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Decreased CD1a$^+$, CD83$^+$ and factor XIIIa$^+$ dendritic cells in cervical lymph nodes and palatine tonsils of AIDS patients

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Aims: The purpose of this study was to quantify and compare the density of dendritic cells (DCs) in cervical lymph nodes (LNs) and palatine tonsils (PTs) of AIDS and non-AIDS patients.

Methods and results: Factor XIIIa, CD1a and CD83 antibodies were used to identify migratory DCs by immunohistochemistry in LNs and PTs of 32 AIDS patients and 21 HIV-negative control patients. Quantification was performed by the positive pixel count analytical method. AIDS patients presented a lower density of factor XIIIa$^+$ cells ($P < 0.001$), CD1a$^+$ cells ($P < 0.05$) and CD83$^+$ cells ($P < 0.001$) in cervical LNs and PTs compared to the non-AIDS control group.

Conclusion: Overall depletion of DCs in lymphoid tissues of AIDS patients may be predictive of the immune system's loss of disease control.

Keywords: AIDS, dendritic cell, immunohistochemistry, lymph node, palatine tonsil

Introduction

Dendritic cells (DCs) are a heterogeneous population of cells with high phagocytic activity as immature cells and high cytokine-producing capacity as mature cells that work to maintain a balance between tolerance and protective immunity.[1,2] DCs are the most potent professional antigen-presenting cells and are required to initiate immune responses. After antigen capture, DCs migrate to regional lymph nodes (LNs) via afferent lymphatics and endothelial venules, regulating T cell responses in the steady state and during infections.[3,4]

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Depending on location, function and level of maturation, DCs can present variable phenotypes. Immature DCs express CD1a and in the steady state are located at the epithelial surface of the skin, the gastrointestinal, respiratory and genitourinary tracts[5] and in lymphoid organs.[6] Factor XIIIa$^+$ DCs are located in the dermis as dermal DCs, in the interstitial areas of solid organs, and admixed with lymphocytes in the interfollicular area of the LNs.[7] During the maturation process, DCs express CD83 as thymic DCs, circulating DCs, interdigitating reticulum cells present in the T cell zones of lymphoid organs, some germinal centre cells and monocyte derived DCs.[8] However, CD1a$^+$, factor XIIIa$^+$ and CD83$^+$ cells have rarely been investigated in LNs[9,10] or palatine tonsils (PTs) of AIDS patients.

DCs are susceptible to infection by human immunodeficiency virus (HIV) *in vitro* and *in vivo*.[11,12] HIV

causes a marked depletion of DCs in HIV-1-positive patients, and impaired cytokine secretion and ability to stimulate T cells.[13,14]

We have previously reported the depletion of immature DCs in the intraepithelial areas of tongues from AIDS patients with different opportunistic infections.[15] In this work, we describe the effects of HIV infection on DCs in cervical LNs and PTs.

Materials and methods

The Ethics Committee of the University of Campinas and University of São Paulo approved this study and the use of autopsy material according to ethical guidelines.

PATIENT POPULATION

Cervical LNs and PTs from 32 autopsied patients with AIDS were collected at the University of São Paulo between 1997 and 2011. In addition, 21 uninfected patients who had LNs and PTs dissected between 2010 and 2012 for diagnostic or therapeutic reasons at the University of Campinas and Instituto de Anatomia Patologica (Piracicaba, SP) were included in the study. Clinical charts and autopsy reports from all patients were reviewed and the last $CD4^+$ and $CD8^+$ T cell counts prior to the deaths of the 32 AIDS patients were obtained.

TISSUE PROCESSING

Cervical LNs and PTs were fixed in 10% formalin for 24 h and six transverse sections were obtained. All fragments were embedded in paraffin and 3-μm thick histological sections were submitted to H&E staining and immunohistochemistry.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry using monoclonal antibodies against CD1a (010; Dako, Glostrup, Denmark; 1:80), CD83 (1H4b; Novocastra, Newcastle, UK; 1:20) and factor XIIIa (E980.1; Novocastra; Newcastle, UK; 1:100) was carried out for the identification of DCs in the cervical LNs and PTs. Antigen retrieval with citrate was performed for all antibodies. The slides were incubated with secondary antibodies conjugated with biotin (LSAB + System-HRP, mouse/rabbit; Dako) for 30 min at 37°C, followed by streptavidin-horseradish peroxidase (Dako) for another 30 min, and developed with chromogenic substrate (3,3′-diaminobenzidine; Sigma, St Louis, MO, USA). The slides were counterstained with Harris haematoxylin. Negative controls were performed for each reaction, omitting the primary antibodies.

VIRTUAL MICROSCOPIC AND IMAGE ANALYSIS

Glass slides were scanned with the ScanScope CS system (Aperio Technologies, Inc., Vista, CA, USA) to produce a high quality resolution digital image. We performed a quality check before scanning to eliminate poor quality sections with faint staining. Quantification was performed by the positive pixel count algorithm method using ImageScope software (Aperio Technologies, Inc., Vista, CA, USA). Positive pixel count is a multipurpose algorithm that quantifies the area and intensity of stains. Pixels were classified according to staining intensity and colour.[16–18] The staining intensity was classified as strongly positive (red), positive (orange), weakly positive (yellow) or negative (blue) (Figure 1). A minimum area of 1 mm^2 of lymphoid tissue was considered and the microscopic analysis was performed at full ×20 magnification. The results were expressed as the area of positive staining as a percentage of the total area analysed.

STATISTICAL ANALYSIS

Statistical differences between groups were estimated using a standard non-parametric test (Mann–Whitney *U*-test). The results are presented as median and interquartile ranges. Differences at $P < 0.05$ were considered as statistically significant. The statistical software SPSS version 17.0 was used for the analyses.

Results

CLINICAL FINDINGS

The control group consisted of lymphoid tissue from 12 male patients and nine females. The mean age was 38.7 ± 17.2 years (range 15–84 years). The main clinical diagnoses in control patients were: diabetes, hepatitis B, cardiovascular disease, asthma and obesity.

The AIDS group consisted of 24 males and eight females. The mean age was 37.4 ± 10.5 years (range 15–69 years). The mean $CD4^+$ T lymphocyte blood counts were 74 ± 104 cells/μl. The mean $CD4^+/CD8^+$ T cell ratio was 0.21 ± 0.35. The main autopsy findings in the AIDS patients were: pneumonia, pneumocystosis, pulmonary thromboembolism, septic shock, tuberculosis and meningitis.

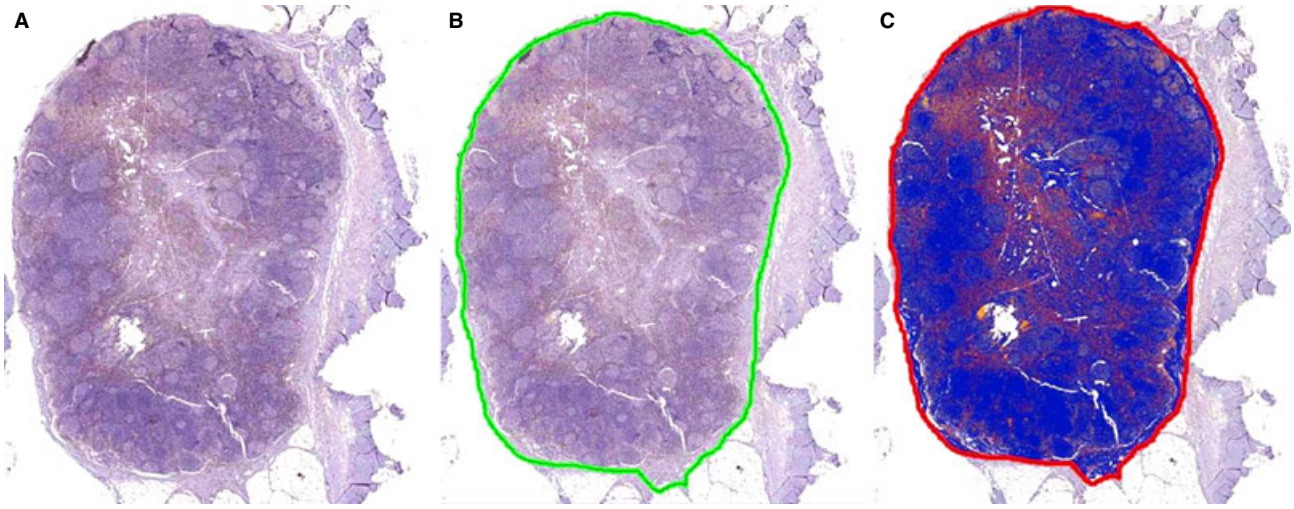

Figure 1. Immunostaining evaluation method using Image Scope software. **A**, Factor XIIIa$^+$ cells in a cervical lymph node from a control patient. **B**, Delimitation of the area to be analysed. **C**, Positive pixel count function measuring the intensity of staining according to specific colour (blue = negative, yellow = weakly positive, orange = medium positive, red = strongly positive).

MICROSCOPIC FINDINGS

Microscopically, factor XIIIa, CD1a and CD83 staining showed positive cells with abundant cytoplasm, ill-defined borders and interdigitating processes. These cells were located mainly in the T cell areas of secondary lymphoid organs. In control individuals, we observed a higher density of all markers in cervical LNs and PTs (Figures 2 and 3).

DENDRITIC CELL QUANTIFICATION

Patients with AIDS had a smaller percentage of DCs in cervical LNs (factor XIIIa$^+$ cells, $P < 0.001$; CD1a$^+$ cells, $P = 0.048$; and CD83$^+$ cells, $P < 0.001$) and PTs (factor XIIIa$^+$ cells, $P < 0.001$; CD1a$^+$ cells, $P = 0.027$; and CD83$^+$ cells, $P < 0.001$) compared to the control group (Figure 4).

Within the AIDS group, there was no statistically significant difference in the percentage of DCs between cervical lymph nodes and palatine tonsils (Factor XIIIa$^+$ cells, $P = 0.989$; CD1a$^+$ cells, $P = 0.998$; and CD83$^+$ cells, $P = 0.999$). A similar finding was observed comparing the two anatomical regions for the control group (Factor XIIIa$^+$ cells, $P = 0.659$; CD1a$^+$ cells, $P = 0.994$; and CD83$^+$ cells, $P = 0.665$). The data are shown in Table 1.

Discussion

The continuous migration of DCs into secondary lymphoid organs such as LNs represents a fundamental element of immune surveillance and homeostasis.[19] Within LNs, DCs present antigens to cognate T cells, resulting in the induction of protective immunity.[20,21] In this study, we showed that the density of Factor XIIIa$^+$, CD1a$^+$ and CD83$^+$ DCs in cervical LNs and PTs from AIDS patients was less than in non-AIDS patients. These findings extend the results seen in a previous study from our group that analysed the Langerhans' cells population, a subset of DCs, in the tongues of AIDS patients,[15] demonstrating that the HIV virus promotes the overall depletion of DCs in lymphoid organs as well as oral tissues.

In the current study, we worked with automated counting for analysing the immunoreactive cells. Computational analysis programmes offer the possibility to eliminate the inherent variability of pathologist-based scoring and to increase the sensitivity of quantitative studies. Some researchers have introduced computerized analysis for counting immunoreactive cells. Slodkowska *et al.*[22] analysed the accuracy of automated Ki-67 labelling index counting in central nervous system tumours after digital copies of entire sections were created automatically by the Aperio ScanScope CS device. Their data indicated that the remote automated analysis of slide images could be used successfully as an alternative method to manual reading. Alvarenga *et al.*[17] used automated evaluations (ScanScope XT, Aperio; and ACIS III, Dako, Glostrup, Denmark) of tissue microarray patterns of protein expression, and compared these with the findings from manual analysis. The results showed better positive pixels or nuclei determination and labelling intensity identification by digital systems than manual reading. In addition, Lloyd *et al.*[23] assessed the

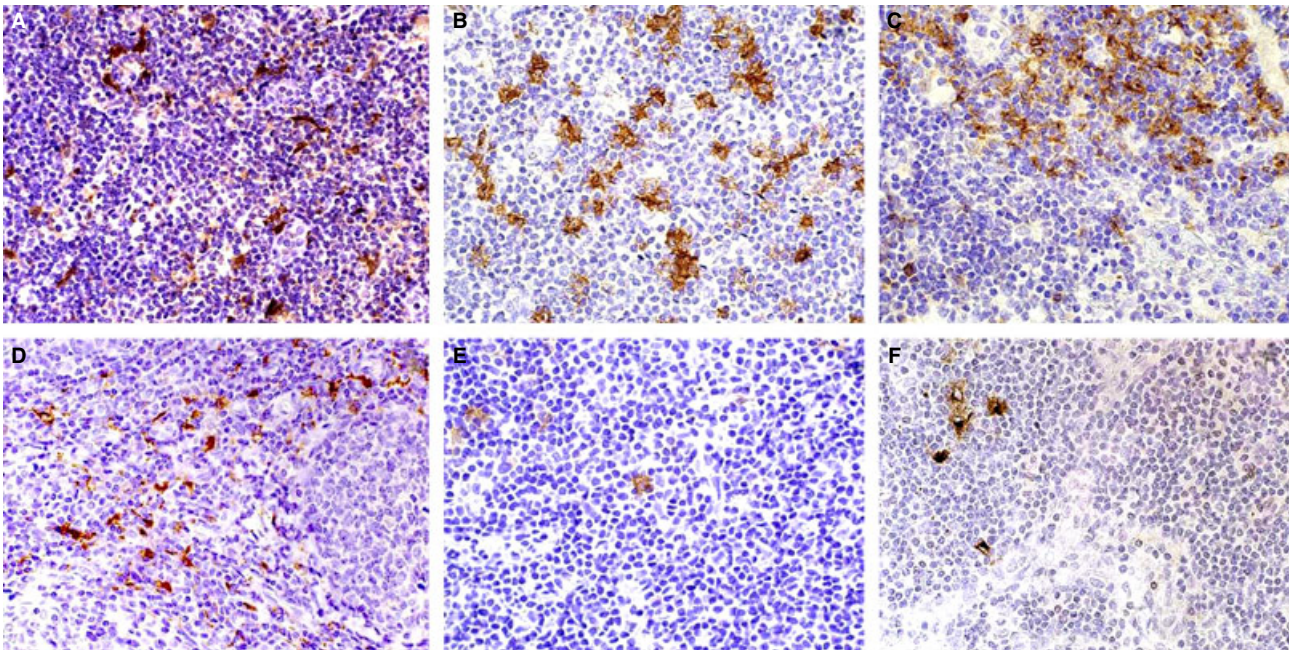

Figure 2. **A**, **B**, **C**, Immunohistochemical detection of factor XIIIa$^+$, CD1a$^+$ and CD83$^+$ cells, respectively, in cervical lymph nodes in the control group. High concentrations of positive cells in the paracortical T cell zone through interfollicular areas of the cortex. **D**,**E**,**F**, Factor XIIIa$^+$, CD1a$^+$ and CD83$^+$ cells, respectively, in the cervical lymph nodes in the AIDS group showing profound depletion of dendritic cells.

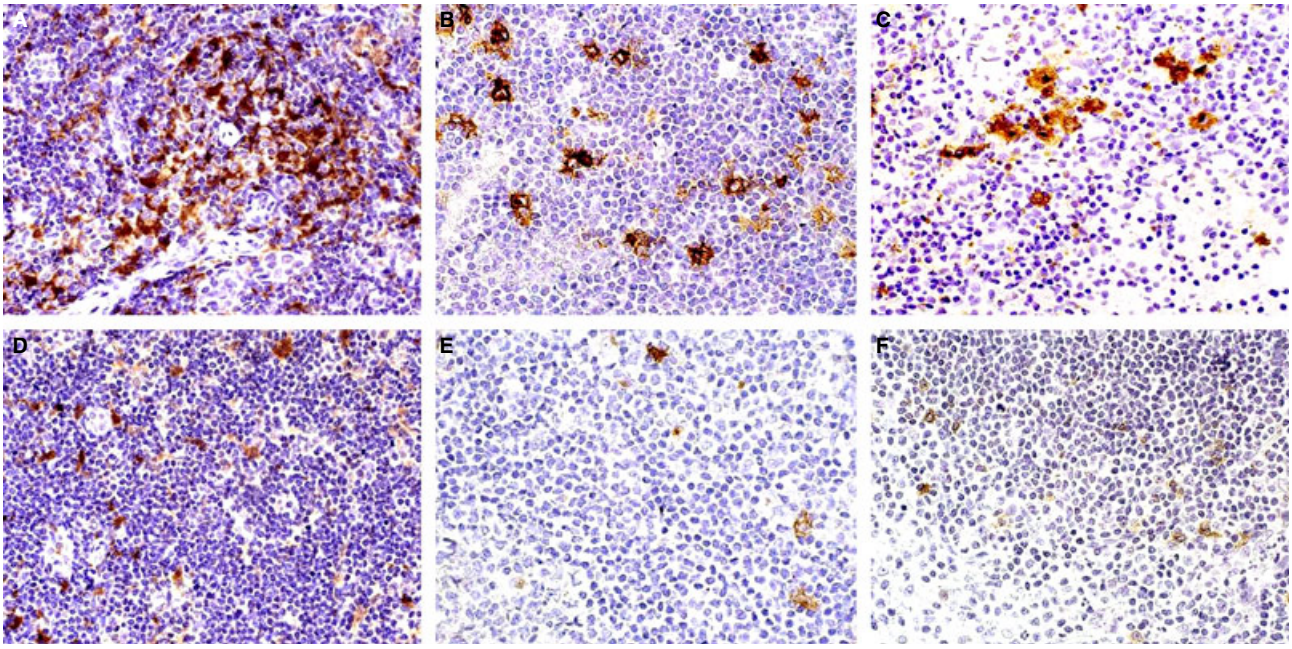

Figure 3. Representative immunohistochemical staining of palatine tonsils. **A**, **B**, **C**, Factor XIIIa$^+$, CD1a$^+$ and CD83$^+$ cells, respectively, in the control group. **D**,**E**,**F**, Depletion of factor XIIIa$^+$, CD1a$^+$ and CD83$^+$ cells, respectively, in the AIDS group.

accuracy and reliability of two image analysis systems, Aperio ScanScope XD (Aperio) and Definiens Tissue Studio version 1.2 (Munich, Germany), in the evaluation of prognostic and predictive immunomarkers for breast cancer, and compared the results directly with semi-quantitative scores. The automated

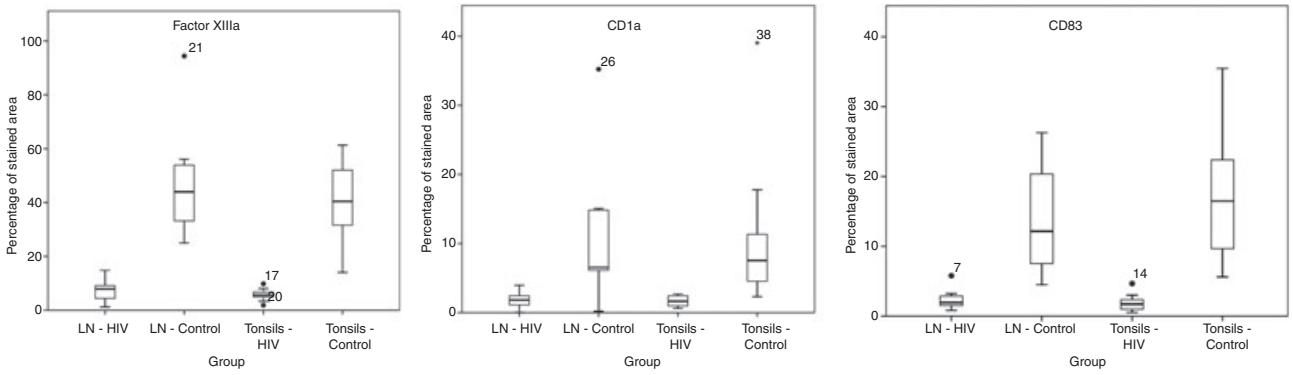

Figure 4. Comparative analysis of factor XIIIa$^+$, CD1a$^+$ and CD83$^+$ cells in cervical lymph nodes and palatine tonsils between the study groups. LN, lymph nodes.

Table 1. Cell density in cervical lymph nodes and palatine tonsils

	Cell density* (%) in			
	Cervical lymph node		Palatine tonsil	
Cell types	AIDS patients	Control group	AIDS patients	Control group
Factor XIIIa	7.52 ± 3.83	44.53 ± 20.18†	5.76 ± 2.24	38.03 ± 13.70†
CD1a	2.05 ± 0.98	11.83 ± 9.25‡	1.62 ± 0.77	11.28 ± 12.13‡
CD83	2.31 ± 1.44	15.48 ± 9.66†	1.94 ± 1.21	16.47 ± 11.28†

*The percentage of stained area to the total area of section observed.
$^\dagger P < 0.001$ in relation to AIDS group.
$^\ddagger P < 0.05$ in relation to AIDS group.

evaluations were highly reproducible, and removed the subjectivity associated with manual or visual inspection of the stains.

According to Donaghy *et al.*,[12] the decline in circulating DCs correlates with an increase in HIV-1 viral load and this depletion correlates with disease progression. HIV-1-exposed DCs retain their ability to mature phenotypically, which is crucial to their transition into potent antigen-presenting cells.[24] Moreover, the loss of DCs from blood could be a consequence of the increased migration of DCs to lymphoid organs or insufficient repopulation of blood DCs.[25]

DC biology suggests that half of the DCs within LNs enter from the bloodstream and the other half are believed to arise from lymph-migrating tissue DCs.[26] Within interfollicular areas of the lymph node cortex, the fibroblastic reticular cell network extends close to the subcapsular sinus floor and serves as a port of entry for cells arriving with the afferent lymph.[27,28] Activated DCs arrive largely via afferent lymph and remain confined to the subcapsular sinus of the afferent side of the lymph node, migrating towards the deeper paracortical T cell zone through interfollicular areas of the cortex.[19,29,30] Only a minority of lymph node DCs in the steady state arise from DCs residing in upstream non-lymphoid organs. Instead, DCs that migrate through lymphatics from upstream tissues contribute substantially to the pool of lymph node DCs, mainly during inflammatory states.[31]

Immature and mature DCs were thought to occur initially in specific anatomical locations: immature DCs capture antigens in peripheral tissues, whereas mature DCs present those antigens on their MHC molecules in the T cell areas of the draining lymphoid organs.[32] However, it has been shown that lymphoid organs such as tonsil, lymph node, thymus and spleen also contain large cohorts of immature DCs.[6,33] These DCs can either respond to infections reaching those organs and mature *in situ*, or play a role in the maintenance of immunological peripheral tolerance.[34,35] This condition was observed in our study as the high density of factor XIIIa$^+$ cells (immature DCs) in lymphoid tissues compared to CD83$^+$

cells (mature cells). Moreover, factor XIIIa antibody can also be detected in a number of monocyte-derived macrophage lineages, including macrophages, histiocytic and dendritic reticulum cells, in LNs.[36,37] Conversely, the density of $CD1a^+$ cells was less than that shown by the other antibodies because mature DCs, which are common in secondary lymphoid organs, express low levels of CD1a.[38]

After HIV crosses the mucosa or skin, it reaches the lymphoid tissue and permanent infection is established. The interaction between DCs and T cells in lymphoid tissue is critical for the generation of immune responses. DCs are suggested to play a crucial role in the early events of HIV transmission by transporting the virus from the peripheral tissues to the lymphoid system and creating a perfect microenvironment for HIV-1 replication.[39,40] Some studies have suggested that DCs maintain their ability to stimulate T cells after HIV exposure.[41,42] During HIV infection, the majority of DCs can most probably respond to stimulation and present antigen to T cells, although the quality of the response may be altered and the expression of molecules lower than that normally found in lymphoid tissues.[25,43]

Nevertheless, factor $XIIIa^+$, $CD1a^+$ and $CD83^+$ cells were located mainly in the cortical areas of LNs, in agreement with other authors,[44,45] who reported that DCs were located in the T cell-dependent areas of lymphoid tissues, in the skin, lungs and gut where antigens enter the body. The main DCs located in the T zone are designated interdigitating DCs, representing a population of mature DCs with high potential to stimulate lymphocytes and with high-level expression of MHC class II and cytokines, including IL-6, IL-10 and IL-13.[1,46,47]

Several investigations have demonstrated that DCs are also involved in regulating T cell-mediated humeral immune responses, indicating that naive B cells interact with DCs in human tonsils.[46,48] Interdigitating DCs interact with the large number of resting naive B cells directly in the T cell area of the lymph node and are positive for mature-DC markers such as CD80, CD83 and CD86.[49,50]

DCs may influence B cell responses against HIV through contact or production of B cell growth factors, such as B lymphocyte stimulator (BLyS/BAFF), that modulate the outcome of CD4 T cell HIV effectors.[51] Some longitudinal studies involving HIV patients with different rates of disease progression have shown that DC levels were reduced in the blood, beginning in the acute phase of infection and persisting throughout the course of disease despite successful therapy.[52] This condition has been correlated with increased serum levels of DC-trophic chemokines and drainage to peripheral sites, and has also been associated with inflammatory conditions.[53,54]

The present study had some limitations. We studied the population of DCs in lymphoid tissues of patients with AIDS and severe immunosuppression because most of the patients died before highly active antiretroviral therapy (HAART) initiation. Hence, it would be interesting to study the distribution of DCs in LNs of HIV-positive patients at different stages of disease or on HAART. A better understanding of the tissue distribution of DCs in HIV-infected patients may contribute to the development of new therapeutic strategies and to inducing favourable adaptive anti-HIV immunity.[43]

In conclusion, advanced-stage AIDS patients have a profound depletion of DCs in cervical LNs and PTs that may be predictive of the immune system's loss of disease control. Advanced understanding regarding the role of DCs in HIV-1 infection will be important for establishing antiviral mechanisms and/or control of viral spread.

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