Exploration of the Histogenesis of Congenital Granular Cell Epulis: An Immunohistochemical Study

EMAN A. ABO-HAGER, Ph.D.*; DINA S. KHATER, Ph.D.** and MOHAMED M. AHMED, Ph.D.***
The Departments of Oral & Dental Pathology, Faculty of Dental Medicine for Girls, Al-Azhar University*; Oral & Dental Pathology, Faculty of Oral & Dental Medicine, Cairo University** and Oral & Dental Pathology, Faculty of Dental Medicine for Boys, Al-Azhar University***.

ABSTRACT

Objective: Congenital granular cell epulis is a benign soft tissue lesion of the neonate that arises from the alveolar ridges of the jaws in newborns. The aim of this study is to define the histogenesis of congenital granular cell epulis by using several immunohistochemical markers.

Materials and Methods: A series of six cases of congenital granular cell epulis were immunostained with a panel of antibodies (NSE, CD68, CD99, Mesothelin, Inhibin-α, GFAP, Dystrophin, NGFR/p75 and TLR1). The percentage of positive cells was measured in the form of area percent using an image analysis software (Leica-Qwin) system. Analysis of Variance (ANOVA) was used to compare between means of positive immunostaining antibodies.

Results: Granular cells of all cases showed positive cytoplasmic immunostaining for NSE and CD68. The interstitial cells of five cases showed immunopositivity for CD99. Granular and interstitial cells in all cases were negative with the remaining antibodies. The immunoreactivity of the granular cells to NSE showed the statistically significantly highest mean area percent. This was followed by CD68 which showed lower values. The immunoreactivity of the interstitial cells to CD99 showed the statistically significantly lowest mean area percent.

Conclusion: The expression of NSE and CD99 in all cases of the present study support a neuroectodermal derivation of congenital granular cell epulis.


INTRODUCTION

Congenital granular cell epulis (CGCE) (synonyms: congenital epulis of newborn, congenital gingival granular cell tumor, and congenital granular cell myoblastoma) was first described by Neumann [1] in 1871, as a benign soft tissue lesion of the neonate that always arises from the alveolar ridges of the jaws in newborns. This terminology was suggested by the newest histological classification of the World Health Organization [2], but the lesion is also known in recent literature under a variety of names, including congenital granular cell tumor, congenital granular cell lesion, and gingival granular cell tumor of the newborn.

CGCE is a very rare lesion that appears as a sessile or pedunculated lesions protruding from the neonate’s mouth. It has a normal or reddish color and varies in size from several millimeters to a few centimeters. The tumor occurs eight times more frequently in females than males indicating a hormonal component in its development [3], and three times more frequently in the maxilla than mandible, mainly on its anterior portion. It usually presents as a single lesion, however multiple lesions have been reported [4]. The tumor usually shows a benign behavior with no further growth after birth, showing no recurrence or malignant transformation [5,6]. Microscopic examination shows nests of large polygonal cells with abundant eosinophilic granular cytoplasm and small basophilic nuclei and spindle cells resembling fibroblasts. Pseudoepitheliomatous hyperplasia of the overlying mucosa does not occur in CGCE as in granular cell tumors [7-11].

In the past, some pathologists assumed that CGCE and the granular cell tumor (GCT) are
one lesion because of their similarity under light microscopy [12,13]. However, further ultrastructure and immunohistochemical investigations support their being two distinct lesions [14]. As such, CGCEs and GCTs are now considered to represent separate entities based on their clinical manifestations and behavior, certain histopathologic features, and immunophenotypic markers.

Although the existence of CGCE has been known for many years, the histogenesis of CGCE has remained controversial in spite of a vast number of immunohistochemical and ultrastructural studies. One of the main deficiencies of immunohistochemical studies on CGCEs is due to its rarity, with most studies having been performed on one or two lesions and only a few on 3-5 lesions. Reaching a meaningful conclusion from a single lesion or a very small series is invariably difficult [14]. Numerous theories of its histogenesis have been proposed including epithelial [12], undifferentiated mesenchymal cells [15-17], fibroblasts [18], histiocytes [11,15], pericytes [19], smooth muscle differentiation [20], nerve related cells [21,22] and myofibroblasts [23]. In search of the origin of the granular cells, numerous epithelial, myogenic, neurogenous, neuroendocrine, vascular, fibroblastic, histiocytic, and hormonal immunostains have been tested. With the exception of vimentin, they were all found to be negative in most of the studies, including the S-100 protein that is positive in GCT, meanwhile neuron-specific enolase (NSE) was found to be positive in some studies of CGCE [22,24-26] and negative in others [22,23-29].

The main objective of the present study is to elucidate the cell derivation of CGCE using a panel of antibodies against different antigens in an attempt to approach its origin on an immunoprofile basis.

MATERIAL AND METHODS

This study consisted of a series of six cases of CGCE which were retrieved as paraffin blocks from the archives of the Oral Pathology Department, Faculty of Oral and Dental Medicine, Cairo University; Oral and Dental Pathology Department, Faculty of Dental Medicine, Al-Azhar University and El-Riyadh College for Dentistry and Pharmacy, Kingdom of Saudi Arabia. All the patients were females: All cases were from the anterior maxillary alveolar ridge.

For immunohistochemistry (IHC), four-micron serial sections were obtained from each formalin-fixed paraffin-embedded tissue block, mounted on electrically positively charged slides and dried. To enhance immunoreactivity, sections were subjected to microwave heat treatment. The preparation procedure was as follows: the slides were first deparaffinized, dehydrated in graded ethanol concentrations, and incubated with 0.6% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase activity. After rinsing with water, the slides were placed in a glass dish filled with 10mmol/L sodium citrate buffer, pH 6.0. Tissue sections were boiled in a microwave oven twice for 5 minutes each to enhance immunoreactivity. The slides were allowed to cool and rinsed with phosphate-buffered saline (PBS), pH7.2.

The immunoreactivity of the granular cells was examined by a panel of nine commercially available primary antibodies, both traditional and new ones: NSE, CD68, CD99, GFAP (monoclonal, ready to use, Zymed, USA), Inhibin-α (monoclonal, 1:20, Dako, Denmark), NGFR/p75 (monoclonal, 1:20, Diagnostic BioSystems, Pleasanton, CA, USA), Mesothelin, Dystrophin (monoclonal, ready to use, Biogenix, Canada) and TLR1 (monoclonal, 1:20, ProSci, USA). This extensive panel of immunostains was chosen to determine the immunoprofile of CGCE lesions and to enable us to compare it to a large series of oral CGCTs, which we recently examined using a similar panel of immunostains. Immunohistochemical staining was done according to the manufacturer’s instructions. Immunostaining was detected by using a SuperPicTure™ polymer kit (Zymed-Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s recommendations. For negative controls, the slides were treated following the same procedures, including antigen retrieval, except for the application of the primary antibodies.

Immunohistochemical assessment:

The immunostained sections were examined using light microscopy to assess the prevalence of positive cases and the localization of immunostaining within the tissues. Tumor cells with unequivocal staining of the granular cytoplasm were considered positive. The percentage of
positive cells was measured in the form of an area and area percent inside a standard measuring frame of area 11434.9 micrometer$^2$ per 10 fields using a x 200 magnification, and an image analysis software (Leica-Qwin) system, at the Oral and Dental Pathology Department, Faculty of Dental Medicine for Girls, Al-Azhar University.

**Statistical evaluation:**
Quantitative data of the image analyzer were first summarized and presented as means and standard deviation (SD) values. Analysis of Variance (ANOVA) was used to compare the area percent of the three genotypes. Tukey’s post-hoc test was used for pair-wise comparison between the means when ANOVA test was significant. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 16.0® (Statistical Package for Scientific Studies) for Windows (SPSS, Inc., Chicago, IL, USA).

**RESULTS**

I- **Histopathological findings:**

The microscopic examination of the five lesions was similar, showing that the tumors were uncapsulated, and covered by a stratified squamous epithelium without rete ridges. The overlying epithelium appeared to be separated in most instances from the underlying tumor cells by a zone of fibrous connective tissue of varying thickness. Marked, but focal, acute and chronic inflammatory cell infiltrates were present. The tissue was composed mainly of nests of extremely large cells with abundant pale acidophilic granular cytoplasm. The cells contained a single, round to oval, usually centrally and occasionally eccentrically located nucleus with a single and deeply stained nucleolus. The cell membranes were prominent in most areas, while they were delicate and indistinct in others, thereby creating an impression of syncytium.

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Fig. (1): Photomicrograph of congenital granular cell epulis (A) H&E stain (original magnification X100) (B) granular cells showed intense immunopositivity for NSE, (C) granular cell were positive for CD68, (D) only interstitial cells were positive for CD99 (original magnification X200).
Occasionally, non-granular, medium-sized spindle shaped and markedly elongated cells were seen around the granular cells (interstitial cells). There was no cellular pleomorphism or increased mitotic activity throughout the lesion.

A prominent and complex network of vascular channels, ranging from small capillaries to dilated vessels, was regularly dispersed between the granular cells. Some vessels had well-developed walls, but the majority—even those of fairly large caliber—were composed of a single layer of endothelium. Fibrous stroma was minimally present and appeared to be completely lacking for the most part. Vascular channels were abundant in the pedicles.

II- Immunohistochemical findings:

All tumors were positive for NSE, only granular cells showed a cytoplasmic immunostaining for NSE (Fig. 1B). The granular cells of all cases showed positive immunostaining for CD68 (Fig. 1C). The interstitial cells of five cases showed immunopositivity for CD99 (Fig. 1D). Granular and interstitial cells in all cases were negative with the remaining antibodies (Mesothelin, Inhibin-α, GFAP, Dystrophin, NGFR/p75 and TLR1).

III- Statistical analysis:

The immunoreactivity of the granular cells to NSE showed the statistically significantly highest mean area percent. This was followed by CD68 which showed lower values. The immunoreactivity of the interstitial cells to CD99 showed the statistically significantly lowest mean area percent; shown in Table (1) & Fig. (2).

![Bar chart comparing the mean positivity area percent of the three antibodies.](image)

**Table (1):** The means, standard deviation (SD) values, results of ANOVA and Tukey’s test for comparison between area percent of the three antibodies.

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>NSE</th>
<th>CD68</th>
<th>CD99</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>49.7</td>
<td>32.5</td>
<td>3.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SD</td>
<td>10.2</td>
<td>17.6</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at p<0.05. Means with different letters are statistically significantly different according to Tukey’s test.

**DISCUSSION**

In the present study, an attempt to explore the histogenesis of CGCE by using several immunohistochemical markers was made. All cases of our work showed strong positive neuron-specific enolase (NSE) immunoexpression in the cytoplasm of granular cells. NSE is a dimeric glycolytic enzyme that is composed of three distinct subunits (alpha, beta and gamma). The alpha subunit is expressed in most tissues, the beta subunit only in muscle, whereas the gamma subunit is expressed primarily in neurons in both normal and neoplastic neuroendocrine cells [30]. This finding is consistent with several reports [21,22,24,26] which may suggest a possibility for a neural origin for CGCE. Recently, Vered et al. [14] found fewer cells with moderate immunoreactivity for NSE and did not support a neuroendocrine differentiation of the granular cells as experience of NSE has shown lack of neural specificity in tumors [31,32]. Meanwhile, other investigators used NSE to show that the cells may be related by neural origin [21,22] and hence caution should be exercised in relying on NSE as a marker of histogenesis. Furthermore, the present work showed negative immunostaining for NSE in interstitial cells in all studied cases although one study reported that they were positive for NSE [22].

All examined cases in this study showed positive immunostaining of the granular cells for CD68, which is not consistent with Pietro Leocata et al. [7]. Light and electron microscopy showed that CD68 is associated with phagolysosomes [33], mostly associated with macrophages. Kaiserling et al. [28] investigated the ultrastructural and the immunohistochemical features of both CGCE and the granular cell tumour (GCT), and found that the phagolysosomes were much more uniform in appearance.
We can interpret the positive immunostaining of CD99, and to NSE and S-100 to a lesser extent. and the neuroendocrine phenotype was con-
noexpression by the vast majority of tumor cells. The presence of strong membranous CD99 immu-
diagnosis of Ewing’s sarcoma/PNET on the basis of their differenti-ation. Colovic et al. used to diagnose tumors with neuroectodermal differentiation. Colovic et al. [44] based their diagnosis of Ewing’s sarcoma/PNET on the presence of strong membranous CD99 immunoexpression by the vast majority of tumor cells and the neuroendocrine phenotype was confirmed immunohistochemically by positivity to CD99, and to NSE and S-100 to a lesser extent. We can interpret the positive immunostaining of interstitial cells for CD99 as the reflection of some sort of neuroectodermal differentiation, and this is in accordance with Takahashi et al. [22].

Glial fibrillar acid protein was negative in the present study. This is not consistent with most of the investigators who believed that the granular cell of CGCE is derived from undifferentiated mesenchymal cells; however, it is in agreement with Takahashi et al. [22]. On the other hand, some investigators accepted that CGCE is derived from muscle cells [20,24], whereas other ultrastructural studies failed to support this theory [19,36]. The absence of desmin and myoglobin in CGCE reported by Urgas et al. [26] and the lack of dystrophin in our study have not supported the theory that it is derived from muscle cells.

In accordance with Vered et al. [14], the present results showed that both granular and interstitial cells were negative to NGFR/p75. NGFR/p75 was originally discovered as a low affinity nerve growth factor receptor. Later, it was found that it was the receptor for all neurotrophins. It mediates signals of neurotrophins for neuronal survival, apoptosis, neurite outgrowth and synaptic plasticity. It can mediate cell survival as well as cell death of neural cells and is highly expressed in a number of non-neuronal and neuronal cells including motor neurons during development and also in damaged neurons [45].

Inhibin-α is a sensitive and relatively specific marker of sex cord-stromal tumors of the ovary. The lack of inhibin-α expression in this work is in agreement with Lack et al. [15] and Tucker et al. [23] who detected an absence of estrogen and progesterone receptors in CGCE. This gives us no clue for the striking female predominance.

Despite limited understanding of mesothelin’s biological function, its restricted expression by normal tissues combined with frequent high level expression in several tumor types including ovarian and pancreatic adenocarcinomas, led to considering it as a reliable tumor marker [46]. The present study revealed negative immunoreactivity to mesothelin, supporting the non-neoplastic nature of CGCE [47]. This can be also supported by the clinical observations that the lesion has no growth potential after birth, its ability to regress spontaneously (when small lesions are involved), and the lack of recurrence
even after incomplete excision [48]. In light of these features, it has been assumed that a CGCE is either reactive in nature or that it is a manifestation of a degenerative process [49].

In summary, we believe that neuroectodermal differentiation may play a role in the histogenesis of CGCE because of the positive staining of CD99 which is known to be a marker for neuroectodermal tissues [41-43] and positive immunostaining to NSE in spite of the negative staining of S-100 and NGFR/p75 reported in the literature [5,14,24]. In addition, the positive immunoperoxidase of granular cells to CD68 similar to granular cells of GCT, which is known to be of neural origin, infers that these cells contain an intracytoplasmic accumulation of phagolysosomes of neural origin [50-52].

REFERENCES


