Immunophenotype of Dental Implant-Associated Peripheral Giant Cell Reparative Granuloma in a Representative Case Report

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We report the case of a 74-year-old white male patient who had worn an overdenture for the previous 6 years, retained by 4 screwed implants and a bar, who presented with an exophytic multilobed lesion of 2.5 × 2.0 cm on the anterior aspect of 1 implant neck, which was surrounded by pink-reddish tissue. All of the soft tissue around the implant was removed until the periosteum was reached. Histologic examination of the lamina propria revealed a cellular proliferation with imprecise boundaries, dense stromal component composed of spindle- to round-shaped mononucleated cells (fibroblasts and monocytes/macrophages), abundant multinucleated giant cells surrounding microscopic hemorrhagic foci, and deposits of hemosiderin; the diagnosis was peripheral giant-cell reparative granuloma (PGCG). Giant cells share the immunohistochemical expression of monocyte/macrophage markers (CD68, calprotectin [Mc387]) and osteoclastic cell markers (tartrate-resistant acid phosphatase, cathepsin K, and microphthalmia-associated transcription factor). After 6 months of follow-up, no bone resorption or recurrence of implant loss was observed. There have been only 12 case reports on dental implant–associated PGCG. Research results to date indicate that there may be little difference in immunophenotype among the giant cells of PGCG, central giant cell reparative granuloma, and peri-implant osteolysis. In conclusion, the immunohistochemical study confirms an osteoclast like giant cells phenotype differentiation in PGCG.

Key Words: peripheral giant cell reparative granuloma, dental implant

INTRODUCTION

Giant cell reparative granuloma (GCRG) was first described by Jaffe in 1953 1 and was subsequently divided between 2 distinct clinical forms: peripheral (PGCG), involving the gingiva and alveolar mucosa; and central (CGCG), mostly involving the facial bones and promoting osteolytic destruction at intraosseous level. 2

PGCG is an exophytic tumor-like pathologic condition arising on the buccal or lingual attached gingival or alveolar mucosa and on the crest of the edentulous alveolar ridge. Although the etiology is uncertain, it is likely to be a reactive lesion caused by chronic local irritants or trauma rather than a true neoplasm; these could include plaque, calculus, chronic infections, chronic irritation, tooth extraction, improperly finished fillings, or an unstable dental prosthesis. 3

Only 13 cases of dental implant–associated PGCG have been published to date. 4–12 However, in our knowledge, there are no immunohistochemical reports about possible histogenetic origin of these lesions.

According to the literature, PGCG associated with dental implants clinically manifests as a gingival lobulated mass similar to pyogenic granuloma (PG), which is the preliminary clinical diagnosis in 56.3% of PGCG cases. Three-quarters of affected patients are female (10/13), the mean age at diagnosis is 50 years (range, 21–69 years), and the localization is the posterior maxillary area in 76.9% of cases (10/13). PGCG associated with dental implant has been reported to induce bone resorption in 76.9% (10/13), implant loss in 38.4% (5/13), and single or multiple relapse in 38.4% of cases (5/13).

CASE REPORT

We report the case of a 74-year-old white male patient, ex-smoker for 14 years, and with no other harmful habits, with a history of periodontitis but no systemic disease. The patient...
reported a previous history of inflammatory cyst in the anterior region of the maxilla, although after surgical removal, 10 years earlier, no signs of recurrence had been observed, neither clinical nor radiologically. For the previous 6 years, he had worn an overdenture retained by 5 screwed implants. Another implant was placed in the surrounding area, but it was not used in the bar because a fracture in its neck (Microdent System, Barcelona, Spain). An exophytic multilobed lesion of 2.5 × 2.0 cm (Figure 1a) was observed at the interface between 1 implant and the bar. Yellow lines crossed the lesion, which was attached to pink-reddish keratinized gingiva that surrounded the implant neck but was also observed at some other sites. The lesion was ulcerated in some areas. The patient reported that the lesion had grown over the previous month. It was not hard to the touch.

Total extirpation of the lesion was conducted under local anesthesia (Ultracain, Normon S.A., Tres Cantos, Madrid, Spain) (Figure 1b and c). Healthy margins were obtained from the adjacent keratinized mucosa. All of the soft tissue around the implant was removed until the periosteum was reached. The resulting wound was irrigated with chlorhexidine, which was then also applied as a gel. The patient was instructed to perform a saline mouth-rinse from the first day post surgery every 8 hours for a 7-day period, during which he was prescribed 1 g amoxicillin and 600 mg ibuprofen every 8 hours (tablet form). The patient was recalled every 7 days to follow-up the healing, which proved uneventful. The bar was removed from the beginning of the surgical treatment until the healing was totally completed. After 4 weeks, the bar and overdenture were reinstalled (Figure 1d). After 6 months of follow-up, no evidence of relapse has been observed.

The radiographic examination showed no marginal bone loss related to this implant, ruling out peri-implantitis, and the peri-implant bone had a normal appearance. No marginal bone loss was observed for any of the 4 implants, although some surrounding clinical inflammation was observed due to the inadequate cleaning of bar-implant interfaces (Figure 2a and b).

Histologic examination of the PGCG revealed a partially ulcerated exophytic lesion coated by polystratified mucous epithelium with spongiosis (Figure 3a). The lamina propria showed cellular proliferation with imprecise boundaries and a dense stromal component made up of spindle-shaped to round-shaped mononucleated cells (fibroblasts and monocytes/macrophages), with abundant multinucleated giant cells surrounding microscopic hemorrhagic foci, and deposits of hemosiderin (Figure 3b). Varying proportions of hemosiderin remains and small, negatively polarized refractive particulates were detected within the macrophages and giant cells. In the deep lamina propria, a moderate chronic inflammatory infiltrate
was observed, composed of plasma cells, macrophages, and lymphocytes.

For the immunohistochemical study, buffered 10% formaldehyde-fixed, paraffin-embedded sections were dewaxed, hydrated, and heat-treated in 1 mM EDTA buffer for antigenic unmasking in a PT module (Thermo Fisher Scientific, Kalamazoo, Mich) at 95°C for 20 minutes. Sections were incubated for 10 minutes at room temperature with different prediluted antibodies (Table). Immunohistochemical staining was performed with an automatic immunostainer (Autostainer480, Thermo Fisher Scientific) using the micropolymer-peroxidase-based method (Ultravision Quanto, Thermo Fisher Scientific, Kalamazoo, Mich), followed by development with diaminobenzidine. All reagents and antibodies were purchased from the same company (Master Diagnostica, Granada, Spain).

The immunophenotypes of mono-nucleated and multi-nucleated cells are reported in the Table. Giant cells share the expression of monocyte/macrophage markers (CD68, calprotectin [Mc387]) and osteoclastic cell markers (tartrate-resistant acid phosphatase [TRAP], cathepsin K, and microphthalmia-associated transcription factor [Mitf]). We observed translocated cytoplasmic p63 expression in the giant cells (Figure 4).

### DISCUSSION

Although PGCG is the most common giant cell lesion of the jaws, its association with implants is rarely observed.4–12 We report a new case of dental implant–associated PGCG and describe the clinical appearance, morphology, and immunophenotype of the reactive cells induced in the gingiva.

It can be difficult to clinically differentiate between PGCGs and PGs, and peripheral odontogenic tumors and CGCGs can perforate the cortex and mimic a PGCG. Although CGCGs are usually associated with bone expansion,13 they should be considered in the differential diagnosis of PGCG. Because of the similar clinical appearance of all these lesions, the definitive diagnosis is based on the histologic study, and the microscopic examination of a biopsy is mandatory. In fact, the preliminary

![FIGURES 2 AND 3.](image-url)
clinical diagnosis of peri-implant PGCG was PG in 7 out of the 13 reported cases,\(^4\)\(^–\)\(^12\) although PG is rarely associated with a dental implant (only 3 cases to date).\(^14\)

Histologically, both PGCG (associated or not with dental implant) and CGCG show similar features (ie, a variable proportion of multinucleated giant cells in a background of proliferative ovoid to spindle-shaped mesenchymal cells). Nevertheless, these lesions differ in their biological activity.\(^15\),\(^16\) Peripheral lesions that are not associated with a dental implant affect the bone in only 10% of cases, whereas implant-associated PGCG produces bone resorption in >75% of cases (although not in the present patient), and CGCG is characterized by a more rapid growth, root resorption, and cortical perforation.\(^15\)

The etiology of dental implant-related reactive lesions has not been fully elucidated. The close association between the lesion and the titanium implant strongly suggests that the metal-like particles histologically observed in the tissue correspond to titanium and might be attributed to a corrosive process,\(^5\) although unequivocal metallosis was not observed in the present PGCG case. Other causes can contribute as recognized chronic irritative effect such as maladjusted prosthetic, excessive proximity between implants, response to peri-implantitis, entrapment of food debris, or dental calculus by lack of hygiene in the surrounding area to the implants.\(^5\)

Recently, Peñarrocha-Diago and coworkers\(^1\),\(^2\) have reported a similar case related to implants. However, a main difference can be noted. In their case, a fixed prosthesis cemented over implants and horizontal bone loss was reported, which is not present in our case. Anyway, as in the majority of the previous cases reported, they do not study the phenotype of the giant cells.

The histogenesis of giant cell lesions is also unclear. Although it has been proposed that PGCG may originate from elements of the periodontal ligament or periosteum,\(^16\) it is mainly composed of monocyte/macrophage cells.\(^17\),\(^19\) Our immunohistochemical study confirmed a monocyte/macrophage cell origin of the PGCG, with massive positivity for CD68, numerous calprotectin (Mc387)-positive giant cells, and scattered CD14-positive giant cells, similar to reports in PGCGs not related to a dental implant\(^17\),\(^18\) and in CGCGs, as previously suggested.\(^16\) Shen et al\(^19\) reported that the macrophages, multinucleated foreign body giant cells, and osteoclasts in peri-implant osteolytic tissue derive from a common hematopoietic lineage, and the mild CD34 expression in some giant cells in the present case may reflect this provenance. Various phenotypic markers have been utilized to differentiate among these cells, including gene products that give the osteoclast its unique capacity to recognize and bind to the bone surface for resorption of mineralized bone matrix. The attachment and activation of the osteoclast has been shown to involve several integrins, including the vitronectin receptor \(\alpha v \beta 3\).\(^20\),\(^21\) Expression of this integrin has served as a useful marker to identify osteoclasts and to distinguish them from their colony-forming unit-macrophage precursors, which do not express the \(\beta 3\) gene.\(^22\) Additional gene products that are essential for creating an acidic environment for mineral dissolution and resorption of the organic matrix of bone are induced during osteoclast differentiation. Cathepsin K and TRAP are among the enzymes that are expressed in these cells and contribute to resorption of the extracellular matrix component of bone.\(^23\)\(^–\)\(^25\)

Mitf, a member of the helix-loop-helix transcription factor subfamily with at least 9 isoforms, which is abundantly expressed in mononuclear and multinucleated osteoclasts, is involved in the terminal differentiation of osteoclasts.\(^26\) However, no osteoclast-specific Mitf isoform has been reported,\(^27\) and this factor is variably expressed in various types of monocyte-derived giant cells and/or adjacent mononuclear cells/histiocytes, including osteoclast-like giant cells observed in various tumors (eg, in 89% of giant cell tumors of the bone, 100% of GCRG cases, and 80% of foreign-body giant cell reactions).\(^26\)

In our case, the massive expression of TRAP, cathepsin K, and Mitf indicates an osteoclastic-like differentiation. Nevertheless, it should be borne in mind that expressions of cathepsin K, TRAP, and Mitf genes have served as markers of the osteoclast phenotype and, under certain conditions, cathepsin K and TRAP activity has been detected in cells that are not directly involved in bone resorption,\(^28\) indicating that their expression is not restricted to osteoclasts. For instance, macrophages have been shown to express both of these enzymes.\(^29\) However, the occurrence of bone loss in a high percentage of PGCG cases (10/13) suggests that these giant cells possess true resorption capacity.

Despite being a single case, our findings provide further evidence that cells expressing the full repertoire of osteoclast phenotypic markers are involved in the pathogenesis of PGCG. Konttinen et al\(^25\) also demonstrated that foreign-body giant

### Table

<table>
<thead>
<tr>
<th>Antibody (Clone)</th>
<th>Multinucleated Cells Distribution, %</th>
<th>Intensity (1–3+)</th>
<th>Mononucleated Cells Distribution, %</th>
<th>Intensity (1–3+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68 (KP-1)</td>
<td>100</td>
<td>+++</td>
<td>40</td>
<td>+++</td>
</tr>
<tr>
<td>Calprotectin (Mc387)</td>
<td>4</td>
<td>+</td>
<td>68</td>
<td>+++</td>
</tr>
<tr>
<td>CD14 (7)</td>
<td>18</td>
<td>+</td>
<td>&lt;1</td>
<td>+</td>
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<tr>
<td>CD34 (QBEND/10)</td>
<td>70</td>
<td>+</td>
<td>&lt;5</td>
<td>+</td>
</tr>
<tr>
<td>TRAP (26(^v))</td>
<td>99</td>
<td>+++</td>
<td>20</td>
<td>+++</td>
</tr>
<tr>
<td>Cathepsin K (3F9)</td>
<td>100</td>
<td>+++</td>
<td>85</td>
<td>+++</td>
</tr>
<tr>
<td>Mitf (C5/DS)</td>
<td>50</td>
<td>++</td>
<td>&lt;1</td>
<td>++</td>
</tr>
<tr>
<td>p63 (4A4)</td>
<td>68*</td>
<td>++</td>
<td>&lt;5</td>
<td>+</td>
</tr>
</tbody>
</table>

*Cytoplasmic immunostaining; Mitf indicates microphthalmia-associated transcription factor; TRAP, tartrate-resistant acid phosphatase.
FIGURE 4. Immunophenotype expression of peripheral giant cell granuloma. Intense immunohistochemical staining of (a) tartrate-resistant acid phosphatase, (b) cathepsin K, (c) microphthalmia-associated transcription factor, (d) cytoplasmic p63, and variable expression of monocyte/macrophage markers: (e) intense CD68 positivity and (f) scattered calprotectin and (g) CD14 positivity in giant cells and mononuclear cells of peripheral giant cell granuloma. (h) CD34 intense positivity in endothelial cells and weak positivity in giant cells (original magnification ×20).
cells, although believed to be phenotypically and functionally distinct from osteoclasts, can express many osteoclast-associated proteins. However, the levels and expression patterns of these proteins differ between the 2 cell types. We speculate that, in addition to the role of cytokines and growth factors, the substrate with which these cells interact plays a critical role in their differential phenotypic and functional properties. Based on other reports in the literature, it appears that PGCG, CGCG, and peri-implant osteolysis can have similar immunophenotypes. In conclusion, the immunohistochemical study confirms an osteoclast-like giant cells phenotype differentiation in PGCG.

ABBREVIATIONS

CGCG: central giant-cell reparative granuloma
GCRG: giant cell reparative granuloma
Mittf: microphthalmia-associated transcription factor
PG: pyogenic granuloma
PGCG: peripheral giant-cell reparative granuloma
TRAP: tartrate-resistant acid phosphatase

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