pDCs, as well as their percentage in total PBMCs, were reduced in HIV-1. The diminished proportion of blood DC subsets in HIV-1 infected individuals can be attributed to direct cytopathic effect of the virus as the replicating virus affects DC survival by activating the proapoptotic pathway, and activation and migration of the DCs from peripheral blood into lymphoid tissue. In addition, HIV infection also interferes with DC generation by infecting monocytes, the precursors of blood DCs, and impairing their differentiation into DCs[21].

We assessed the relation between peripheral $CD4^+$ T cell counts and numbers of mDCs and pDCs in HIV-infected subjects, and found a strong positive correlation for both DC subsets as well as total blood DC counts, as has been documented by several others[22,23]. This correlation of blood DC subsets with $CD4^+$ T cell counts highlights the importance of these cells as measure of immune status during HIV-1 infection, and indicates increased depletion of these cells in the HIV-infected individuals with a decreased $CD4^+$ T cell pool, and hence progressive disease. We further examined the association of DC subset numbers with plasma viraemia, and found a significant negative correlation of DC subset numbers with plasma viraemia[12,24], thereby demonstrating the deleterious effect of viral replication on the blood DC population. This enhanced depletion of peripheral DCs in conjunction with the reduction in $CD4^+$ T cells, and an increasing viral load leads to an increasingly impaired immune system, which renders an individual vulnerable to opportunistic infections.

DCs on encountering antigens get activated leading to a change in their surface expression of various molecules like CD40, CD80, CD86, and MHC, involved in T cell activation. Recently, DCs have been implicated in the overall state of immune activation observed in HIV infection[25]. To assess this,

we studied the expression pattern of co-stimulatory molecules CD40 and CD86 on the surface of both mDCs and pDCs in our study subjects. Significantly increased level of CD86 expression was noted on the DC subsets in HIV infected subjects as compared to the uninfected controls. Similarly, expression of CD40 on the two DC subsets was also higher in HIV infected subjects, though the difference was not significant. This alteration in surface molecule expression may indicate an activated state of DCs in infected subjects as has been documented earlier[12,20,26].

These levels of co-stimulatory molecules on DC surface correlated positively with plasma viraemia suggesting that HIV triggers activation and/or maturation in the blood DCs. This activation can be a result of direct effect of the replicating virus, or due to the altered cytokine milieu with increased concentrations of pro-inflammatory cytokines like TNF-α. The increased activation state of the DCs, specifically mDCs with their higher expression of co-stimulatory molecules, can in turn cause bystander activation of T cells, and may contribute to the state of chronic T cell activation and dysfunction observed in HIV infection[12]. Other possible reasons for this altered state of DC activation can be due to direct or indirect activation by HIV-1[27], or through microbial products like lipopolysaccharide (LPS)[28]. Further, it has been shown that some HIV antigens inhibit HIV maturation or only partially activate DCs[29,30].

We studied the expression of HIV-1 co-receptors CXCR4 and CCR5 on the two DC subsets. The mDCs and pDCs from HIV infected subjects expressed higher levels of these co-receptors than the HIV uninfected controls.

We attempted a cross-sectional analysis of the effect of antiretroviral therapy on blood DCs. When compared to ART naïve subjects, higher numbers of blood DC subsets were observed in ART receiving subjects. The increases were significant for both the absolute blood DC counts and number of circulating DC subsets. When segregated on basis of ART duration and on basis of effective viral replication suppression, a higher increase was seen in the DC numbers and percentages in subjects who had longer ART experience with effective viral control. Subjects with still detectable viral loads had comparatively lower DC subset percentages and counts suggesting long term effective suppression of viral load to below detectable limits can restore DC populations to almost normal levels. The ART receiving subjects had reduced expression of CD40 and CD86, with this decrease most apparent in the mDC subset. A longitudinal characterization of these parameters on ART receiving subjects would further aid in our understanding of the effect of treatment on HIV induced DC defects.

In conclusion, our findings showed the effect of HIV disease progression on blood DC subsets: mDCs and pDCs in HIV-1 subtype C infected Indian individuals. The depletion of blood DCs is associated with a host of phenotypic changes which make these cells more susceptible to viral infection, and implicates them in HIV associated immune activation. Though a limitation in the present study, assays correlating immune activation with DC alteration would help in better understanding of the role of DCs in HIV induced immune activation. Since blood DCs play an important role in generation of immune response, future studies in this population should aim at analysing the T cell immune response *vis-à-vis* DC defects to further clarify the interplay between DCs and T cells.

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## References

1. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, *et al*. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; *18* : 767-811.
2. Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, Young RA, *et al*. The plasticity of dendritic cell responses to pathogens and their components. *Science* 2001; *294* : 870-5.
3. Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* 1997; *388* : 782-7.
4. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat Immunol* 2000; *1* : 305-10.
5. Macatonia SE, Knight SC, Edwards AJ, Griffiths S, Fryer P. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. *J Exp Med* 1987; *166* : 1654-67.
6. McMichael AJ, Rowland-Jones SL. Cellular immune responses to HIV. *Nature* 2001; *410* : 980-7.
7. Fong L, Mengozzi M, Abbey NW, Herndier BG, Engleman EG. Productive infection of plasmacytoid dendritic cells with human immunodeficiency virus type 1 is triggered by CD40 ligation. *J Virol* 2002; *76* : 11033-41.

8. Patterson S, Rae A, Hockey N, Gilmour J, Gotch F. Plasmacytoid dendritic cells are highly susceptible to human immunodeficiency virus type 1 infection and release infectious virus. *J Virol* 2001; *75* : 6710-3.

9. Rubbert A, Combadiere C, Ostrowski M, Arthos J, Dybul M, Machado E, *et al*. Dendritic cells express multiple chemokine receptors used as coreceptors for HIV entry. *J Immunol* 1998; *160* : 3933-41.

10. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat Immunol* 2000; *1* : 311-6.

11. Patterson S. Flexibility and cooperation among dendritic cells. *Nat Immunol* 2000; *1* : 273-4.

12. Barron MA, Blyveis N, Palmer BE, MaWhinney S, Wilson CC. Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals. *J Infect Dis* 2003; *187* : 26-37.

13. Pacanowski J, Develioglu L, Kamga I, Sinet M, Desvarieux M, Girard PM, *et al*. Early plasmacytoid dendritic cell changes predict plasma HIV load rebound during primary infection. *J Infect Dis* 2004; *190* : 1889-92.

14. Vajpayee M, Kaushik S, Sreenivas V, Mojumdar K, Mendiratta S, Chauhan NK. Role of immune activation in CD4(+) T-cell depletion in HIV-1 infected Indian patients. *Eur J Clin Microbiol Infect Dis* 2009; *28* : 69-73.

15. Agarwal A, Sankaran S, Vajpayee M, Sreenivas V, Seth P, Dandekar S. Correlation of immune activation with HIV-1 RNA levels assayed by real-time RT-PCR in HIV-1 subtype C infected patients in Northern India. *J Clin Virol* 2007; *40* : 301-6.

16. [No authors listed]. Interim proposal for a WHO staging system for HIV infection and disease. *Wkly Epidemiol Rec* 1990; *65* : 221-4.

17. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992; *41* : 1-19.

18. Chehimi J, Campbell DE, Azzoni L, Bacheller D, Papasavvas E, Jerandi G, *et al*. Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. *J Immunol* 2002; *168* : 4796-801.

19. Hasskamp JH, Zapas JL, Elias EG. Dendritic cell counts in the peripheral blood of healthy adults. *Am J Hematol* 2005; *78* : 314-5.

20. Almeida M, Cordero M, Almeida J, Orfao A. Different subsets of peripheral blood dendritic cells show distinct phenotypic and functional abnormalities in HIV-1 infection. *AIDS* 2005; *19* : 261-71.

21. Chougnet C, Gessani S. Role of gp120 in dendritic cell dysfunction in HIV infection. *J Leukoc Biol* 2006; *80* : 994-1000.

22. Donaghy H, Pozniak A, Gazzard B, Qazi N, Gilmour J, Gotch F, *et al*. Loss of blood CD11c(+) myeloid and CD11c(-) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. *Blood* 2001; *98* : 2574-6.

23. Pacanowski J, Kahi S, Baillet M, Lebon P, Deveau C, Goujard C, *et al*. Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. *Blood* 2001; *98* : 3016-21.

24. Soumelis V, Scott I, Gheyas F, Bouhour D, Cozon G, Cotte L, *et al*. Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood* 2001; *98* : 906-12.

25. Boasso A, Shearer GM. Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. *Clin Immunol* 2008; *126* : 235-42.

26. Jones GJ, Watera C, Patterson S, Rutebemberwa A, Kaleebu P, Whitworth JA, *et al*. Comparative loss and maturation of peripheral blood dendritic cell subpopulations in African and non-African HIV-1-infected patients. *AIDS* 2001; *15* : 1657-63.

27. Fonteneau JF, Larsson M, Beignon AS, McKenna K, Dasilva I, Amara A, *et al*. Human immunodeficiency virus type 1 activates plasmacytoid dendritic cells and concomitantly induces the bystander maturation of myeloid dendritic cells. *J Virol* 2004; *78* : 5223-32.

28. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, *et al*. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006; *12* : 1365-71.

29. Fantuzzi L, Purificato C, Donato K, Belardelli F, Gessani S. Human immunodeficiency virus type 1 gp120 induces abnormal maturation and functional alterations of dendritic cells: a novel mechanism for AIDS pathogenesis. *J Virol* 2004; *78* : 9763-72.

30. Majumder B, Janket ML, Schafer EA, Schaubert K, Huang XL, Kan-Mitchell J, *et al*. Human immunodeficiency virus type 1 Vpr impairs dendritic cell maturation and T-cell activation: implications for viral immune escape. *J Virol* 2005; *79* : 7990-8003.

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