

Density of Langerhans cells in the keratocystic odontogenic tumor

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Densidade das células de Langerhans no tumor odontogênico queratocístico

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key words	abstract
Odontogenic cysts	<p>Introduction: Keratocystic odontogenic tumors (KOTs) are distinct odontogenic lesions commonly affecting the mandible bones. Langerhans cells (LCs) are specialized dendritic cells responsible for the presentation of antigens to T lymphocytes in mucosal and cutaneous surfaces. Objective: This study analyzed the immunohistochemical expression of LCs in KOTs. Material and method: Fifteen cases of KOTs were studied using the anti-CD1a marker. Results: LCs were observed in all 15 cases analyzed. They were found to be concentrated in areas of cystic epithelial hyperplasia, mainly in those areas presenting higher concentration of inflammatory cells. Furthermore, a significant association between the number of LCs and areas of cystic epithelium presenting hyperplasia (Mann-Whitney test, $p = 0.0223$) was observed. The shape and location of these cells in KOTs epithelium were variable. Conclusion: The lower number of LCs observed on atrophic cystic epithelium of KOTs may be due to decreased epithelial immunosurveillance and this may result in locally aggressive invasiveness.</p>
Langerhans cells	
Immunohistochemical	
Keratocystic odontogenic tumors	

resumo	unitermos
<p>Introdução: Tumor odontogênico queratocístico (TOQ) é uma lesão odontogênica de caráter distinto que afeta frequentemente ossos maxilares. Células de Langerhans (CLs) são células dendríticas especializadas, responsáveis pela apresentação de antígenos aos linfócitos T nas superfícies cutânea e mucosa. Objetivo: Este estudo analisou a expressão imuno-histoquímica das CLs em lesões de TOQ. Material e método: Quinze casos de TOQ foram estudados utilizando o marcador anti-CD1a. Resultados: As CLs foram observadas em todos os 15 casos analisados. Essas células estavam concentradas em áreas de hiperplasia do epitélio cístico, especialmente naquelas que apresentavam alta concentração de células inflamatórias. Em adição, foi encontrada associação significativa entre número de CLs e áreas do epitélio cístico que apresentavam hiperplasia (Mann-Whitney test, $p = 0.0223$). O formato e a localização dessas células no epitélio dos TOQs foram variáveis. Conclusão: O menor número de CLs encontrado no revestimento cístico atrófico dos TOQs pode ser atribuído à imunovigilância deficiente e isso pode resultar em comportamento biológico localmente agressivo.</p>	<p>Cistos odontogênicos Células de Langerhans Imuno-histoquímica Tumor odontogênico queratocístico</p>

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Introduction

Keratocystic odontogenic tumors (KOTs) arise from the rests of the dental lamina and are characterized by a locally aggressive biological behavior and a high rate of recurrence⁽²⁷⁾. In general, KOTs affect the posterior region of the mandible in young adults⁽¹⁶⁾. When multiple sites of the jawbones are involved, KOTs are frequently associated with nevoid basal cell carcinoma syndrome (NBCCS). Studies have shown that disorders in the PTCH gene, mapped on chromosome 9q22.3, might be associated with the pathogenesis of NBCCS and sporadic KOTs⁽²⁶⁾. The alteration in the gene PTCH results in the aberrant activation of the Sonic hedgehog (Shh) signaling pathway⁽³⁰⁾.

Histologically, KOTs consist of a parakeratinized and atrophic stratified squamous epithelium comprising six to eight layers of cells and a corrugated surface^(20, 22). Secondary inflammation is not uncommon and contributes to the typical morphological alterations observed, including a hyperplastic epithelium^(8, 15).

Langerhans cells (LCs) are dendritic cells derived from the bone marrow, which present tropism to most stratified squamous epithelia, as the epidermis and epithelium of the oral mucosa. LCs are involved in the presentation of antigens during the induction of the immune response, particularly the presentation of antigens to T lymphocytes. As the skin and mucosa are continuously exposed to a variety of stimuli and are under the influence of antigenic factors, LCs represent the first-line defense, mediating the early capture of antigens and the activation of the immune system by presenting these antigens to T lymphocytes, triggering different pathological processes^(1, 4, 17).

Alterations involving LCs have also been associated with a variety of human diseases, including oral lichen planus, actinic cheilitis, oral cancer, periodontal disease, condyloma acuminatum, and cervical neoplasia^(1, 7, 9, 10, 13, 17, 21, 23). Despite these features, there have been only few small series indicating differences in the density of LC among odontogenic cysts and tumors^(21, 24). More specifically, these reports have noted a decrease density of LCs in KOTs⁽²¹⁾ and an increase in density of LCs in radicular cysts (RCs)⁽²⁴⁾, however, they have not hypothesized possible explanations for this. Thus, the goal of the present investigation was to study the density of LC in KOTs in an attempt to establish possible explanation for such occurrence.

Material and method

After Institutional Ethics Committee approval, 15 cases of KOTs obtained from the files of the Surgical Pathology Service of the School of Dentistry of the Federal University of Bahia (FOUFBA) were studied. The cases were selected based on the histopathological criteria adopted by the World Health Organization (WHO)⁽²⁰⁾.

For immunohistochemistry a monoclonal antibody against CD1a (clone NCL-CD1a, Novocastra Laboratories, UK) was used with the EnVision™ System (Dako Corporation, Carpinteria, CA, USA). For antigen retrieval, conditions included sections boiled in EDTA, pH 8, at a constant temperature of 97°C. Endogenous peroxidase activity was blocked using peroxidase, the paraffin-embedded material was cut into 3 µm thick sections. The tissue sections were deparaffined and block solution provided in the EnVision™ kit, for 10 minutes and the slides were washed with tris-buffered saline.

The tissue sections were then incubated with antibody CD1a at 1:30 dilution for 30 minutes at room temperature, using an antibody diluent with background reducing components (Dako Corporation, Carpinteria, CA, USA). Immunohistochemical reactions were developed with diaminobenzidine as the chromogenic peroxidase substrate and the slides were counterstained with Harris hematoxylin. Human tonsil tissue sections were used as a positive control. Negative controls included replacement of the primary antibody with non-immune bovine serum.

Cases in which LCs exhibited a brownish immunostaining, irrespective of intensity, were considered to be positive. CD1a-positive cells were counted by two observers (TMM and JNS) under a light microscope (E200 Nikon, Melville, NY, USA) at high magnification. The counting was performed in up to 10 high-power fields (400×), using a standard light microscope, and average Langerhans cell count in individual cases was calculated.

In addition to the density of LCs, the shape and location of these cells in the epithelial layers were also evaluated. KOTs lining epithelium was classified as atrophic when exhibited up to eight cell layers and as hyperplastic if the number of cell layers was higher. These morphological patterns were related to the density of CD1a-positive LCs and with the presence or absence of an underlying inflammatory process.

Inflammatory infiltrate density in the connective tissue wall was evaluated by manual count of inflammatory cells. The degree of inflammation in the lesions studied was classified according to the criteria proposed by Kaplan and

Hirshberg⁽¹⁵⁾: grade 0 – absence of inflammation; grade 1 – < 15 inflammatory cells/field; grade 2 – 15 to 50 inflammatory cells/field; grade 3 – > 50 inflammatory cells/field.

Data were analyzed statistically using the Bioestat 3.0 program (Sociedade Civil Mimirauá, MCT-CNPq, Conservation International, Brasília, DF, Brazil, 2003). Since the data presented an asymmetric distribution on descriptive analysis, nonparametric statistical tests were used. To determine the association of CD1a-immunostained LCs with the degree of inflammation and epithelial thickness, the Mann-Whitney and Kruskal-Wallis tests were used for the comparison of two or more independent samples, respectively. A level of significance (*p*) of less than 0.05 was considered to be significant.

Results

Among the 15 cases of KOTs, eight (53.3%) were males, seven (46.6%) were females. They predominated in the third decade of life, and 13 (86.6%) lesions were located in the region of the body and ramus of the mandible.

Immunohistochemistry for detection of the anti-CD1a antibody resulted in a brown staining of LCs. Positive

staining for this antibody was observed in all cases of KOTs, although it was not abundant in those atrophic areas (**Figure 1**).

With respect to inflammation, focal areas of this phenomenon were observed. In addition, according to the criteria adopted, no inflammation (grade 0) was noted in three (20%) cases. Two (13.3%) cases presented inflammation grade 1, four (26.6%) grade 2, and six (40%) grade 3. Areas exhibiting hyperplastic keratinized stratified squamous epithelium was detected in 11 cases of OKs, and in this location, grades 2 and 3 inflammation in the cystic wall were observed in eight (72.7%) of them (**Table 1**).

Although a higher density of CD1a-positive LCs was observed at sites exhibiting inflammation, no significant association could be demonstrated between the degree of inflammatory infiltration and CD1a-positive LCs (Kruskal-Wallis test, *p* = 0.3236) (**Table 2**), independent of the cystic epithelium thickness.

A higher density of CD1a-positive LCs was observed in KOTs in those areas exhibiting hyperplastic epithelium. Moreover, an association was observed between the density of CD1a-positive LCs and hyperplastic epithelium (Mann-Whitney test, *p* = 0.0223) (**Table 3**), except for specimens whose cystic wall did not exhibit inflammation (Mann-Whitney test, *p* = 0.7133) (**Table 4** and Figure 1).

Table 1 Frequency of cases of KOTs according to thickness of the cystic epithelium and degree of inflammation

	Grade 0 – <i>n</i> (%)	Grade 1 – <i>n</i> (%)	Grade 2 – <i>n</i> (%)	Grade 3 – <i>n</i> (%)	<i>P</i> value
Atrophic KOTs	1 (6.6)	1 (6.6)	1 (6.6)	1 (6.6)	0.8164
Hyperplastic KOTs	2 (13.3)	1 (6.6)	3 (2)	5 (33.3)	

Fisher's exact test, *p* < 0.05. KOTs: keratocystic odontogenic tumors.

Table 2 Mean number of CD1a-positive LCs in KOTs according to the degree of inflammation

KOTs	Grade 0	Grade 1	Grade 2	Grade 3	<i>P</i> value
LCs	3.4	2.7	2.5	11.8	0.3236

Kruskal-Wallis test, *p* < 0.05.

LCs: Langerhans cells; KOTs: keratocystic odontogenic tumors.

Table 3 Mean number of CD1a-positive LCs in KOTs according to thickness of the cystic epithelium

KOTs	Hyperplastic epithelium	Atrophic epithelium	<i>P</i> value
LCs	8.3	1.4	0.0223

Mann-Whitney test, *p* < 0.05.

LCs: Langerhans cells; KOTs: keratocystic odontogenic tumors.

Table 4 Mean number of CD1a-positive LCs in the epithelial lining of KOTs in the absence of inflammation

Epithelium	Positive cases – <i>n</i> (%)	LCs	Negative cases – <i>n</i> (%)	<i>P</i>
Hyperplastic	4 (26.6)	2.1	1 (6.6)	0.7133
Atrophic	6 (40)	1.3	4 (26.6)	

Mann-Whitney test, *p* < 0.05. LCs: Langerhans cells; KOTs: keratocystic odontogenic tumors.

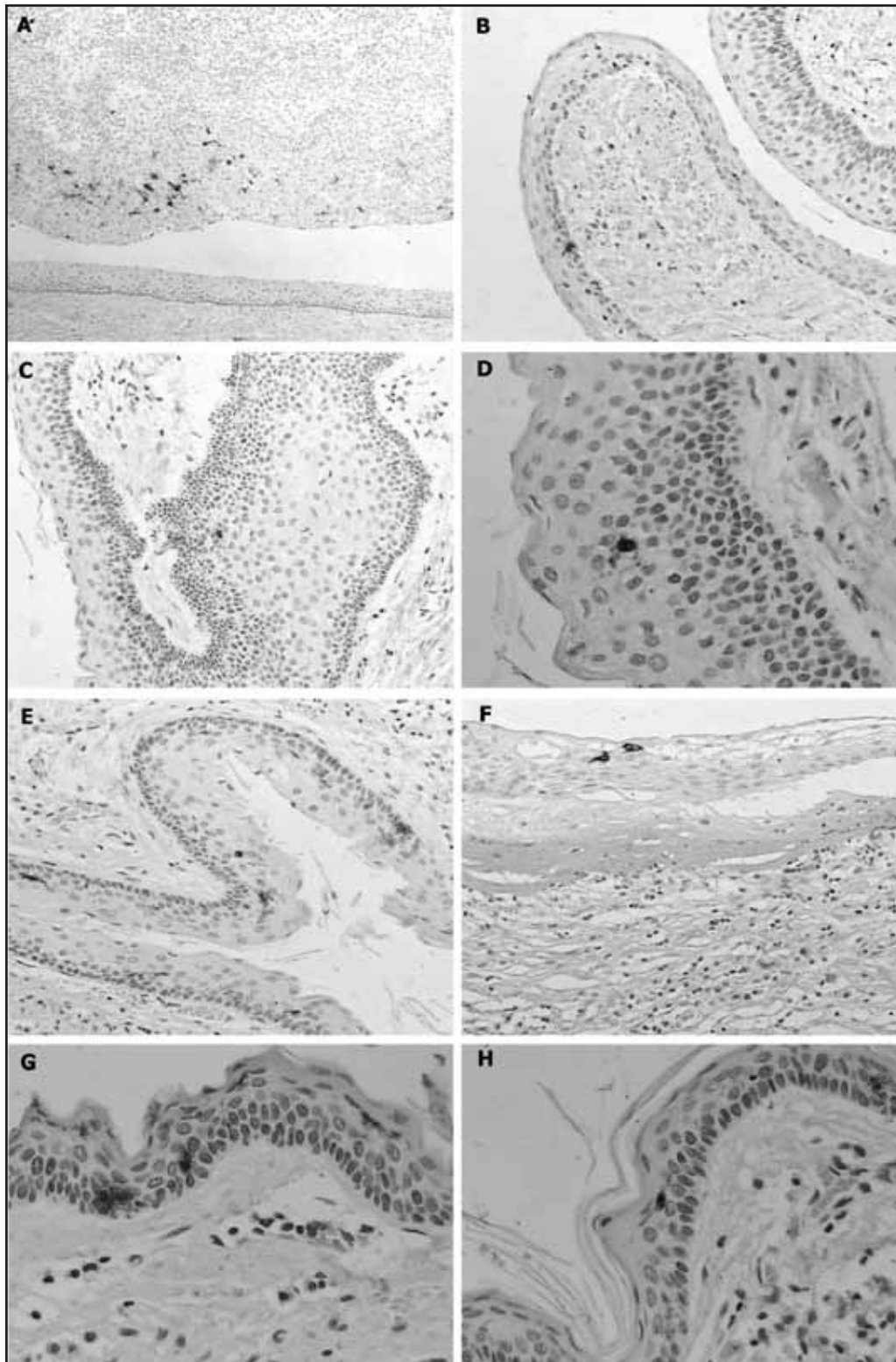


Figure 1 – Immunohistochemical expression of Langerhans cells (LCs) in keratocystic odontogenic tumors (KOTs). (A) Abundant expression of LCs in both basal and suprabasal areas. Note marked chronic inflammation in the adjacent connective tissue and absence of Langerhans cells in the epithelium cystic which covers fibrous wall without inflammation (EnVision™ System, approximately 40×); (B) dendritic cell located in the basal layer of the cystic epithelium (EnVision™ System, approximately 100×); (C) epithelial thickness exhibiting a small LC located in the intermediate layer (EnVision™ System, approximately 100×); (D) LC exhibiting round shape in the intermediate layer (EnVision™ System, approximately 200×); (E) LCs exhibiting different shapes in both basal and intermediate areas (EnVision™ System, approximately 100×); (F) dendritic cells located in the suprabasal and superficial layers. Note absence of LC in the area corresponding to the subepithelial split (EnVision™ System, approximately 100×); (G, H) Dendritic cells in different epithelial layers (EnVision™ System, approximately 200×)

Regarding the shape and location of CD1a-positive LCs in the epithelium of the KOTs, these cells mainly presented a dendritic shape but were more commonly located in the intermediate and superficial layers (Figure 1).

Discussion

Langerhans cells are dendritic cells which exert different immunological functions in different tissues. The role of LCs in diseases affecting the oral mucosa has not been completely established. However, it is known that these cells are involved in the presentation of antigens to T lymphocytes in both pathological or non-pathological situations^(1, 17). Thus, the presence of these cells suggests their participation in important cellular events, with lymphocytes activated by LCs producing soluble mediators and lymphokines involved in a variety of cellular responses, such as cell proliferation, migration and differentiation⁽¹⁰⁾. These processes, in turn, may influence the biological behavior of odontogenic lesions, such as RCs and KOTs^(1, 21, 24).

LCs cannot be identified by light microscopy in routinely stained specimens. Therefore, many investigators have used immunohistochemical markers, such as CD1a, HLA-DR, CD29, CD54 and S-100 antibodies for the identification of these cells^(10, 17, 23). In the present study, we evaluated the distribution of LCs in the epithelium of KOTs by immunostaining using an anti-CD1a antibody, which is considered to be the most specific for the identification of LCs because of the presence of this protein on the surface of these cells^(10, 17, 23). CD1a represents a molecule that belongs to a group of non-polymorphic, B2-microglobulin-associated, transmembrane glycoproteins. It is well conserved and strongly related to classical major histocompatibility antigen-presenting molecules^(6, 29).

In the present study, LCs were detected in all cases of KOTs studied, suggesting that these cells may participate in the cellular response underlying the formation of these lesions. Akhlaghie *et al.*⁽¹⁾ and Piattelli *et al.*⁽²¹⁾ also identified LCs in these lesions, suggesting that immune reactions are important for the proliferation and differentiation of the cystic epithelium. Although, we observed CD1a-positive cells in all cases of KOTs no significant association was observed in cases of inflamed lesions showing atrophy. However, areas exhibiting hyperplastic epithelium in KOTs were significantly associated with a higher density of CD1a-positive LCs. These areas corresponded to those exhibiting

inflammation for the majority the cases and influenced the hyperplasia encountered in the cystic epithelium^(8, 15).

It is important to state that Piattelli *et al.*⁽²¹⁾ did not detect differences in the density of CD1a-positive LCs between sites with or without inflammation when studying RCs and KOTs. We agree that the marked expression of LCs in inflamed areas is an expected finding since these cells mediate the signaling for early capture and presentation of antigens to T lymphocytes^(2, 7, 17, 28). In addition, growth factors and cytokines produced during the inflammatory process might be responsible for the higher proliferative activity of the cystic epithelium observed in these lesions^(8, 15, 18).

KOTs usually do not present an important inflow of inflammatory cells and, as observed in the present study, CD1a-positive LCs are absent or often markedly reduced in areas without inflammation. The lower number of LCs in these areas can be attributed to the phenomenon of apoptosis mediated by the accumulation of immunosuppressive cytokines, such as IFN- γ and IL-10^(2, 3). This phenomenon also occurs in malignant neoplasms, such as melanomas, being this due to TGF β -1⁽¹⁴⁾. The study of these cytokines in recurrent, syndromic or non-syndromic KOTs will probably contribute to the elucidation of their biological behavior since they may influence the differentiation and attachment of LCs^(2, 4, 14).

LCs seem to influence the maturation of keratinocytes⁽¹¹⁾. Schweizer *et al.*⁽²⁵⁾ analyzing normal epithelial tissue in the tail of rats, reported a lower expression of these cells in parakeratinized areas compared to orthokeratinized areas, indicating a distinct histological pattern of this cell type. Although we did not study orthokeratinized odontogenic cysts, this finding was observed in the present study which showed a lower density of CD1a-positive LCs in KOTs. A similar result was also obtained when comparing areas of inflammation found in KOTs. Taken together, these data suggest that a loss of LCs could stimulate an altered differentiation and contribute to the pathogenesis and invasiveness of KOTs, as other lesions, such as skin and oral squamous cell carcinoma, intraepithelial neoplasia of the cervix and condyloma acuminatum, also show decreased density of LCs^(2, 9, 19). In addition, Gurgel *et al.*⁽¹²⁾ demonstrated high levels of p63 in the cystic epithelium of KOTs, suggesting that p63-positive keratinocytes may represent immaturity of this lesion as well as influence the regulation of epithelial cell differentiation.

Depending on their degree of maturation and location in the epithelium, LCs present two distinct

shapes. Mature cells are composed of numerous dendritic prolongations, present an irregular and stellate shape, and are generally found in the suprabasal layer of the epithelium. In contrast, immature LCs possess few epithelial projections, present a round shape, and are usually located in the basal layer^(5, 17). It has been well established that LCs undergo these structural and morphological changes during migration to the regional lymph nodes after capture of antigens to promote greater mobility^(2, 4). In the present study, we observed a larger number of LCs, with a predominantly dendritic shape, in the intermediate and superficial layers of KOTs. This finding agrees with Piattelli *et al.*⁽²¹⁾ who reported a higher density of these cells in intermediate layers corresponding to areas of hyperplastic epithelium.

Conclusion

Thus, it is possible that the lower number of LCs observed on atrophic cystic epithelium of the KOTs may be due to decreased epithelial immunosurveillance and this may result in locally aggressive invasiveness.

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