Morphometric analysis of dendritic cells from anal mucosa of HIV-positive patients and the relation to intraepithelial lesions and cancer seen at a tertiary health institution in Brazil[1]

Análise morfométrica das células dendríticas da mucosa anal de pacientes HIV-positivos e relação com as lesões intraepiteliais e o câncer numa instituição de saúde terciária no Brasil

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ABSTRACT

PURPOSE: To morphometrically quantify CD1a+ dentritic cells and DC-SIGN+ dendritic cells in HIV-positive patients with anal squamous intraepithelial neoplasia and to evaluate the effects of HIV infection, antiretroviral therapy and HPV infection on epithelial and subepithelial dendritic cells.

METHODS: A prospective study was performed to morphometrically analyze the relative volume of the dendritic cells and the relationship between anal intraepithelial neoplasia and cancer in HIV-positive patients from the Tropical Medicine Foundation of Amazonas, Brazil. All patients were submitted to biopsies of anorectal mucosa to perform a classic histopathological and immunohistochemical analysis, employing antibodies against CD1a and DC-SIGN for the morphometric quantification of dendritic cells.

RESULTS: HIV-negative patients displayed a CD1a DC density significantly higher than that of HIV-positives patients (3.75 versus 2.54) (p=0.018), and in patients with severe anal intraepithelial neoplasia had correlated between DC CD1a density with levels of CD4 + cells (p: 0.04) as well as the viral load of HIV-1 (p: 0.035). A not significant rise in the median density of CD1a+ DC was observed in the HIV positive/ HAART positive subgroup compared to the HIV positive/ HAART negative subgroup. The CD1a+ DC were also significantly increased in HIV-negative patients with anorectal condyloma (2.33 to 3.53; p=0.05), with an opposite effect in HIV-positive patients.

CONCLUSIONS: Our data support an enhancement of the synergistic action caused by HIV-HPV co-infection on the anal epithelium,

weakening the DC for its major role in immune surveillance. Notoriously in patients with severe anal intraepithelial neoplasia, the density of CD1a+ epithelial dendritic cells was influenced by the viral load of HIV-1. Our study describes for the first time the density of subepithelial DC-SIGN+ dendritic cells in patients with anal severe anal intraepithelial neoplasia and points to the possibility that a specific therapy for HIV induces the recovery of the density of epithelial DC.

Keywords: Dendritic Cells. HIV. Anus Diseases. Neoplasms.

RESUMO

OBJETIVO: Quantificar morfometricamente as células dendríticas DC CD1a+ e DC DC-SIGN+ em pacientes HIV positivos portadores de neoplasia escamosa intraepitelial anal e avaliar os efeitos da infecção pelo HIV, da terapia antirretroviral e da infecção pelo HPV sobre as células dendríticas epiteliais e subepiteliais.

MÉTODOS: Um estudo prospectivo foi realizado para analisar morfometricamente o volume relativo das células dendríticas e as relações entre neoplasia intraepitelial anal e o câncer em pacientes HIV positivos da Fundação de Medicina Tropical do Amazonas, Brasil. Todos os pacientes foram submetidos a biópsia da mucosa retal para realizar uma análise clássica histopatológica e imunohistoquímica utilizando anticorpos contra anti-CD1a e anti-DC-SIGN, para a quantificação morfométrica das células dendríticas.

RESULTADOS: Os pacientes HIV negativos apresentaram densidade das DC CD1a+ significativamente maior do que a dos pacientes HIV positivos (3,75 versus 2,54) (p:0,018), e os pacientes com severa apresentaram correlação das DC CD1a com os níveis de células TCD4(p:0,04) assim como a carga viral do HIV-1 (p:0,035). Observamos no subgrupo HIV-positivo/HAART positivo elevação não significativa na mediana da densidade das DC CD1a+ em relação ao grupo HIV-positivo/HAART negativo. As DC CD1a+ também se elevaram nos pacientes HIV negativo portadores de condiloma anorretal(2,33 para 3,53; p:0,05), com efeito inverso nos pacientes HIV positivos.

CONCLUSÕES: Nossos dados confirmam a potencialização da ação sinérgica representada pela coinfecção HIV-HPV sobre o epitélio anal, fragilizando as DC em sua função primordial de vigilância imune. Notoriamente nos pacientes com neoplasia intraepitelial anal grave, a densidade das DC CD1a+ epiteliais sofreu influência da carga viral do HIV-1. Nosso estudo descreveu pela primeira vez a densidade das DC subepiteliais DC-SIGN+ em pacientes com neoplasia intraepithelial anal severa e apontamos para a possibilidade de que a terapia específica para o HIV induza a recuperação da densidade das DC epiteliais.

Descritores: Células Dendríticas. HIV. Doenças do Ânus. Neoplasias.

Introduction

Anal cancer is a rare pathology among the population in general. The incidence of this cancer has been increasing in the last few decades and has risen by thirty times in patients who are practitioners of receptive anal sex; with incidence of 70/100,000 in HIV positive (+) men who have sex with men (MSM)[1,2].

Among the factors causing anal cancer in immunodeficient patients is a compromised cell immunity, particularly to the dendritic cells (DC)[3,4] These cells are strategically located at the interaction sites between the individual and the environment; because of their characteristic function of capturing and presenting antigens to the CD4+ T cells (i.e., to trigger the specific immune response), DC carry viral particles to the lymphatic stations, favoring CD4+ T cells infection and the deregulation of the immune system by the virus[5].

The infection of DC by HIV seems to hinder the capacity of the DC to stimulate T cells due to the inhibition of cytokine secretion (chiefly IL-12), loss of maturation capacity (through a decrease in the expression of a co-stimulating molecule) and MHC deficiency, which delays the immune response[5,6]. In high degree anal intraepithelial neoplasia (AIN), patients have a decrease in the inflammatory response, TH1 type, characterized by a decrease in IL-2, IFN-γ and TNF-α[7].

The mechanism by which anal cancer is established has not yet been elucidated, but HPV-HIV interactions and the performance of dendritic cells in immune surveillance are known to be crucial in the development of this cancer.

We therefore conducted a comparative study of the density of dendritic cells in HIV-positive patients with anal squamous intraepithelial neoplasia with or without HPV infection. The anal samples were analyzed by histopathology and immunohistochemistry to perform morphometric analysis of the density of CD1a+ DC and DC-SIGN+ DC in the anorectal epithelium. Statistical analysis was used to evaluate the effects of HIV infection, antiretroviral therapy and HPV infection on DC in different groups.

Methods

A nonparametric prospective observational analytic study was performed to assess the relationship between dendritic cells and AIN or anal mucosa cancer of patients at the main institution for the treatment of HIV/AIDS in the Amazon region, Tropical Medicine Foundation of Amazonas (FMTAM), Brazil. The participants were divided into two groups: 1) the case group (n=59), which was comprised of HIV-positive patients of both sexes whom were under specialized medical monitoring in the institution and 2) the control group (n=55), which was comprised of HIV-negative patients referred to the service due to proctologic complaints.

All of the patients were given information regarding the objectives of the project as well as the benefits and risks of the research. Those who agreed to participate were asked to sign a consent form. This study was initiated only following approval by the FMTAM ethical research committee (n:3137105).

Statistical analysis

To assess the association between the categorical variables, the HIV result and the AIN occurrence, a Pearson chi-square statistical test and Fisher exact test were used for 2x2 tables, as indicated. The variance analysis test was used in the comparison of the averages, and when the normality hypothesis was not accepted; the *Kruskal-Wallis test* was used, depending on the number of comparisons[9,10]. Epi-Info 3.3.2 software was used in the analysis, and the level of significance used in the tests was 0.05.

Definition of groups and highly active antiretroviral therapy (HAART) according to the classification of the Center for Disease Control (CDC)

Control patients - patients who were negative for HIV, as determined by performing immunoassay-ELISA and indirect immunofluorescence; **HIV-positive patients without AIDS-**defined as patients with a serologic diagnosis of HIV, but whom did not show any of the defining illnesses suggestive of AIDS or have a CD4+ T cells count greater than 201 mm^3 (i.e., classified as A1, B1, A2 or B); and **AIDS patients-**patients who displayed one or more aids defining illness with CD4+ T counts below 200 and/or were being treated with HAART.

We analyzed the antiretroviral drugs taken by patients with AIDS and noted the large number of drug combinations. For the purposes of analysis, the most frequent associations were studied as outcome variables. ZDV or AZT: zidovudine, 3TC: Lamivudine, EFV: Efavirenz, NVP: Nevirapine, TDF: Tenofovir,

LOP/R or LPV/R: Lopinavir / Ritonavir.

Anal examination

All of the patients were submitted to high resolution anoscopy. The digital rectal examination was performed and was followed by an inspection of the mucosa with the use of a 16 to 40 times magnification optical colposcope after gauze soaked in 3% acetic acid was introduced for two minutes. The anoscope was reintroduced for the performance of the anal canal and rectum examination under image magnification. The findings were registered and the predominant acetowhite areas were biopsied.

Histological analysis

The samples were submitted to a pre-fixation with zinc (*IHC Zinc fixative*, ref 550523, BD Pharmingen®) and prepared for histological analysis, followed by processing for inclusion in paraffin blocks. Histological sections of 4 μm were stained with Hematoxylin-Eosin and examined under a light microscope[11].

The purpose of the histological exam was to assess the presence of AIN I, AIN II/III, squamous cell carcinoma, adenocarcinoma and inflammatory diseases[12,13].

HPV typing

Genotyping of HPV present in the samples of the anal canal was performed using a nested PCR technique. Amplification of HPV-specific DNA was first performed using the consensus primer MY09/MY11 and was sequentially performed from the amplicons of positive reactions using a mix containing the consensus primer GP5/GP6. DNA sequencing of amplicons from positive reactions was performed with 5 μL of primer MY09 or MY11 or GP5 or GP6 mixed with 4 μL of premix DYEnamic ET Terminator Kit (Amersham Bio- sciences, Piscataway, New Jersey). The product of this reaction was sequenced by electrophoresis, aligned (CLUSTAL W; Bio- Edit, Carlsbad. California) and the nucleotide sequences were compared to the GenBank sequences by the BLAST program. A known sequenced HPV-positive sample served as a positive control and water as a negative control.

Immunohistochemical analysis

The anti-CD1a (IgG1, κ, MTB1, ref. 235-L-CE, Novocastra®) and the anti-DC-SIGN (CD-209, mouse IgG$_{2b}$, κ, ref. 551249, BD Pharmingen®) primary antibodies (diluted in PBS in the validated proportions of 1:30 and 1:50, respectively) were used on distinct slides from the same patient and incubated overnight in a humidified chamber at 4°C. The sections were incubated with the ready-to-use biotinylated secondary antibody (Novocastra®,

ref. 103) or a biotinylated goat anti-mouse polyclonal Ig (ref. 550337, BD Pharmingen®) at a 1:150 dilution for 60 minutes, followed by application of streptavidin (Novocastra®, ref 104) for 30 minutes. Diaminobenzidine (DAB) 60% was used to reveal the stages the light shelter. The specificity of the immunoreaction was investigated by omitting the primary antibody.

Determination of the volume density (Vv) of the dendritic cells

The volume density, or relative volume, of the dendritic cells was determined using Delesse's Principle, which estimates the volume fraction of a phase of interest (dendritic cells) within a reference volume (epithelial or subepithelial region) of an anal biopsy[14]. A test system (point grid) that combines two sets of points of different densities in the same grid was used for this propose. The fine points (crosses and circled crosses) were used for counting the CD1a+ DC and DC-SIGN+ DC cells, while the coarse points (circled crosses only) were used for counting the reference space (epithelial and subepithelial regions of the biopsy)[14].

The cellular points which touched the test system, fine points hitting dendritic cells, were quantified as having a maximum of 7 consecutive fields and a minimum of 4 fields when 200 cells of interest were counted[14] at an optical magnification of 100 x (Figures 1 and 2). To avoid confusion with the melanocytes of the basal layer, the marked cells in this region were computed separately.
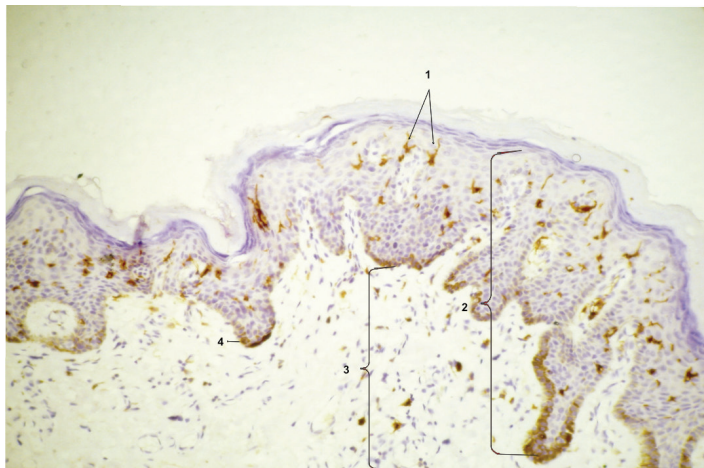

FIGURE 2 - Immunohistochemical staining of a serial section of rectum. **3**=cells of lamina propria, **5**=columnar epithelium, **6**=rectal glands, **7**=DC-SIGN+DC, DC=dendritic cells. Magnification=400X.

Results

The study included 114 patients; 59 patients were male and 51.7% were seropositive for HIV. The characteristics of the group and the main behavioral factors implicated in anal carcinogenesis were analyzed. The mean age was 38.3 years (range of 14-78) and 77.5% (31/40) of patients said that they were HIV-positive homosexual / bisexual men (OR=0.18; p<0.001). Among these patients 81.8% reported having had more than 10 partners in the last 5 years (p <0.001).

Analysis of co-infection with HIV-HPV

PCR was performed to genotype for HPV in 91 samples, and no significant difference between the HIV-positive and -negative group was shown (p=0.09; n=47). The most prevalent type was HPV type 6, which was detected in 18 patients; 10 of these patients had AIN I and 2 had AIN II/ III. We identified the highly oncogenic HPV serotypes 53, 58 and 16; the latter serotype was detected in five patients, among whom four were HIV + / HAART + and three had AIN. Many other less prevalent HPV types were found, including the serotypes 11 (n=4), 70 (n=4), 61 (n=3), 58 (n=3) and 33 (n=3). Types 31, 66, 85, 82, 71, 18 and 72 were each found in only one sample.

The analysis of anal intraepithelial neoplasia revealed a high rate of severe AIN in HIV-positive patients

The histopathological analysis of both groups indicated that 49/114 patients had AIN; of these patients with AIN, 71.9% showed AIN I and 22% showed AIN II/III. Fifty-nine (51.8%) examinations were normal. HIV-positive patients are admittedly

FIGURE 1 - Immunohistochemical staining of a serial section of positive skin control. **1**=CD1a+ DC, **2**=anal squamous epithelium, **3**=cells of lamina propria, **4**=melanocyte or CD1a+DC in basal layer, DC=dendritic cells. Magnification=100X.
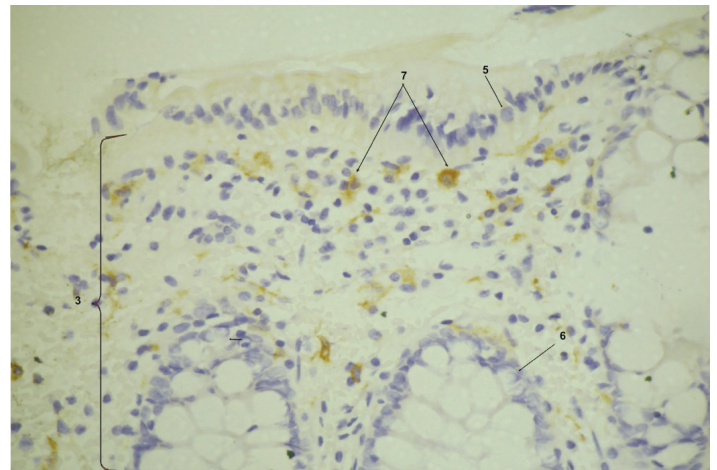
more susceptible to anal cancer and its precursor lesion (AIN), but this study showed no difference in the prevalence of AIN among HIV-positive and HIV-negative individuals. AIN was present in 40% of HIV-negative cases (22/55), with 77.2% and 22.7% AIN I and AIN II/III, respectively. In the 53.8% (28/53) of cases representing the HIV-positive group with AIN, 71.4% (20/28) showed low AIN and 28.6% (8/28) showed severe AIN (p=0.09). The prevalence of AIN differed significantly in relation to the practice of receptive anal sex (p <0.006) and the occurrence of STDs (p <0.001). Only one case of anal squamous cell carcinoma was described in an HIV-negative elderly woman.

Influence of antiretroviral therapy on CD4+ T cells

The average duration of HAART was 25 months (range 2-103 months) in this study. No difference was observed in the incidence rates of HPV and AIN when comparing groups with a treatment time of 18 months, 19-36 months and more than 37 months. A comparison of the CD4+ T levels demonstrated a significant difference, pointing to the efficacy of prolonged treatment (p= 0.001, data not shown). The AZT + 3TC + EFV/ NVP treatment regimen was used by 18 patients and 4T3C + DDI + NVP/RTV was used by 14 patients. Patients who received the first drug combination had a median CD4 + T cell count that was 50% lower than those using TDF +3 TC + LOP / R (p= 0.041) (data not shown).

The mean CD4+ T count was 369.6 x 10^6 cells/liter (l) in HIV-positive patients and the viral load of HIV-1 was 40,152 copies/mm (n=41). No difference was present in the time of use of HAART or the occurrence of AIN in patients on HAART (p=0.08, p= 0.52); only the log of viral load of HIV-1 levels and CD4+ T cells were different (p=0.012, r=-0.40, n=38; data not shown).

Analysis of CD1a+ dendritic cells and DC-SIGN+DC anorectal mucosa

We analyzed the CD1a+ and DC-SIGN+ dendritic cells of anorectal mucosa. The analyses of the division and the sum of the median density of these cells constituted the main objectives of this study. Analysis of CD1a+ DC showed a median density of 2.91 in the HIV-negative without AIN samples (n=33) (the control group), 1.74 in the HIV+/HAART- subgroup (n=6) and 2.54 in the HIV+/ HAART+ subgroup (n=51) (p=0.371). A difference was present in the CD1a+ DC when comparing the whole HIV-negative group (median of 3.75, n=46) with the whole HIV-positive group (median of 2.54, n=51) (p=0.018) (n=97) (Table 1).

TABLE 1 - Significant findings involving dendritic cells.

GROUP/ DC (density)	Variable 1(n)	Variable 2(n)	p-value/n
Pat. HIV+ and HIV-: CD1a+ DC (median)	HIV+= 2.54(51)	HIV-: 3.75(n=46)	0.018 (n=97)
Pat. HIV+ and HIV-: CD1a+ DC (median)	No STD: 2.33 (n=53)	STD: 3.53 (n=50)	0.05(n=103)
Pat. with condyloma: CD1a+ DC (median)	HIV- 5.3	HIV+ 1.9	0.04
Pat. HIV+: T CD4+(x10^6 cell/l)	CD1a+ DC <3.14: 498	CD1a+ DC >3.14 218	0.02 (n=18)
Pat. HIV+: CD1a+ DC (median)	Viral load of HIV-1 < 30,000 viral copies/mm Between 30,001 and 50,000 > 50,000		0.035
Pat. HIV+/AIN III: CD1a+ DC (median)	T CD4+(x10^6 cell/l) R= -0.74		0.035 (n=8) (FIGURE 4)
Pat. HIV+/HAART+: DC-SIGN+ (median)	T CD4+(x10^6 cell/l) R=-0.28		0.018 (n=46)

Pat=patients, DC=dendritic cells, HAART=highly active antiretroviral therapy, CD1a=Cluster of differentiation type 1a, DC-SIGN+=dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin.

The study of the distribution of DC-SIGN in DC anal or rectal mucosa has previously been performed by other authors[15-17]. In our study, differences between the median DC-SIGN+ DC (p=0.481), sum of the cells (CD1a+ DC plus DC-SIGN+ DC) (p=0.605) and the cell ratio (CD1a+ DC/DC-SIGN+ DC) (p=0.605) among HIV-positive and negative groups were not significant. However, among those with severe AIN (n=8), only one patient had a DC-SIGN+ DC density below 3.0, which was the opposite of the result in patients with AIN I. These data demonstrate the relationship between the accumulation of DC-SIGN+

DC and the occurrence of severe AIN.

Several factors influenced the density of DC

Significant analyses involving dendritic cells are briefly shown in Table 1, in which there is variability in the median CD1a+ DC in relation to the occurrence of STD and HIV status in patients with condyloma (p=0.005 and p=0.04, respectively). Patients with a median of CD1a + DC above the average of the control patients showed a variation in the CD4+ T cells greater than 50%. The CD4+ T cells negatively correlated with the DC-SIGN DC in patients on HAART+ (p=0.018) and in HIV+ patients with severe AIN. The median of CD1a+ DC (p=0.035) too was significantly correlated with the viral load of HIV-1 (p=0.035).

Partial recovery of CD1a + DC and DC-SIGN + DC in patients on HAART

The analysis of the relationship between DC and the degrees of severity of AIN indicated that in the group with severe AIN (n=8), a strong negative correlation (r=-0.74; p=0.035) was observed between the density of CD1a + DC and CD4+ T cells (Figure 4).
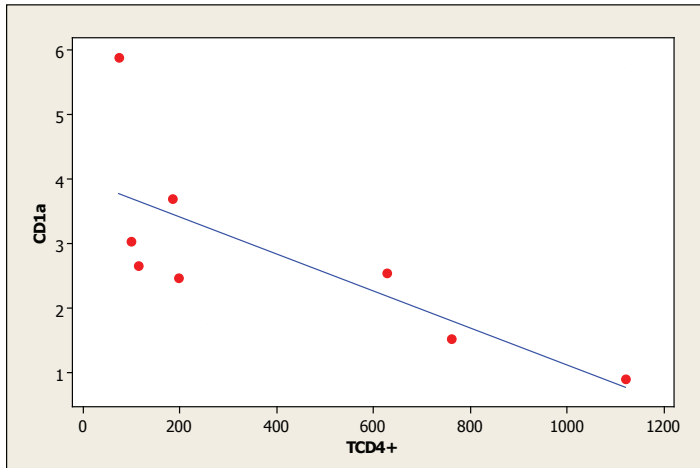

FIGURE 4 - Correlation between CD1a+DC and the levels of CD4+T (10^6 cells/l) in patients with AIN II/III. N=8, l=liter, DC=dendritic cells, CD1a=cluster of differentiation type1a.

In the HIV+/HAART+ subgroup, an increase in the median density of DC subsets in relation to the HIV+/HAART- subgroup was observed, which, although not significant, can be seen in the main variables (Table 2).

TABLE 2 - Median of dendritic cells in the study of subgroups.

	Groups			
	HIV-/Histo Normal	**HIV + / HAART-**	**HIV+ / HAART+**	**P-value***
CD1a+ DC				
Mean±SD	3.11 ± 1721	1.81 ± 1.24	3.3 ± 2.78	
Median	2.91	1.74	2.54 ↑↑	0.371
N	9	6	51	
DC-SIGN+ DC				
Mean ± SD	5.84 ± 1.4	4.7 ± 2.7	7.02 ± 4.72	
Median	5.78	3.62	6.41 ↑↑	0.495
N	10	5	49	
CD1a+ DC/ DC-SIGN+ DC				
Mean ± SD	0.50 ± 0.22	0.51 ± 0.43	0.65 ± 0.59	
Median	0.52	0.56	0.49	0.939
N	8	5	48	
CD1a+ DC+ DC-SIGN+ DC				
Mean ± SD	7.61 ± 2.99	6.51 ± 3.35	10.63 ± 6.10	
Median	7.54	5.55	10.09 ↑↑	0.130
N	45	5	48	

Kruskal-Wallis Test. SD=standard deviation, Histo=histopathology analysis, DC=dendritic cells, HAART=highly active antiretroviral therapy, CD1a=cluster of differentiation type 1a, DC-SIGN=dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin.

Elevation of the density of CD1a+ DC in HIV-negative patients with condyloma and the opposite effect in co-infected patients

The impact of STDs in DC of the anal mucosa was confirmed in patients with HIV-STD. Variations in the density of CD1a+ DC of 2.33 (n=53) to 3.53 (n=50) (p=0.05) in CD1a+/DC-SIGN+ DC (p=0.03) were observed among patients infected by HPV in the groups of HIV-positive and -negative patients with anal or perianal warts. In HIV-HPV co-infected patients, we found clear effects on the density of CD1a+ DC compared to HIV-negative patients with condyloma (Table 3).

TABLE 3 - Median CD1a+ dendritic cells in relation to the occurrence of condyloma and serology for HIV.

HIV/CONDYLOMA	N	Mean	DP	Median	p-value
CD1a+ DC					
HIV-/cond-	35	4.48	3.18	3.71	
HIV-/cond+	10	4.66	3.63	5.3	0.04
HIV+/cond-	36	2.79	2.27	2.37	
HIV+/cond+	18	3.14	2.94	1.92	

N=number of subjects, SD=standard deviation, DC=dendritic cells, HIV=human immunodeficiency virus, cond=condyloma, CD1a=cluster of differentiation type 1a.

Discussion

In this study of 114 patients, the variation in the density of dendritic cells in patients with AIN was examined. We found that the incidence of STD was significantly concentrated in the HIV + / HAART + subgroup. No significant difference in the prevalence of HPV was present between the HIV-positive and HIV-negative groups (p=0.09), probably due to the practice of anal intercourse by HIV-negative gay men (MSM) in this study, which is discordant with studies by Palefsky et al.[18]. However, Roka et al.[19] have described a high prevalence of HPV DNA of low (58.6%) and high oncogenic potential (51.4%) in samples from MSM regardless of HIV status.

In 2003, Palefsky and Holly[20] described HPV-induced genomic instability as essential for the progression of AIN to cancer in HIV patients. In our study, the prevalence of AIN in HIV-positive patients (53.8%) was slightly higher than in the studies by Abramowitz et al.[21], Gimenez et al.[22] and less than 81% among HIV-positive MSM described by Palefsky et al.[23] and Manzione et al.[24]. The authors are unanimous in asserting the importance of the presence of HIV infection in the occurrence of AIN[3,4,18,21]. In our study, the difference in the rates of AIN in HIV-positive and HIV-negative patients was not significant, this can be attributed to the presence of HIV-negative patients adherents of the practice of receptive anal intercourse, since the prevalence of AIN in this study was different in these patients (p <0.006) and patients with sexually transmitted diseases (p<0.001). Regarding severe AIN, our prevalence (28.6%) was similar to studies by Manzione et al.[24] (30%) and was greater than that reported by others[4,22,23], including a study at the same institution with a reported 7.1% II/III AIN and 35.7% I AIN.

The relationship between the occurrence of AIN and peripheral levels of CD4+ T cells has been described by Palefsky et al.[18] in pre-HAART patients and more recently[20], we have found a strong negative correlation (r= -0.74) between the density of CD1a+ DC and the number of T CD4+ cells in patients with severe AIN. Sobhani et al.[3] and Abramowitz et al.[21] have described the association of low levels count of CD4+ T cells only with the occurrence of condyloma. Yaghoobi et al.[25] have observed a higher risk of recurrence of anal cancer and AIN III in patients with low CD4+ T cells, but they suggested that the levels of peripheral CD4+ T cells are not an adequate tool for predicting cancer associated with HPV.

The mean peripheral HIV-1 viral load of our patients was high but was close to that described by Abramowitz et al.[21]. In patients with severe AIN, viral load had a negative influence on the median CD1a+ DC, which has been described by Sobhany et al.[4], showing the pressure of viral association with cellular immunity in these patients. However, Palefsky and Holly[20] have reported the occurrence of AIN and cervical intraephitelial neoplasm (CIN) in these patients as more closely related to the levels of CD4+ T cells than to the HIV viral load.

Upon measuring the density of dendritic cells, we observed a decrease in the median CD1a+DC from the HIV-positive group. These data are in agreement with data from Nadal et al.[26], which reported an odds ratio (OR) of 6 for the presence of HIV as a factor for the reduction of CD1a+DC. In 1997, Steinbrink et al.[27] described, in HIV-positive patients, the immune changes with decreased migration of dendritic cells for activation of the immune dysfunction of cellular maturation, where, according to Blauvelt et al.[28], HIV would subvert the primary function of Langerhans cells. Yaghoobi et al.[25] have shown an increased occurrence of III AIN and anal cancer that is associated with decreased tissue presence of CD1a+ Langerhans cells and CD3 T lymphocytes and increased expression of FoxP3, IL-23 and IL-8; these changes allow a regulatory T response that facilitates tumor outcome.

In HIV-negative patients with condyloma, an increase in the median CD1a+ DC and CD1a+ DC/ DC-SIGN+ DC was observed, which differs from the HIV-positive group with condyloma (which showed a decrease in these cells). These findings are corroborated by Uchimura[29], who have described a similar effect on S100+ DC in patients with squamous neoplasia, severe AIN and immunosuppressive therapy, and by Sobhani et al.[3,4] in the HIV-positive group, revealing a potentiation of the effects on the anal epithelium.

Here, we describe for the first time the density of subepithelial DC-SIGN+ DC in patients with AIN and the relationship between the accumulation of DC-SIGN+ DC and the occurrence of severe AIN. According to Gurney et al.[15], in patients with viral and tumor diseases related to AIDS, the increase in DC-SIGN associated with the blockade of CD83 and CD86 co-

stimulatory molecules is related to a regulatory DC response that is mediated by IL-10; these responses facilitate the spread of disease in these patients.

In the analysis of HIV-positive patients, we observed no significant persistent increase in the median CD1a+ DC, DC-SIGN+ DC or CD1a+ DC + DC-SIGN+ DC in patients on HAART compared to the control group (despite recognizing that the significance of this finding may have been affected by the small number of HIV+ HAART- patients). Although previous studies have pointed to the not restructuring of cellular immune response to HPV in patients on antiretroviral-specific therapy[20] (i.e., identifying these changes as additional risk factors for the development of severe AIN and anal cancer[21]), this study suggests that these patients could be recover the density of skin anal DC, especially of CD1a+ DC. Recent studies have reported similar rates of survival among patients on HAART and HIV-negative patients with anal cancer[30], a pointing that change in patients' prognosis for some diseases associated with HIV[31] would be encouraged by the reduction of pro-inflammatory cytokines induced by HAART[32] and translated by a favorable response and tolerance to chemotherapy[30]. Therefore, we hypothesize that HAART could allow the CD1a+ DC in the anal mucosa to recover. However, in a scenario in which the cellular maturation is ineffective, the formation of DC regulators[15] and high survival afforded by HAART[33] could further substantiate the high incidence of anal cancer in these patients, but with a better prognosis[30,31].

In this study, the elevation in the density of CD1a+ DC and DC-SIGN+ DC reflects the disparate role of these cells in relation to protection and vulnerability of the body to infections and tumors. Nagorsen *et al.*[34] have shown that S100+ DC infiltrate colorectal cancer, with a positive correlation between regulatory T cells and better survival. However, studies by Witte *et al.*[35] have demonstrated that DC-SIGN+ DC are associated with the facilitation of HIV infection in the body, while the langerin+ DC are effective in killing the virus, showing disparate functions performed by strategically positioned mucosal DC.

Conclusions

The synergistic action between the HIV and HPV viruses on anal epithelium in weakening the major role of DC in immune surveillance. Furthermore, we demonstrated the immunosuppressive effect of HIV-1 viral load on epithelial DC, particularly in patients with severe AIN.

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