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Langerhans Cells in Oral Mucosa from Patients with Acquired Immunodeficiency Syndrome

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Abstract

Background: Oral manifestations are common in patients with acquired immunodeficiency syndrome (AIDS).

Objectives: Compare the number of Langerhans cells and intensity of anti-CD1a expression in the mucous membranes of the oral cavities of the same patients with AIDS and HIV-negative individuals.

Materials and methods: Sixteen autopsied adults were investigated, including 11 with AIDS, and 5 HIV negative. We identified Langerhans cells in three oral regions of the same subject using an anti-CD1a antibody and quantified them in cells/mm2. Were applied normality tests and Mann-Whitney.

Results: The numbers of Langerhans cells in the AIDS patient group were less than in the control group, but didn't differ significantly between the two groups. The intensity of anti-CD1a expression was lower in patients with AIDS. Of the three areas, the greater intensity of CD1a cells were found in the masticatory mucosa.

Discussion: We observed a reduction of Langerhans cells in the oral mucosa of patients with AIDS and this is the first report, to our knowledge, that evaluate three different oral mucosal membranes in the same subject.

Conclusion: Our study suggests that AIDS influences on the depletion of Langerhans' cells, particularly in the specialized mucosa, and the intensity of expression of anti- CD1a regardless of the type of oral mucosa.

Keywords: Acquired Immunodeficiency Syndrome; Langerhans cells; Mononuclear phagocyte system; HIV; CD1a antigen

Introduction

In individuals affected by acquired immune deficiency syndrome (AIDS), the digestive system is a frequent target of changes as a result of infection with human immunodeficiency virus (HIV) and several other pathogens. Although the mucosal surfaces are natural sites of penetration and probable reserves of HIV, the oral cavity is not primarily considered as a route of transmission of the virus, with exceptions of transmission through breastfeeding or oral sex. However, due to the diversity of infectious processes and perioral and oral manifestations related to this particular chronic infection, this site has been studied by some authors [1-6].

The oral mucosa is made up of stratified squamous epithelial cells that vary morphologically by region, being called specialized mucosa in the tongue, masticatory mucosa in gingival tissues and hard palate, and lining mucosa in the cheeks, floor of the mouth, soft palate, the deep portion of the vestibule, the ventral portion of the lingual and internal lips [7]. Among the cell types found in the oral mucosa are Langerhans cells, which originate from bone marrow and reside in the stratum spinosum, where their cell bodies exhibit extensions that permeate surrounding epithelial cells. They belong to the mononuclear phagocyte system (MPS), which are processors and presenters of antigens, and they act as peripheral components of the immune system, expressing CD1a antigen molecules on their cell surfaces [8-13].

The epithelium interfaces between the superficial oral tissues and deeper tissues and Langerhans cells are key components of the immune response in the oral mucosa, and people with AIDS show an impaired immune response. Therefore, this study investigated Langerhans cells in the different mucous membranes of the oral cavity of autopsied individuals with AIDS [6,14]. The aim of the present

study was to compare the numbers Langerhans cells and the intensity of their CD1a expression in the masticatory, lining, and specialized of oral mucosal of individuals with AIDS versus those in HIV-negative individuals (controls).

Material and Methods

This study was approved by the Research Ethics Committee under protocol number 879. Specimens were obtained from autopsies performed at the Department of Surgical Pathology and Department of General Pathology at the Clinical Hospital of the Federal University of Triângulo Mineiro, Uberaba, MG or the Clinical Hospital at the School of Medicine of Ribeirão Preto, SP between April 2007 and July 2010.

The ages ranged from 25 to 54 years, with a mean age of 40.5 ± 7.9 years in the AIDS group and a mean age of 35.8 ± 6.9 years in the control group. The mean body mass index (BMI) for these groups was 22.3 and 27.6 kg/m^2, respectively. The majority (54.5%) of patients in the AIDS group had malnutrition (BMI<18 Kg/m^2). All patients were immunocompetent in control group, not undergoing chemotherapy or radiotherapy and were dentate at least in the region where the masticatory mucosa fragment was removed. All patients in the AIDS group had defining criteria of this syndrome [15]. Considering the inclusion criteria, was chosen by a non-probabilistic sample of convenience, according to the accessibility used the Mann-Whitney test [16]. Samples from three regions of the buccal mucosa (lining, masticatory, and specialized) were examined for each of the 16 subjects. The AIDS group included 11 individuals and the control group 5. Only 2/11 of the AIDS group subjects were receiving antiretroviral therapy and none were receiving chemotherapy or radiotherapy. Small fragments (~1 cm^2) were harvested from each of the three regions of interest of the mucosa and were fixed in Carnoy's solution for 30 minutes, processed, and embedded in paraffin. Thereafter, the specimens were subjected to immunohistochemistry simultaneously. They were incubated with an anti-CD1a. Primary antibody (Cell Marque ®) at a dilution of 1:20 for 1 hour and 30 minutes Immunolabeling was enhanced with the streptavidin-biotin complex technique (LSAB+ System-HRP, DAKO ®) according to the manufacturer's intructions. Images of Langerhans cells were captured at 400× magnification with a video camera attached to a brightfield microscope and a computer system with Q Win Capture software (Leica). Cells were identified and quantified throughout length of the fragments and expressed in cell numbers per mm^2, considering only those with defined cell bodies and at least one identifiable dendritic extension.

The intensity of anti-CD1a expression was classified subjectively as absent, weak, moderate or intense. Statistical analysis was performed using Biostat ® version 5.0. A normality test and Mann-Whitney test were applied. Values were considered significant at $p \leq 0.05$.

Results

Langerhans cells labeled with anti-CD1a were observed in the fragments of all three types of mucosa in individuals from both groups, and were always in the suprabasal location (Figure 1). The lining mucosa was the region with the highest number of Langerhans cells per mm2, while the masticatory mucosa was lower. Comparing groups AIDS and control, the distribution wasn't normal and the statistical test used was the Mann-Whitney test. Groups lining mucosa and masticatory aren't statistically significant different, since the specialized mucosa showed a statistically significant difference between groups (p=0.01) (Table 1). There was a wide variation in the number of Langerhans cells between individuals and the results were illustrated in Table 1. The intensity of anti-CD1a expression was greatest in the masticatory mucosa, although the number of cells was low in this region. Expression of anti-CD1a was lower in AIDS patients (weaker positivity) in all regions of the oral mucosa (Table 2). Anti-CD1a expression was illustrated in Figure 1.

Group	Median Langerhans cell number in mucosa (LC/mm^2) (25-75%)		
	Lining	Masticatory	Specialized
AIDS	12 (4.8-60.3)	2 (0-10.5)*	8 (5.3-10)**
Control	34 (22-67)	10 (7.8-34)	33 (21-52.8)
*p=0.06; **p=0.01			

Table 1: Langerhans cells of lining, masticatory and specialized oral mucosa tissues from autopsied AIDS patients and controls individuals at HC/UFTM or FMRP/USP from 2007 to 2010.

	L+	L-	M+	M-	S+*	S-*
Absense/rare	5	3	7	3	10	1
Moderate/intense	6	2	4	2	1	4
+ positive; - negative; *S+/S- p=0.01						

Table 2: Expression of CD1a in Langerhans cells of lining (L), masticatory (M) and specialized (S) oral mucosa tissue from autopsied Aids patients and controls patients at HC/UFTM or FMRP/USP from 2007 to 2010.

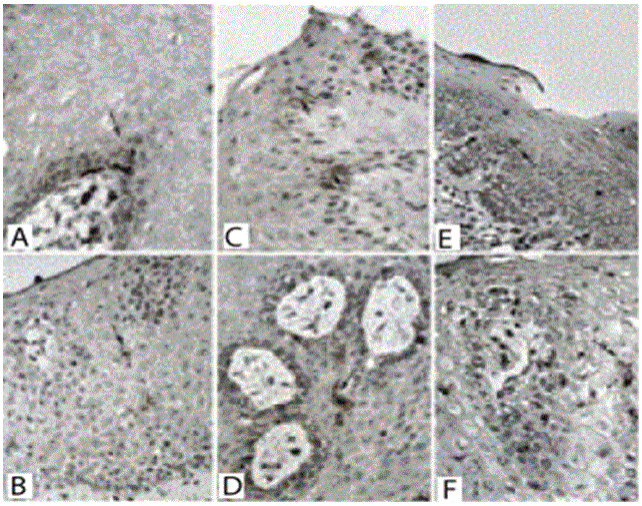

Figure 1: CD1a immunolabeling of Langerhans cells in representative samples from the lining, masticatory, and specialized mucosa from autopsied individuals with AIDS and controls.

A-B. Mucosal lining of HIV-negative control (A) and AIDS patients (B). C-D Masticatory mucosa from HIV-negative control (C) and an

AIDS patient (D). E-F Specialized mucosa from HIV-negative control (E) and an an AIDS patient (F) (400×).

Of the 11 AIDS patients, 9 had viral load available and 4 of these over 50 000 viral copies/ml. Among the 9 patients with available viral load, only one had CD4 counts greater than 200 cells/mm^3 and this was not on ART.

Discussion and Conclusion

Our observation of fewer Langerhans cells in AIDS patients, particularly in the specializated mucosa, fits with observations by other authors in the oral mucosa and gastrointestinal tract [2,17-22]. However, we found no prior reports in the literature evaluating three different mucous membranes of the oral cavity of the same individuals, as in the present study.

HIV have tropism of Langerhans cells, in addition to its effects on T lymphocytes, macrophages, dendritic cells, and endothelial cells. Chronic HIV infection is characterized by a progressive depletion of Langerhans cells, as evidenced in this study. HIV infection likely has direct effects on oral mucosal immunity as well as indirect effects related to its effects on the immune response [13].

Regional variations in the distribution and density of Langerhans cells in the mucosa in different locations of the oral cavity with similar functions have been described previously, but from different individuals [23-25]. In the present study, we observed similar regional variations in the same individuals, with a greater number of Langerhans cells in the lining mucosa, followed by the specialized and the masticatory, in both groups.

It was described recently that more Langerhans cells are present in HIV-positive individuals with periodontitis, using anti-S100 immunohistochemistry, compared to HIV-negative individuals. The lower specificity of the anti-S100 antibody relative to the anti-CD1a antibody used in this study may have contributed to their observation of a larger effect [25,26]. Moreover, we evaluate patients with AIDS and, in the previous study, patients had an HIV infection, without immunosuppression declared.

Periodontitis is an inflammatory infectious disease, common in the general population, including among HIV-positive and SIDA individuals. Kato Segundo et al (2011) conducted a study in patients with AIDS, antiretroviral therapy (ART) and periodontal disease. These authors categorized the oral disease in severity. The authors observed that the use of ART decreases the viral load and consequently the destruction of Langerhans cells in gingiva of patients with periodontitis [27]. In the present study, only 2 of AIDS group subjects were receiving ART and most had high viral load and low CD4 count.

Another factor that could contribute to there being fewer Langerhans cells in patients with AIDS is malnutrition, which can impair the immune response to various inflammatory and infectious processes [28,29].

In conclusion, by comparing different regions within the same individuals, our study provides evidence that AIDS probably influences the number of Langerhans cells in the oral mucosa, particularly in the specialized mucosa, and the intensity of expression of anti-CD1a regardless of the type of oral mucosa. However, further studies with a larger number of subjects are needed to confirm our findings.

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