Blood Myeloid Dendritic Cells and slanDC in Antiretroviral Therapy-Suppressed HIV-Infected Patients

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Abstract: Myeloid dendritic cells (mDCs) play a complex role in HIV infection regardless of viral replication. The aim of our study was to analyse mDCs in long term antiretroviral therapy (ART)-suppressed HIV-infected patients. We evaluated the numbers of mDCs and slanDC in the context of different degree of $CD4^+$ T cell recovery, persistent T cell activation (as $HLA\text{-}DR^+/CD38^+$ expression) and monocyte-macrophage activation assessed in terms of circulating levels of both sCD14 and sCD163. We enrolled 72 aviremic patients under effective ART and 34 healthy donors (HD). Patients were divided into two groups on the bases of ΔCD4, indicating the difference between the value of CD4 at the time of sampling and CD4 nadir. Higher levels of mDCs and slanDC were found in patients with ΔCD4>200/mmc in comparison to HD. In those patients also an increased level of sCD14 was found, whereas sCD163 seemed to be at normal levels. An augmentation of activated CD4 T lymphocytes was found, although less pronounced in patients with ΔCD4<200/mmc. In conclusion, our findings showed an expansion of mDCs with a shift to inflammatory slanDC that could sustain both microbial translocation and HIV latency in CD4 T cells.

Keywords: slanDC, Myeloid Dendritic Cells, HIV, sCD163, sCD14, immune activation, ART.

1. INTRODUCTION

HIV infection is characterized by an immune activation that results in increased cell turnover, immune system exhaustion and progression to AIDS. The general contribution of HIV replication is uncertain, because there is immune activation found also in patients with undetectable viremia [1] and a lack of immune activation in the presence of high viral load in simian immunodeficiency virus infection [2], suggesting that plasma virus replication is not an absolute requirement for systemic immune activation. Moreover, low levels of immune activation persist overtime also in HIV-infected patients under effective antiretroviral therapy (ART) despite CD4 T cells recovery and viral suppression [3]. Causes and mechanisms of this chronic inflammatory status are poorly understood, but are likely to be multifactorial and encompass innate and adaptive immune responses, with T-cell- and monocyte/macrophage-associated markers as well as inflammatory soluble plasma molecules being predictive of disease progression [4]. In this regard, the analysis of HLA-DR/CD38 expression on T-cells and the measurement of circulating levels of soluble CD14 (sCD14) and CD163 (sCD163) are increasingly being recognized as important parameters to assess the degree of T-lymphocytes and monocyte/macrophages activation during the course of HIV infection [5,6].

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Persistent T and myeloid cell activation are present even in patients with successful HIV suppression, and are linked to non-AIDS morbidity and mortality [7,8]. In this context the contribution of dendritic cells (DCs) in sustaining HIV-associated immune activation is not completely understood.

DCs participate in the immune dysregulation characteristic of chronic HIV infection, through the secretion of inflammatory cytokines and interferons, and DCs also alter T cell proliferation and differentiation [4]. Myeloid (mDCs) and plasmacytoid dendritic cells (pDCs) decrease rapidly during the first weeks of HIV infection and their circulating levels are only partially restored after the beginning of ART, especially for pDCs [9,10]. In addition, a low baseline pDCs count predicts virological failure in ART treated patients, despite CD4+ T cells recovery [11].

A third subset of DCs was identified in peripheral blood using the monoclonal antibody M-DC8, which binds to 6-sulfo LacNac (slan), a carbohydrate moiety of the P selectin glycoprotein ligand 1 (PSGL-1) [12,13]. SlanDC cells seem to derive from the pro-inflammatory CD14+CD16+ monocytes [14] and to contribute to the pathogenesis of chronic inflammatory diseases such as Crohn's disease, rheumatoid arthritis and psoriasis, since they infiltrate the inflamed ileal mucosa, skin and synovial tissue [15,16].

Recent data suggest a critical role of mDCs and in particular of slanDC in driving immune activation in viremic HIV-infected patients [17]. On the other hand, the increased microbial translocation with release of LPS and inflammatory cytokines could represent one of the major

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stimuli for mDC generation and activation during HIV infection. The high degree of plasticity associated with the high capacity of recruitment at tissue-level, frame the mDCs and slanDC as potential key effector cells involved in inflammatory HIV-mediated disorders.

In the present study, we analysed the mDC compartment in a cohort of patients who have undetectable HIV viremia following long term treatment with ART. In particular, we evaluated the numbers of mDCs and slanDC in the context of different degree of CD4+ T cell recovery, persistent T cell activation (percentage of HLA-DR+/CD38+ double positive T Lymphocytes) and monocyte-macrophage activation assessed in terms of circulating levels of both sCD14 and sCD163.

2. METHODS

2.1. Study Population

The study was approved by the local ethics committee and all the recruited subjects provided a written informed consent to participate.

The study population was selected from all patients who presented consecutively to the Outpatients Clinic of the Department of Public Health and Infectious Diseases, Sapienza University of Rome. Inclusion criteria were patients (i) with at least 2 undetectable (<37copies/ml) determinations of HIV-RNA levels in the previous 12 months, (ii) on a stable ART for at least 2 years and (iii) free from any acute infections at the time of blood collection. Exclusion criteria were pregnancy, age <18 years, hepatitis B and C infection. Based on the selected criteria 72 HIV infected subjects were enrolled in the study. Moreover a group of 37 healthy donors (HD) was included in the study as controls. Demographic, clinical and laboratory data as well as therapies were collected for all participants.

The CD4+ T lymphocytes count at the time of sampling and the CD4 nadir were used to calculate ΔCD4, as the difference between the two parameters. According to ΔCD4 value, patients were divided into two groups: the first with 56 subjects with ΔCD4>200 cells/mmc and the second one with 16 subjects with ΔCD4<200 cells/mmc. The value of 200 cells/mmc, as a cut-off for ΔCD4, was chosen on the basis of studies that have shown a correlation between this parameter and the risk of clinical progression of the disease [18,19].

2.2. Enumeration of DCs

To identify the number of DCs subsets was used a whole blood assay with a protocol no lyse no wash. This method allowed us to quantify the absolute number of cell subsets using the MACSQuant Analyser. 50 µl of whole blood were stained with a mixture of antibodies following titration: CD3-PerCP, CD20-PerCP, CD14-PerCP, CD56-PerCP, HLA-DR-APC and M-DC8-FITC (all from Miltenyi Biotec, Germany), CD235a-PerCP and CD123-BV (BioLegend, Inc, USA), CD11c-PE (BD Biosciences Pharmagen, Italy). After 30 minute incubation, at room temperature, in the dark, the lyse solution was added and, after an additional 10 minute incubation, data were acquired using the flow cytometer. Before data acquisition began a calibration was performed, using MACSQuantTM Calibration Beads (Miltenyi Biotec, Germany). Cells were run on a MACS-Quant Analyser and analysis was performed using MACSQuantifyTM software (Miltenyi Biotec, Germany). Peripheral blood mononuclear cells (PBMCs) (R1 gate) were gated in a dot plot of Side Scatter Channel (SSC) *versus* Forward Scatter Channel (FSC). CD3, CD20, CD14, CD56 and CD235a (lineage) surface markers were used to exclude T and B lymphocytes, monocytes, NK cells and erythrocytes, respectively. From R1 gate, total DCs were defined as HLA-DR+ Lineage- (R2 gate). On R2 gate, dendritic cell markers CD123 and CD11c were used to define pDCs (R3 gate) and mDC (R4 gate) respectively. On mDCs gate we identified slanDC as cells M-DC8+ and CD11c+ positive.

2.3. CD4+ and CD8+ Activation

Activation markers of CD4+ and CD8+ T lymphocytes were evaluated by staining 50 µl of whole blood with a mix of pre-titred monoclonal antibodies containing CD45-VioBlue, HLA-DR-FITC, CD38-APC, CD8-PerCP and CD4-PE (all from Miltenyi Biotec, Germany). After 20 minute incubation, at room temperature and in the dark, lyse solution was added and after additional 10 minute incubation, data were acquired using MACS-Quant Analyser and then analysed by MACSQuantifyTM software (Miltenyi Biotec, Germany). SSC and CD45 were used to identify lymphocytes. In lymphocytes, CD4 and CD8 were gated. Finally, we gated HLA-DR+/CD38+ double positive CD4+ and CD8+ T cells to identify activated lymphocytes.

2.4. sCD14 and sCD163 Plasma Levels

Plasma samples were frozen at -80° until analysed. Soluble immune activation markers sCD14 and sCD163 were measured by ELISA (Quantikine ® ELISA, R&D Systems, Inc. Minneapolis, USA), according to manufacturer's instructions. All samples were tested in double.

2.5. Statistics

For statistical analysis, SPSS version 20.0 for windows (SPSS Inc., Apache Software Foundation, Chicago, Illinois) was used. Statistical comparison between groups was done using the Kruskal–Wallis test followed by Dunnett's post hoc test to detect differences between all groups. Spearman's rank correlation coefficient was calculated to determine associations between variables. P value <0.05 was considered statistically significant.

3. RESULTS

3.1. Demographical, Clinical and Laboratory Findings

72 HIV infected subjects (40 males and 32 females) were enrolled into the study, with the following characteristics (data are expressed as median and ranges): age 51 years (23-79), CD4 nadir 107/mmc (4-613), actual CD4 550/mmc (92-1550), estimated years to be living with HIV infection 14 (2-26). Regarding ART, 26 patients were under a triple combination therapy containing a protease inhibitor, 34

containing a non nucleoside reverse transcriptase inhibitor and 12 containing an integrase inhibitor. The median (range) period of continuous HIV viral suppression was 3 years (1-7). The HD were 37, 18 males and 19 females, with a median age of 42 (20-54).

3.2. Enumeration of mDC and slanDC

In all HIV-infected patients, mDC levels were not different compared to HD (cells/ml median, range, 27000/ml, 531-92135 *vs* 20008/ml, 7760-57900 p=0.09).

Stratifying subjects according to ΔCD4, we found that the absolute number of mDCs was significantly lower in patients with ΔCD4<200 cells/mmc, compared to patients with ΔCD4>200 cells/mmc (16750/ml, 1657-42787 *vs* 32271/ml, 531-92135; p<0.001). Interestingly, the group of ΔCD4> 200 cells/mmc showed a number of mDCs higher than HD (32271/ml, 531-92135 *vs* 20008/ml, 7760-57900 p=0.01) (Fig. **1A**). The median percentage of mDCs, within total PBMCs, was higher in patients with ΔCD4>200 cells/mmc compared to those with ΔCD4<200 cells/mmc (0,63% *vs* 0.3%, p=0.006) and with HD (0,63% *vs* 0.41%, p=0.004).

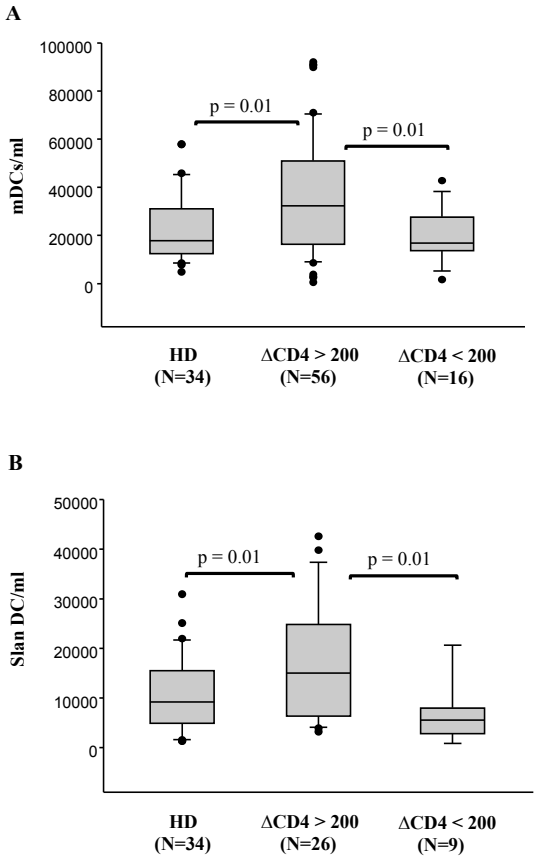

Fig (1). Enumeration of myeloid DC and slanDC in the study population according to ΔCD4. DCs were measured using a whole blood assay no lyse no wash. Box Plots represent circulating mDCs (**A**), M-DC8$^+$ (**B**) in patients with ΔCD4>200 cells/mmc and ΔCD4<200 cells/mmc in comparison to healthy donors. Boxes show 25th, 50th (median) and 75th percentile and wiskers 10th and 90th percentile. Dots represent outlying values. Statistical significance was analyzed by using Kruskal-Wallis test. HD: healthy donors.

In a subgroup of 35 patients we evaluated slanDCs, that were increased in patients with ΔCD4>200 compare to HD and in patients with ΔCD4< 200 (15050/ml, 3200-42600 *vs* 8830/ml, 1300-30900 p=0.01 and 5600/ml, 840-20700 p=0.01) (Fig. **1B**). The median percentage of slanDCs was higher in patients with ΔCD4>200 cells/mmc compared to those with ΔCD4<200 cells/mmc (0,24% *vs* 0.08%, p=0.02) and to HD (0,24% *vs* 0.12%, p=0.01).

Moreover, we defined the proportion of mDC coexpressing M-DC8 markers, computing the percentage of slanDC and M-DC8 negative cells on mDC. We found an increasing trend of slanDCs in patients with ΔCD4>200 (49%, 9-75) in comparison with HD (39%, 5-93) and subjects with ΔCD4< 200 (34%, 8-57) (Fig. **2A**).

No differences were observed in pDCs count in patients and HD (6040, 640-20826 *vs* 6222, 3100-14900, p= 0.8), even stratifying according to ΔCD4 (ΔCD4<200 *vs* ΔCD4>200, 4735, 640-19115 *vs* 6579, 1120-20826) and between HD and ΔCD4< and >200 (p>0.05). The median percentage of pDCs, did not show differences between groups.

3.3. CD8+ and CD4+ Immune Activation

All HIV positive patients showed a significant increase in HLA-DR and CD38 expression on CD4+ cells compared to HD (1.8%, *vs* 1.04%, p=0.003). The group of patients with ΔCD4<200 showed the highest level (2.37% *vs* 1.04%, p=0.003), although even in patients with ΔCD4>200, a higher percentage of CD38 and HLA-DR coexpression compared to HD was observed (1.76%, *vs* 1.04%, p=0.01) (Fig. **3**). There were no significant differences in the percentages of HLA-DR+/CD38+ CD8+ T cells between HIV patients and HD (0.8% *vs* 1.24%, p=0.6), neither according to CD4 recovery (ΔCD4>200 *vs* ΔCD4<200: 0.7%, *vs* 1.67%, p=0.6).

3.4. sCD14 and sCD163 Plasma Levels

sCD163 levels were significantly higher in all HIV subjects compared to HD (574 ng/ml, 58-2364 *vs* 377 ng/ml, 215-1026; p=0.03), sCD163 was significantly increased in patients with ΔCD4<200, compared with subjects with ΔCD4>200 and HD (853 ng/ml,150-2310 *vs* 548 ng/ml, 58-2364 and *vs* 377 ng/ml, 215-1026; p= 0.03 and p=0.01) (Fig. **4A**).

sCD14 levels were higher in HIV patients compare to HD (1989 ng/ml, 210-4799 *vs* 855 ng/ml, 290-2467; p=0.005), and subjects with ΔCD4>200 had even a higher level of sCD14 in comparison with patients with ΔCD4<200 (2139 ng/ml, 210-4799 *vs* 970 ng/ml, 510-2779, p=0.04) (Fig. **4B**).

3.5. Correlation Analysis

We observed a positive correlation between the absolute number of mDCs and slanDC (r=0.773, p<0.001), between ΔCD4 and slanDCs (r=0.413, p=0.02) and pDCs (r=0.379, p=0.001) and between HLA-DR+/CD38+ CD4+ and HLA-DR+/CD38+ CD8+ (r=0.37, p=0.006).

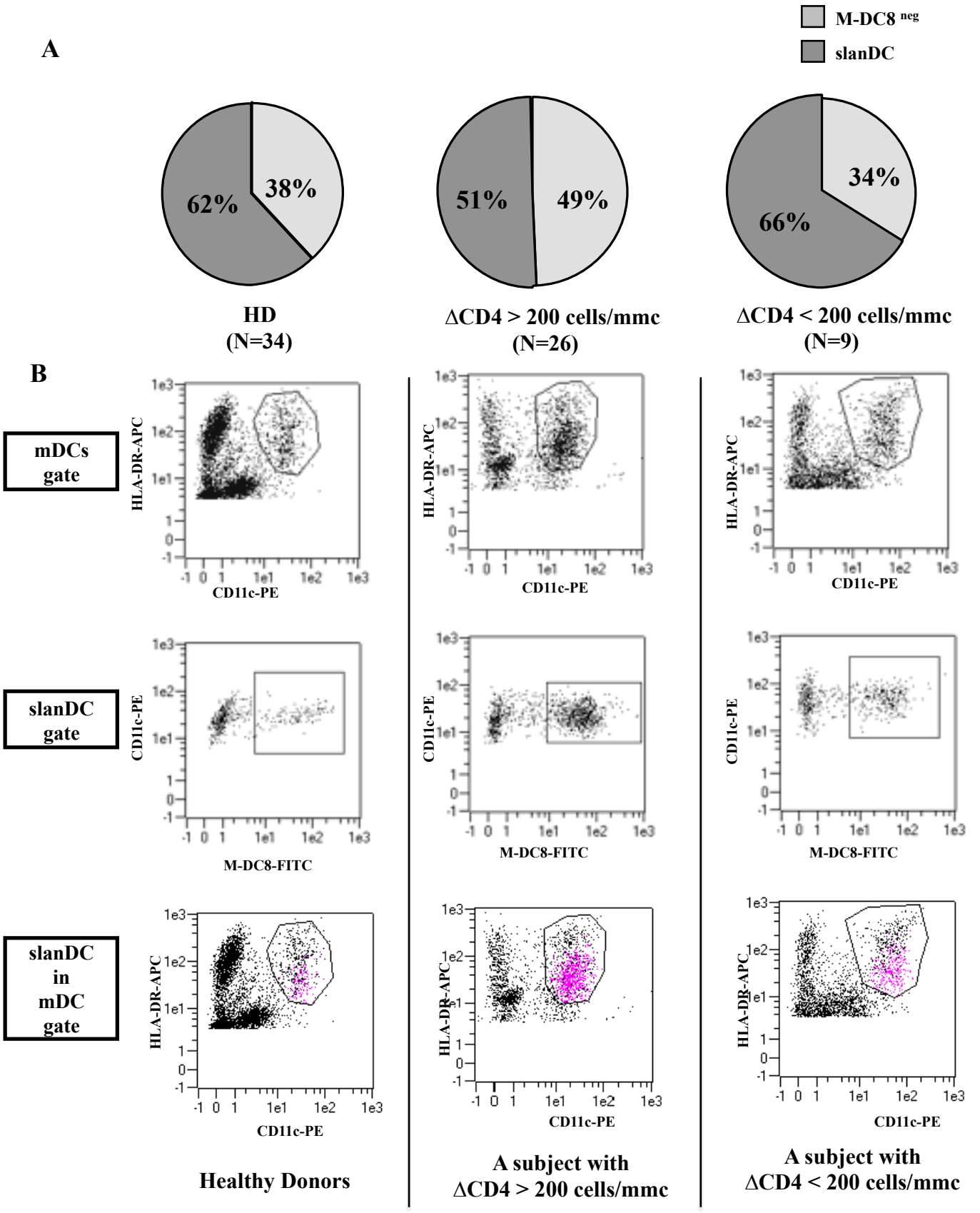

Fig (2). Frequency of slanDC (M-DC8^{+}) cells in mDC gate. Pies represent the percentage of M-DC8^{neg} *versus* slanDC (M-DC8^{+}) in healthy donors and patients with ΔCD4>200 cells/mmc and ΔCD4<200 cells/mmc (**A**). Fig. (**2B**) shows three representative analysis from an healthy donor (left panel), a subject with ΔCD4>200 (middle panel) and a subject with ΔCD4<200 (right panel). Dot plots identify mDC, slanDC, and slanDCs within mDCs gate in a cytofluorimetric backgating analysis using different colour.

Regarding sCD163 and pDCs we found a negative correlation (r=-0.241, p=0.04). No other important correlations were found.

Concerning clinical parameters, we found a positive correlation between the value of sCD14 and the years of HIV infection (r=0.3, p=0.01) and between sCD163 and age (r=0.305, p=0.01). No other correlations were found (Tables **1** and **2**).

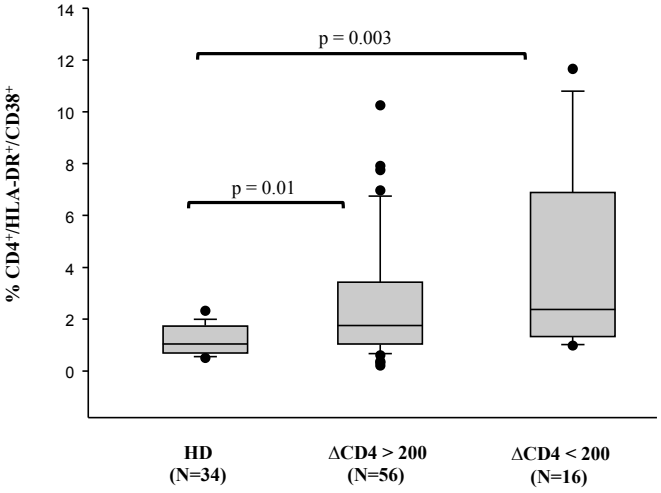

Fig. (3). HLA-DR+/CD38$^+$ CD4$^+$ T cells according to ΔCD4. Box plots represent the percentage of HLA-DR$^+$/CD38$^+$ CD4$^+$ T cells in patients and healthy donors. Activation of CD4$^+$ T lymphocytes was analyzed by using a whole blood assay no lyse no wash. Boxes show 25th, 50th (median) and 75th percentile and wiskers 10th and 90th percentile. Dots represent outlying values. Statistical significance was analyzed by using Kruskal-Wallis test. HD: healthy donors.

4. DISCUSSION

DCs are the most efficient antigen-presenting cells (APCs), expressing high levels of major histo-compatibility complex (MHC) class II molecules, with the ability to regulate both innate and adaptive immune response towards pathogens [20]. They have the capacity to localize to various tissues/organs, and migrate to lymphoid tissues after antigen acquisition, where they secrete cytokines and initiate immune response [21].

There are two major subsets of DCs, mDCs and pDCs, which differentiate from precursors found in the bone marrow and migrate to the periphery as immature cells. The effects of HIV infection on pDC have been widely investigated and previous data indicate that this DC subset is persistently impaired in number and function despite effective ART. On the other hand, there has been a renewed interest in the mDC subset that seems to play a complex role in HIV infection. mDC are involved in multiple processes of the immune response including the stimulation, maintenance and control of both immunity and inflammation. In addition, mDC cells are able to act as immune sentinels to detect and regulate aberrant immune activation, induce tolerance, avoiding autoimmune diseases [21]. A cytotoxic function was also attributed to mDC in both neoplastic [22] and other viral infections beyond HIV, such as Ebola and measles [23,24].

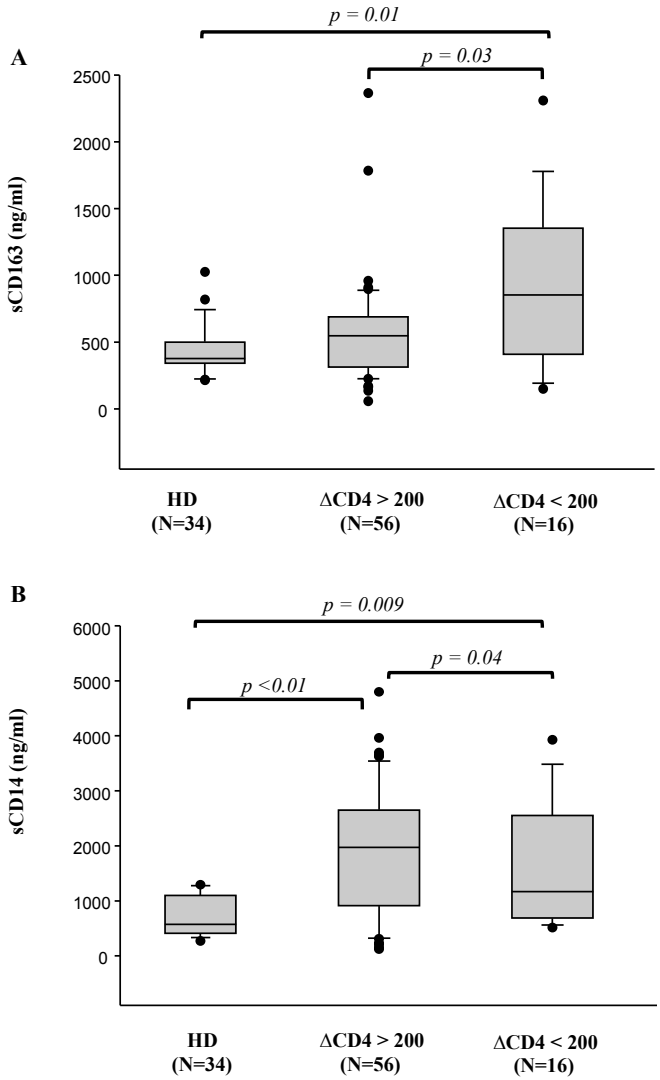

Fig. (4). sCD14 and sCD163 plasma levels. Levels of soluble immune activation markers, sCD14 and sCD163, in plasma were tested by ELISA kit. Dot plots represent the ng/ml of soluble markers in patients and control. Lines indicate the median value. Statistical significance was analyzed by using the Kruskal-Wallis test. HD: healthy donors.

In this study we evaluated the role of mDCs and in particular of slanDCs, in a cohort of HIV-infected patients undergoing effective ART. We found that HIV-infected patients with persistent undetectable viremia following long-term ART treatment had increased circulating levels of mDCs if compared to healthy donors. Interestingly, the most significant increase of these cells was found especially in subjects with a better CD4 recovery (>200 cell/mmc) suggesting a concomitant recovery of both arms of cellular immune system: the innate and the adaptive ones. However, comparing mDC level of patients with ΔCD4>200 to HD we have observed not just a recovery but a truly mDC expansion. It remains to be clarified the role of this finding in the context of HIV pathogenesis, considering the multiple functions of mDCs. In fact, mDCs are pivotal players in HIV infection, promoting transmission and spread and at the same time are critical for recognizing HIV and initiating immune responses to fight infection [25]. Considering these functional features, it can be assumed that in HIV

Table 1. Spearman's correlations between subpopulations of dendritic cells, activated T lymphocytes and soluble factors.

		mDC	slanDC	pDC	$CD4^+$ $DR^+/38^+$	$CD8^+$ $DR^+/38^+$	sCD14	sCD163
mDC	**R**		**0.773**	0.198	-0.166	-0.067	0.052	-0.0007
	p		**<0.001**	0.1	0.2	0.6	0.7	0.9
slanDC	**R**			0.255	0.029	-0.056	0.236	-0.006
	p			0.1	0.9	0.8	0.2	0.9
pDC	**R**				0.201	-0.242	0.162	**-0.241**
	p				0.1	0.08	0.2	**0.04**
$CD4^+$/ $DR^+/38^+$	**R**					**0.37**	0.041	0.234
	p					**0.006**	0.8	0.09
$CD8^+$/ $DR^+/38^+$	**R**						**-0.465**	0.239
	p						**0.01**	0.08
sCD14	**R**							0.21
	p							0.08

Table 2. Spearman's correlations between subpopulations of dendritic cells, activated T lymphocytes soluble factors and clinical parameters.

		mDC	slanDC	pDC	$CD4^+$ $DR^+/38^+$	$CD8^+$ $DR^+/38^+$	sCD14	sCD163
ΔCD4	**R**	0.28	**0.413**	**0.379**	-0.164	**-0.298**	0.096	**-0.258**
	p	0.2	**0.02**	**0.001**	0.2	**0.02**	0.4	**0.03**
Years of HIV	**R**	-0.007	0.015	0.154	0.161	-0.113	**0.3**	0.133
	p	0.9	0.9	0.2	0.2	0.4	**0.01**	0.3
Age	**R**	0.034	0.068	-0.067	-0.011	-0.05	0.145	**0.305**
	p	0.7	0.7	0.6	0.9	0.7	0.2	**0.01**
CD4 Nadir	**R**	-0.169	-0.013	0.18	-0.111	0.058	0.161	0.072
	p	0.1	0.9	0.1	0.4	0.7	0.2	0.5

pathogenesis, in which the disease progression is driven by high degree of immune activation and inflammation, mDCs may have a significant role [26]. Increase in the number and in the differentiation degree of CD11c+ dendritic cells was observed also in several autoimmune [27] and chronic inflammatory diseases [28,29] suggesting a role of these cells in sustaining inflammation in non microbial diseases. Some authors suggest that mDCs elevation was sustained by microbial translocation or asymptomatic low grade infections that act as a trigger to mDC differentiation and recruitment [30]. Enumeration of mDC in blood has been proposed as a predictive tool for monitoring chronic inflammatory disease because the augmentation in blood seems to precede the tissue involvement [31].

Recently, a significant increase in the number of circulating slanDC cells, a major myeloid-derived proinflammatory subpopulation of human blood DCs, has been described in HIV viremic patients compared to both healthy donors and virologically suppressed ART-treated patients [17,32]. Moreover, slanDCs from viremic ART naïve patients are involved in Tumor Necrosis Factor (TNF) -α production in response to LPS, suggesting a pivotal role in the pathogenesis of deleterious immune activation [17]. In a subset of our ART-suppressed HIV-infected patients we also evaluated slanDCs. We observed that the percentage of slanDCs was significantly higher in subjects with ΔCD4>200. Conversely, in subjects with ΔCD4<200 we found a lower absolute number of mDCs with a low percentage of slanDC, although the small number of patients in this group could represent a limitation. As expected, slanDCs positively correlated with mDCs and ΔCD4; moreover a positive correlation with pDCs was found. A higher percentage of slanDC, usually known as inflammatory DC, can represent the potential dangerous face of the observed myeloid expansion [16,17,33,34].

In the present study, the analysis of DC subsets was done according to the CD4+ T cell recovery, but also in the context of persistent T cell and monocytes/macrophages activation. Concerning T cells compartment, we observed higher levels of the immune activation markers HLA-DR and CD38 on CD4+ T cells, indicating persist latent HIV infection of these cells. Several studies showed an increased frequency of HLA-DR+/CD38+ CD4+ and/or CD8+ T lymphocytes in subjects with a persistent viral suppression

[6,35]. Older age and low CD4 count (and as a consequence lower ΔCD4) could be the leading factors for immune activation in our cohort of patients. Moreover in an *in vitro* model of cell to cell contact, resting memory CD4+ T cells seem to be latently infected by HIV only in the presence of mDCs [36]. Thus the high amount of mDCs found in long term ART aviremic patients may facilitate residual ongoing infection of resting T cells and supply the HIV reservoir in the tissues [36]. However, mDCs themselves can serve as reservoir for HIV, in addition to spread the virus [37]. We did not find significant differences in CD8+ T lymphocyte activation between HIV infected subjects and uninfected controls, but we observed a direct correlation between the levels of CD4 and CD8 activation. Moreover, the CD8 immune activation was inversely correlated to ΔCD4.

In order to assess whether the expansion of mDC and slanDC was associated to the residual monocyte/macrophage innate immune activation, we measured circulating levels of sCD14 and sCD163. Both sCD14 and sCD163, were found to be increased in patients with both AIDS and non-AIDS related diseases [7,8,38,39]. sCD14 exerts an inflammatory effect and is produced mainly by monocytes, whereas sCD163 has an anti-inflammatory modulatory effect and is released by monocytes/macrophages and other tissue cells. A sCD14-dependent pathway has been demonstrated in the LPS activation of DCs, especially with low levels of LPS and has been found in aviremic subjects [40]. In aviremic ART treated subject a persistent low level of LPS was described suggesting that in this population plasma sCD14 is fundamental to maintain residual immune activation [40]. Moreover, a subpopulation of circulating mDC expresses CD163, together with CD91, and is expanded in HIV-infected patients showing an immunostimulatory role besides its effect in hemoglobin scavenging and anti-inflammation [41].

In our study we found an increase in sCD14 plasma levels, more pronounced in patients with a good CD4 recovery (ΔCD4>200) suggesting persistent monocyte activation together with mDC increase also in aviremic subjects. sCD14 correlated with the years of HIV infection independently from the duration of therapy. The increase of sCD14, in part due to microbial translocation, could act as a stimulus for mDCs and slanDC production and expansion from circulating monocytes. Concomitantly this myeloid expansion could sustain the persistent bowel mucosa damage. In fact slanDCs are found in abundance in inflamed mucosal tissues [33] and they produce large amounts of TNF-α, which have an important role in the intestinal epithelial cell destruction, leading to LPS translocation [42] by setting up a vicious circle. Conversely in our study, we did not find a significant correlation between sCD14 level and mDC or slanDC number, indicating that other factors could be important in the myeloid expansion.

The other soluble factor related to myeloid activation, sCD163, seems to have an important role as a regulator factor with a quenching role in terminating inflammation, especially at tissue macrophage level [43]. In fact, together with its physiological role, which is the inhibition of cellular growth after sequestering extracellular haemoglobin iron [44], sCD163 inhibits the activation and proliferation of T lymphocytes by limiting the activation of the inflammatory response [43].

In our study sCD163 plasma levels were persistently increased in ART patients with ΔCD4<200 suggesting a potential immunosuppressive role on CD4+ T cells. As already observed [45,46], sCD163 levels correlated with age of patients, suggesting the inability of CD4 recovery in normalizing sCD163 in older HIV subjects. sCD163 levels were not related to mDC and slanDC cells, but were inversely related to pDCs that in our cohort showed a full recovery.

A limitation of our study is the lack of monocyte analysis, however a recent study showed a persistent alteration of CD16+ monocytes in spite of sCD163 normal levels under ART [8]. Besides we did not evaluate soluble plasma markers strictly related with DCs (such as TNF-α, IFN-α, IL-12, GM-CSF) since they are produced in a limited amount in plasma from ART treated subjects [17, 47-48]. Ex vivo stimulation with an intracellular assay should be performed to better characterize DCs functionality.

In conclusion HIV-infected people, who have a persistent viral suppression and good CD4 recovery after long term ART, exhibits a large expansion of mDCs associated with a higher proportion of M-DC8 expression, augmentation of sCD14 and CD4 T cells activation despite a normalization of pDC count, CD8 activation and sCD163 levels. The persistence of microbial translocation and immune activation could represent a good environment for the generation of slanDCs and mDCs from monocytes in long term aviremic ART treated patients. On the other hand, this myeloid expansion could sustain both microbial translocation at bowel mucosa level and HIV latency in long lived CD4+ T cells creating a close and vicious circle. A clinical long term follow up of HIV patients with mDC expansion is needed to understand if this increase is associated with a higher risk of non AIDS events, considering the proinflamatory effect of mDC at tissue level.

Studies that contribute to an overall understanding of the potentially detrimental roles of mDCs during viremic or aviremic HIV infection should be strongly encouraged in order to assess the clinical consequence and possible therapeutic approaches.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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