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Langerhans cells in odontogenic cysts. A retrospective study based on 142 cases

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SUMMARY

Dendritic cells with the ultrastructural and immunocytochemical characteristics of Langerhans cells are commonly identified in lymphoid organs and in keratinized and non-keratinized epithelia, such as oral mucosa. These types of cells were previously described in the epithelial wall of periapical odontogenic cysts.

S-100 protein positive cells were demonstrated immunocytochemically in all of our 142 cases of odontogenic cysts (32 developmental cysts; 110 inflammatory cysts). Their tropism for squamous epithelia may explain their presence in the epithelial wall of odontogenic cysts. Their number is not always correlated with an inflammatory condition.

KEY WORDS:

Langerhans cells; odontogenic cysts.

RÉSUMÉ

Des cellules dendritiques présentent les caractéristiques immunocytochimiques et ultrastructurales des cellules de Langerhans sont communément observées dans les épithélium kératinisés et non kératinisés, telle que la muqueuse orale. Des cellules de ce type ont déjà été décrites précédemment dans la paroi épithéliale des kystes odontogènes périapicaux.

Des cellules protéine S-100 positives ont été démontrées par immunocytochimie dans tous nos 142 cas de kystes odontogènes (32 kystes dus à une anomalie de développement; 110 kystes inflammatoires). Leur tropisme pour les revêtements malpighiens peut expliquer leur présence dans la paroi épithéliale des kystes odontogènes. Leur nombre élevé n'est pas exclusivement en corrélation avec les états inflammatoires.

MOTS CLEFS:

Cellules de Langerhans; kystes odontogènes.

INTRODUCTION

Langerhans cells are dendritic cells whose presence has been demonstrated in several tissues in connection with immunity.

This particular variety of dendritic cells was discovered in the epidermis by Langerhans (1868) according to their strong affinity for gold chloride. After one century, Birbeck *et al.* (1961) described by using electron microscope a typical rodshape granule

with a central linear striated density and an expended end, considered until now as a specific ultrastructural marker for this particular cell described previously by Langerhans.

Thereafter, Langerhans cells (LCs) with demonstrable «Birbeck granules» were recognized in the thymus, in lymph nodes and in several stratified squamous epithelia such as exocervix and oral mucosa (Schroeder and Theilade, 1966; Waterhouse and Squire, 1976; Newcomb *et al.*, 1982; Daniels, 1984; Difranco *et al.*, 1985; Ahlfors *et al.*, 1985. For more details concerning the role of LCs in oral pathological conditions, see Lombardi *et al.*, 1993).

It is actually well-known that these dendritic cells – simultaneously present in lymphoid tissues and stratified squamous epithelia – represent an important group of antigen-presenting histiocytes.

It is possible to localise immunocytochemically LCs using anti S-100 protein antibodies. However its lack of specificity (melanocytes, Schwann cells, chondrocytes are also S-100+), this reaction is commonly used to detect LCs associated or not with other markers such as Ia+, HLA-DR (OK-DR), OKT6+, MT1, LN-3, CD1+, ATPase.

Few publications reported the presence of LCs in the epithelium of odontogenic cysts (Contos *et al.*, 1987; Kalusukoma *et al.*, 1987; Matthews and Browne, 1987; Gao *et al.*, 1988).

The purpose of our study was to investigate retrospectively the presence of LCs in a large variety of odontogenic cysts, using their immunocytochemical protein S-100+ character.

MATERIAL AND METHODS

Formalin-fixed, paraffin-embedded specimens of 142 odontogenic cysts were provided from the department of Pathology and from the department of Stomatology, Hôpital Universitaire St-Pierre, Brussels (Table I). They were classified using the Revisted Classification of the International Association of Oral Pathologists (The Netherlands, 1984) and the Classification proposed recently by Shear (1993).

Tissue sections for routine histological examination were stained with hematoxylin-eosin. Immunocytochemistry was performed on paraffin-embedded sections. Rabbit antibodies to cow protein S-100 were used at the concentration of 50 mM (Dakopatts, Prosan, Ghent). The development of peroxydase was revealed with diaminobenzydine. Control stainings were performed after substitution of non-immune rabbit serum of the specific anti-proteine S-100 serum.

TABLE I: Number and type of odontogenic cysts used in this study.
TABLEAU I: Nombre et types de kystes odontogènes utilisés dans cette étude.

ODONTOGENIC CYSTS			
1. developmental cysts:		2. inflammatory odont. cysts:	
– keratocysts	10	– radicular cysts	98
– dentigerous cysts	21	– residual cysts	12
– odont. calcif. cyst	1		

RESULTS

Conventional histological findings:

1. **Developmental odontogenic cysts:** 32 cysts were observed.

– Odontogenic keratocysts (10 cases) exhibited a thin ortho- or parakeratinized epithelial lining with a prominent polaryzed layer of basal cells (=«primordial odontogenic cysts»). The epithelium was separated from the connective tissue by a prominent basement membrane. Multiple keratocysts are usually observed in the same patient.

– Dentigerous cysts, or follicular cysts (21 cases), are usually associated with an impacted or unerupted tooth. They were coated with a layer of stratified squamous epithelium of irregular thickness. The connective tissue was sometimes infiltrated with inflammatory cells.

– One case of calcified odontogenic cysts (Gorlin's cyst) contained groups of partly calcified ghost cells.

2. **Inflammatory odontogenic cysts:** 110 cases of this kind of cysts were observed.

– Radicular and residual cysts possess an epithelial lining derived from the epithelial cell rests of Malassez. The inflammatory context is extremely important: hyperhemy of the connective tissue, abundant neutrophils around the capillaries, in the epithelium and in the lumen, macrophages

sometimes containing hemosiderine and giant cells granulomas developped around spicules of cholesterol.

We never observed the presence of melanocytes in the epithelial layers of our cysts.

Immunocytochemical observations:

The antibodies against S-100 protein revealed stained cells in the epithelial wall of all the cysts of our material. These cells were irregularly distributed, seldom (Fig. 1) or numerous (Fig. 2). They were more frequently observed in the basal layer of the epithelium (Fig. 2).

They appeared to intermigled between the ghosts cells in our case of calcified odontogenic cyst (Fig. 3).

They possessed dendritic cytoplasmic extentions (Figs 1, 2, 3).

Langerhans cells were usually numerous in most of our cases of inflammatory odontogenic cysts (Fig. 4). These cells were not only located in the basal part of the epithelium but also in the most superficial layers of the epithelium were they were round shaped (Fig. 4). Nevertheless, numerous LCs were also encountered in non inflammatory cases (Fig. 2).

Fig. 2: Odontogenic keratocyst. Desquamated keratinized cells occupie the upper part of the figure. Numerous dendritic S-100 protein + cells are observed in the basal layer of the epithelium. One S-100 protein cell (arrow) was identified in the adjacent connective tissue which appeared to be devoid of any inflammatory infiltrate (Anti-S-100 protein. Magnification ×170).
Fig. 2: Kératokyste odontogène. Présence de cellules kératinisées desquamées dans la partie supérieure de la figure. De nombreuses cellules dendritiques protéine S-100+ sont observées dans la couche basale de l'épithélium de revêtement du kyste. Une cellule protéine S-100+ (flèche) est identifiée dans le tissu conjonctif adjacent qui, par ailleurs, ne montre pas d'infiltrat inflammatoire (Anticorps anti-protéine S-100. Grossissement ×170).

Fig. 1: Dendritic cell, S-100 protein+ (arrow) in the epithelial layer of a follicular (dentigerous) cyst (Anti-S-100 protein antibodies. Magnification ×640).
Fig. 1: Cellule dendritique protéine S-100+ (flèche) dans l'épithélium d'un kyste folliculaire (ou dentigère) (flèche) (Anticorps anti-proteine S-100. Grossissement ×640).

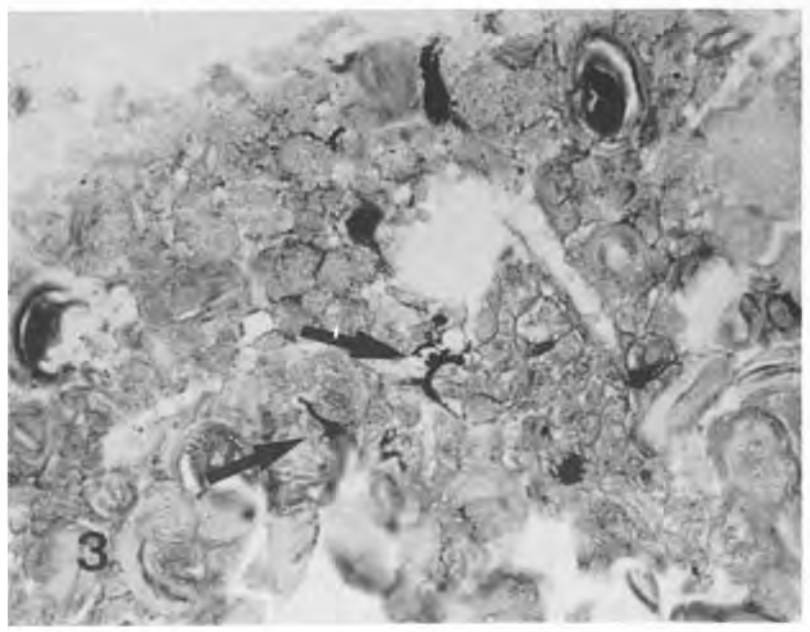

Fig. 3: Calcified odontogenic Gorlin's cyst. 2 Langerhans cells (arrows) were identified among the calcified ghost cells of the cyst (Anti-S-100 protein. Magnification ×400).
Fig. 3: Kyste odontogène calcifié de Gorlin. On identifie 2 cellules de Langerhans (flèches) dans la masse de cellules épithéliales fantômatiques calcifiées du kyste (Anticorps anti-protéine S-100. Grossissement ×400).

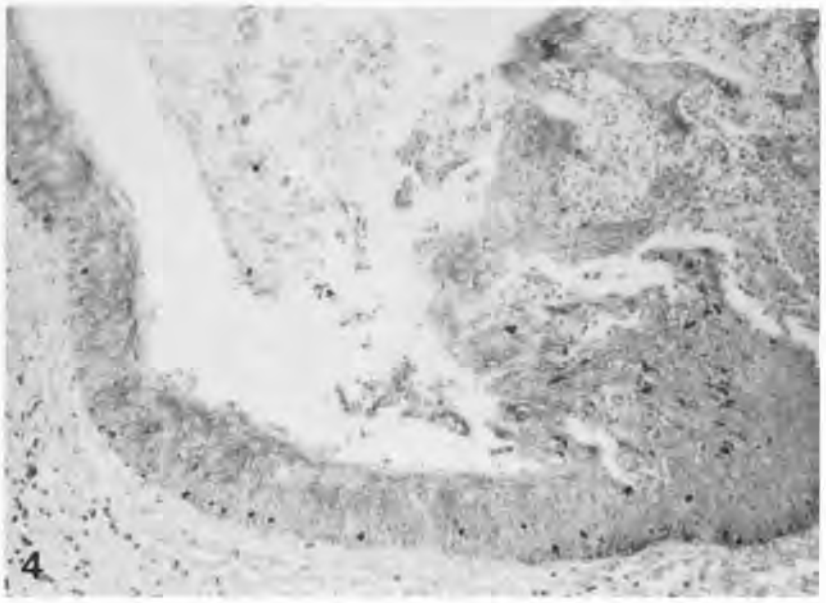

Fig. 4: Inflammatory odontogenic cyst. Numerous S-100 protein + cells in the basal and in the superficial layers of the epithelium. Important inflammatory reaction (Anti-S-100 protein. Magnification ×170).
Fig. 4: Kyste odontogène inflammatoire. Des cellules protéine S-100+ (cellules de Langerhans) sont nombreuses dans les couches basales et superficielles de l'épithélium. La réaction inflammatoire est importante (Anti-corps anti protéine S-100. Grossissement ×170).

Langerhans cells were rarely identified in the connective tissue arround the epithelial wall (Fig. 2). The myelinated nerve fibers were S-100 protein +.

DISCUSSION

It is well known that Langerhans dendritic cells in lymphoid tissues and stratified squamous epithelia represent the major class of antigen-presenting histiocytes and are able to play crucial role in the immunological defenses against some external antigens (Burkhardt *et al.*, 1979; Murphy *et al.*, 1986; and for more recent informations see Teunissen, 1992). Contact zones and desmosomal junctions between LCs and lymphocytes objectivated ultrastructurally might be the morphological expression of such tenuous interaction (Concha *et al.*, 1988).

Langerhans dendritic cells are bone marrow-derived monocytes, but unlike the macrophages, they are non phagocytic. However, when appropriately stimulated, macrophages are capable of migration into the epidermis where they should gradually assume phenotypic characteristics of LCs (Murphy, *et al.*, 1986).

The exact role of LCs in oral mucosa is not fully understood. In oral epithelium, LCs are also involved in reaction to antigen challenge under both and pathological situations (Lombardi *et al.*, 1993). The majority of the oral intraepithelial cell population is made up of lymphocytes (23%) and Langerhans cells (56,8%) (Burkhardt *et al.*, 1979). Two types of LCs are distinguished: more or less «macrophagocytoïd LCs» 80% and «reticuloïd LCs» 20% (Burkhardt *et al.*, 1979).

Quantitative determinations objective in the oral mucosa a density of 890 LCs/mm² (approximate those of epidermis) determined by their ATPase activity (Ahlfors *et al.*, 1985).

LCs are more numerous in the dorsum of the tongue, and their number decrease progressively and respectively in buccal mucosa, lips, lateral borders of tongue, hard palate and finally in the floor of the month (Cruchley *et al.*, 1989).

The age may modify the number of LCs in the murine oral mucosa which falls 30 to 60% of the normal values in old animals (Rittman *et al.*, 1987).

Determined by their OKT6 character in the oral gingival epithelium (OGE), the sulcular epithelium (SE) and in the junctionnal epithelium (JE), the LCs in OGE and in SE are highly dendritic with a density of 8.6 to 21.0/0.1 mm². In JE, LCs are less numerous: 2.8 to 9.4/0.1 mm², with short dendrites or dendrites of moderate lenghts (Juhl *et al.*, 1988). On the other hand, Newcomb and Powell (1986) were unable to demonstrate LCs in the JE. Those variations in the distribution of LCs may reflect a reaction to an initial plaque formation. The relationship between an increased number of LCs and plaque accumulation was subsequently demonstrated (Newcomb *et al.*, 1982: Walsh *et al.*, 1990).

Within the gingiva a relationship between bacterial products and LCs exists (Saglie *et al.*, 1987; Walsh *et al.*, 1990) and the number of LCs increases during the development of gingival inflammation (Newcomb *et al.*, 1992; Difranco *et al.*, 1985) presumably due to locally produced Cytokins on the differentiation of LCs (Walsh *et al.*, 1988).

It is interesting to point here that LCs cells are absent or greatly reduced in the lesions of oral hairy leukoplakia (Daniels *et al.*, 1987), in the lingual epithelium in relation to tobacco and alcohol consumption (Barrett *et al.*, 1991) and in smokeless tobacco-associated oral mucosal lesions (Daniels *et al.*, 1992).

The density of LCs is markedly depressed in median rhomboid glossitis. This localized defect in the immune surveillance may explain the persistant fungal infection of the tongue mucosa (Walsh *et al.*, 1992).

Inversely, the number of LCs is increased in oral cancer (Löning *et al.*, 1982; Kurihara and Hashimoto, 1985), but there are no correlation between LCs population and grading in oral squamous cell carcinoma (Van Heerden *et al.*, 1995). The presence of LCs among the keratinocytes of oral carcinomas raises the question wether these cells play a role in the host resistance against the malignant tumors arising from epidermis or mucosae, contributing in some way to the defense against the neoplasm. Concerning more precisely the odontogenic field, LCs were identified both in the epithelial strands of 2 cases of calcified and non-calcified Pindborg's tumors (Assano *et al.*, 1990; Takata *et al.*, 1993).

LCs were observated in odontogenic cysts (Contos *et al.*, 1987; Kalusukoma *et al.*, 1987; Matthews and Browne, 1987; Gao *et al.*, 1988). LCs are usually increased in areas of intense inflammation. Non specific inflammatory reactions may be working in concert with specific immunological responses in apical periodontal cyst formation (Matthews and Browne, 1986; Contos *et al.*, 1987). The finding of lymphocytes adjacent to LCs support the premise that T lymphocytes act as effector cells of the disease process after receiving information from stimulated LCs (Contos *et al.*, 1987).

On the other hand, LCs distribution seems to be associated with the degree of differentiation of the epithelia (Gao *et al.*, 1988). In fact, LCs have been implicated in the epithelial proliferation control and keratinization (Potten and Allen, 1975). New observations are yet necessary to confirm this particular aspect of the LCs biological significance.

Are the S-100 protein cells identified in our material true Langerhans cells (Charbit *et al.*, 1986)?

Precisely, LCs were identified by their phenotypic expression of S-100 protein. As melanocytes are positive both for S-100 alpha and beta subunit a confusion may be made. But in our experience, melanocytes were never found in our file of odontogenic cysts.

About 20% of the intraepithelial cell population are non yet identified: small to medium sized cells with deeply indented cerebriform nuclei (Burkhardt *et al.*, 1979). Probably other S-100 negative (= « veiled cells ») types of dendritic cells (Barrett and Scully, 1994) may assume functional roles which have to be determined in the future. The possible presence of those cells remain to be identified in odontogenic cysts.

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