REVIEW

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Diagnostic and therapeutic approaches in oral cavity granulomas based on new data concerning their origin and pathogenesis

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Abstract

Giant cell granulomas in the oral cavity are reactive hyperplastic lesions that arise either peripherally in the mucoperiosteum or centrally in the bone. The peripheral giant cell granuloma (PGCG) is a benign lesion induced by local chronic irritation. It may develop at any age, and tends to be more frequent in females. Central giant cell granuloma (CGCG) is a reactive lesion of unknown etiology. It commonly occurs in children and young adults. It is also predominant in females and frequently located in the anterior part of the mandible. Histologically, PGCG and CGCG have similar features. The lesions are non-encapsulated proliferations of oval and spindle-shaped mononuclear cells (MCs) and multiple multinucleated giant cells (MGCs) in a vascular supporting stromal tissue, associated with foci of hemorrhage. Despite the similar microscopic features, PGCG and CGCG have different clinical behavior. PGCG is usually reduced in size and asymptomatic. It grows locally, as an exophytic lesion on the alveolar mucosa, but may become slightly infiltrative in the underlying periosteum and bone. After complete excision and curettage, it has a low recurrence rate. Contrarily, CGCG has an aggressive behavior, with rapid growth and intense osteolytic activity causing perforation of the cortical plate, teeth malposition and pain. Moreover, it is characterized by a high recurrence rate. This review focuses on the origin and activating pathways of MCs and MGCs, discusses the mechanisms underlying their biological activity, tries to explain the variable clinical behavior and proposes therapeutic approaches for the granulomas associated with the jaw bones.

Keywords: oral cavity granulomas, multinucleated giant cells, pathogenesis, diagnosis, therapy.

Introduction

The giant cell granulomas are reactive hyperplastic lesions associated with various tissues in the oral cavity. Two entities have been described, according to the location, etiology and clinical evolution [1–3].

Peripheral giant cell granuloma (PGCG) occurs as an abnormal proliferation of the soft tissues in response to the local aggressions and is located on the gingiva, alveolar mucosa, mucoperiosteum or periodontal ligament. PGCG are commonly associated with the teeth and are caused by chronic mechanical irritation or low intensity repetitive trauma due to food impact, defective dental restorations, occlusal trauma and may be enhanced by the presence of dental plaque and calculus on the retentive dental or prosthetic surfaces [1–3]. Other causes could be the traumatic tooth extractions and the associated inflammatory reactions [1, 4]. On the edentulous alveolar crest, the oral mucosa can proliferate to form granulomatous lesions due to unstable prosthetic pieces [3].

Central giant cell granulomas (CGCG) develop inside the jaw bones as a reaction to unknown factors [5]; however, in some cases, the formation of the CGCG was associated with dental implants and reparative processes after intraosseous inflammations or hemorrhages. A genetic predisposition has also been hypothesized [4, 6].

PGCG can occur at any age, and it is more frequent in females. It develops slowly and asymptomatically as a sessile lesion on the oral mucosa. Occasionally, it can grow deeper into the mucosa to infiltrate the periosteum and to cause the "cupping" or a superficial depression by the erosion of the underlying bone [4, 7]. CGCG is more frequent in children and adults younger than 30 years and predominantly affects females. Frequently, it develops in the anterior region of the mandible [8].

The clinical evolution of the CGCG is aggressive, characterized by significant growth and increased osteolysis, leading to complications such as destruction of the cortical plates and malposition and migration of the teeth, which are accompanied by intense pain. The clinical behavior of the CGCG led to the hypothesis that it could be a neoplasm instead of a reactive lesion [8, 9].

Even though PGCG and CGCG have different etiology and evolution, these two lesions exhibit similar histological features [4, 10–12].

Clinical aspects of the PGCG

Clinically, PGCGs occur as exophytic lesions localized on the oral mucosa, and their presence is usually associated with inappropriate oral hygiene and multiple carious lesions [1–3]. At the intra-oral examination, the lesions appear as sessile masses with a large base or pedunculated (Figure 1), localized on the oral mucosa in zones subjected to occlusal trauma (Figure 1A) or on the alveolar crest and associated with remaining roots or carious cavities with retentive food debris and bacterial plaque (Figure 1C). The lesions may have a smooth or an irregular surface, due to the impressions of the antagonist teeth (Figure 1, A and C).

The consistency can be soft or firm, and the color ranges from pale pink to red or purple in the zones where the contact with the opposing teeth during occlusion occurs (Figure 1, A and C). Radiographically, the interdental septum adjacent to the PGCG can show more intense radiolucency with a normal aspect of the trabecular bone (Figure 1B); or the radiolucency can be seen in the alveolar bone adjacent to the remaining roots, with enlargement of the periodontal space (Figure 1D).

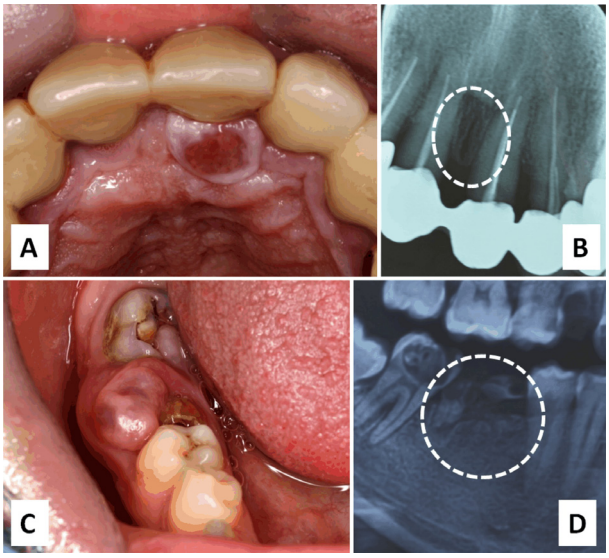

Figure 1 – ***Clinical and radiological aspects of the PGCGs: (A) PGCG located on the hard palate, with smooth surface and pink-red color; (B) Retroalveolar radiography: the radiolucency of the interdental septum between teeth 11 and 21; (C) PGCG associated with the remaining roots of tooth 46, with irregular surface and pink-purple color; (D) Detail of the panoramic radiograph: increased radiolucency of the alveolar crest and the periodontal space (authors collection). PGCG: Peripheral giant cell granuloma.***

Histological features in PGCG

The PGCGs are well-delimited, non-encapsulated masses consisting of numerous mononuclear cells and scattered multinucleated giant cells (MGCs) in a connective tissue stroma, with a rich vascularization (Figure 2A) [1, 4]. At the periphery, the blood capillaries are associated with foci of hemorrhage, extravasated red blood cells and hemosiderin deposits (Figure 2B). On the surface, the epithelium can show zones of dyskeratosis and ulcerations; the zones where the epithelium is ulcerated are covered by fibrinoid necrotic debris, and a rich chronic inflammatory infiltrate is present in the profound areas (Figure 2, C and D) [1, 5, 6].

The mononuclear cells (MCs) form a heterogeneous cell population, and morphologically, they can be ovoid or spindle-shaped, with oval euchromatic nuclei, resembling mesenchymal cells or young fibroblasts. MCs are closely related to the multinucleated cells (Figure 3A) [1, 2].

The MGCs are unevenly distributed in the stromal tissue, among the MCs and inflammatory cells; sometimes, MGCs can be associated with the blood vessels (Figure 3B). MGCs exhibit various morphologies in terms of size, amount and color of the cytoplasm, number and aspect of the nuclei (Figure 3C). Two main types of MGCs are observed; some MGCs are large, irregularly shaped, with abundant acidophilic cytoplasm and multiple euchromatic nuclei scattered in the entire cytoplasm; smaller MGCs are ovoid, with a dark cytoplasm and fewer heterochromatic nuclei, condensed in the center (Figure 3D) [1, 2, 5, 6].

MGCs origin and activating pathways

Numerous studies focused on the microscopic features and histogenesis of oral cavity granulomas. However, MGCs origin and activating pathways are still unclear [11]. Based on the morphological features, it is clear that MGCs are formed by the cytoplasmic fusion of mononuclear precursors. Moreover, the lack of proliferative capacity in MGCs sustains the transition from a mononuclear cell to a multinuclear cell [13].

However, the question that arises is: what cells are the precursors of the MGCs? Theoretically, two possible origins of the MGCs in the peripheral and central granulomas have been proposed: the macrophages/histiocytes or osteoclasts and the stromal MCs.

Based on ultrastructural and immunohistochemical (IHC) findings, several studies sustain both macrophagic/histiocytic and osteoclastic origins for the MGCs. However, these hypotheses are controversial, since MGCs do not have a role in phagocytosis or in bone resorption [1, 14].

Other studies demonstrated that MGCs originate from a subgroup of osteoclast precursors included in the histiocyte/macrophage-like MCs in the stromal tissue [4, 9, 15]. This hypothesis is sustained by evidence showing that functionally, the MCs in the granulomas encompass two groups: the macrophage-like cells, with monocytic origin, which are the precursors for MGCs, and the proliferating spindle-shaped cells, with mesenchymal origin, which are capable to differentiate into the fibroblast/osteoblast lineage, with different roles [16].

Numerous studies showed that some stromal MCs and the MGCs belong to the same lineage, based on the IHC similarities between these two types of cells [13] (Figure 4).

Macrophages are ubiquitary present in the tissues and are implicated in multiple processes: phagocytosis, activation of immune response, and release of cytokines that play important roles in angiogenesis and inflammation, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-β) and tumor necrosis factor-alpha (TNF-α) [17]. In order to identify the macrophages in light microscopy, the IHC methods that reveal the specific markers are recommended [18].

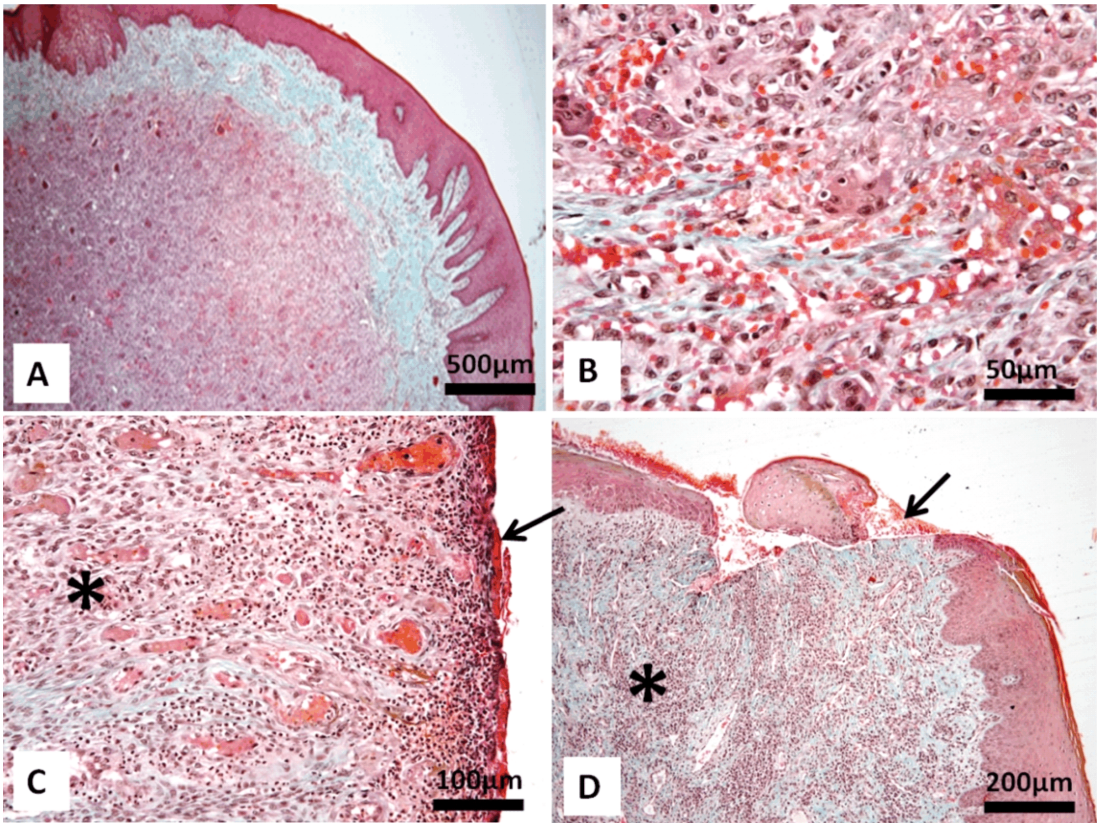

Figure 2 – ***Photomicrograph of the PGCG: (A) The lesion is well delimited and non-encapsulated; (B) Blood vessels associated with hemorrhage and extravasated erythrocytes; (C and D) Superficial ulceration (arrow) and chronic inflammatory infiltrate (asterisk); Goldner's trichrome staining (authors collection). PGCG: Peripheral giant cell granuloma.***

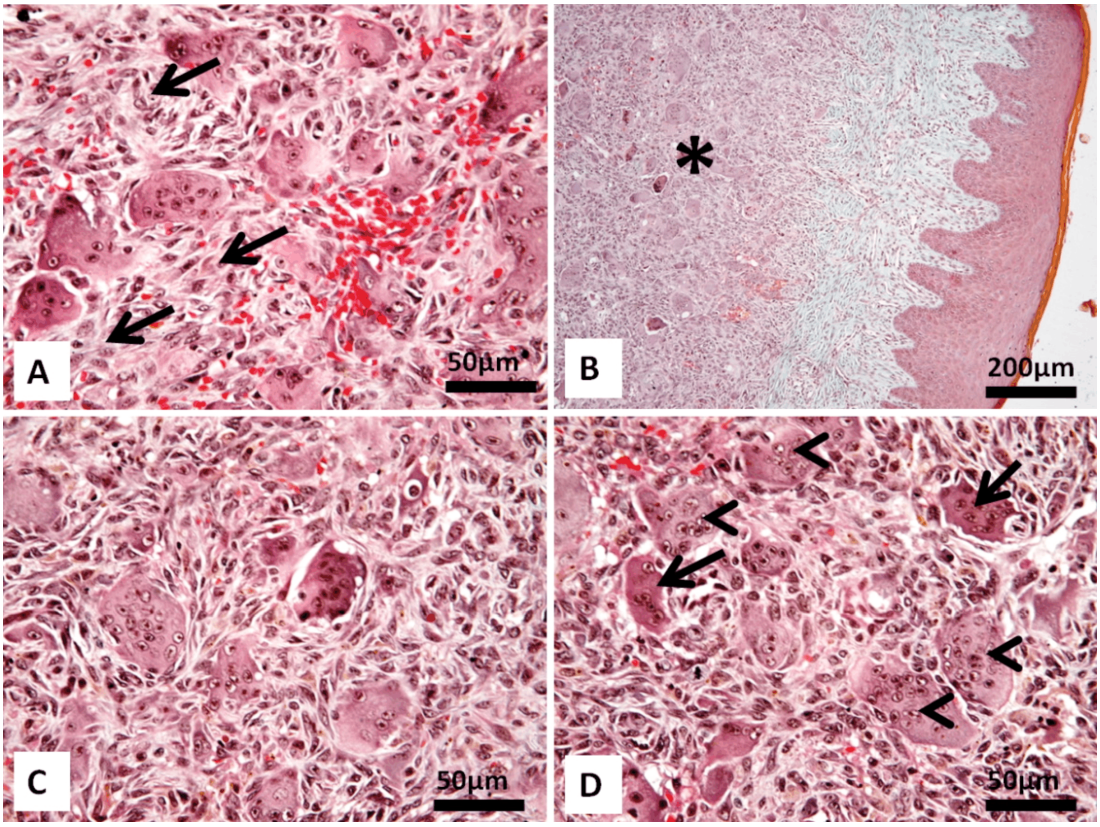

Figure 3 – ***Photomicrograph of the MCs and MGCs in the PGCG: (A) Oval and spindle-shaped MCs in the connective tissue stroma (arrows); (B) MGCs unevenly distributed in the lesion (asterisk); (C) Closely related MCs and heterogeneous MGCs; (D) The two types of MGCs: large, with abundant cytoplasm and euchromatic nuclei (arrowheads); small, condensed cells, with deep acidophilic cytoplasm and heterochromatic nuclei (arrows); Goldner's trichrome staining (authors collection). MCs: Mononuclear cells; MGCs: Multinucleated giant cells; PGCG: Peripheral giant cell granuloma.***

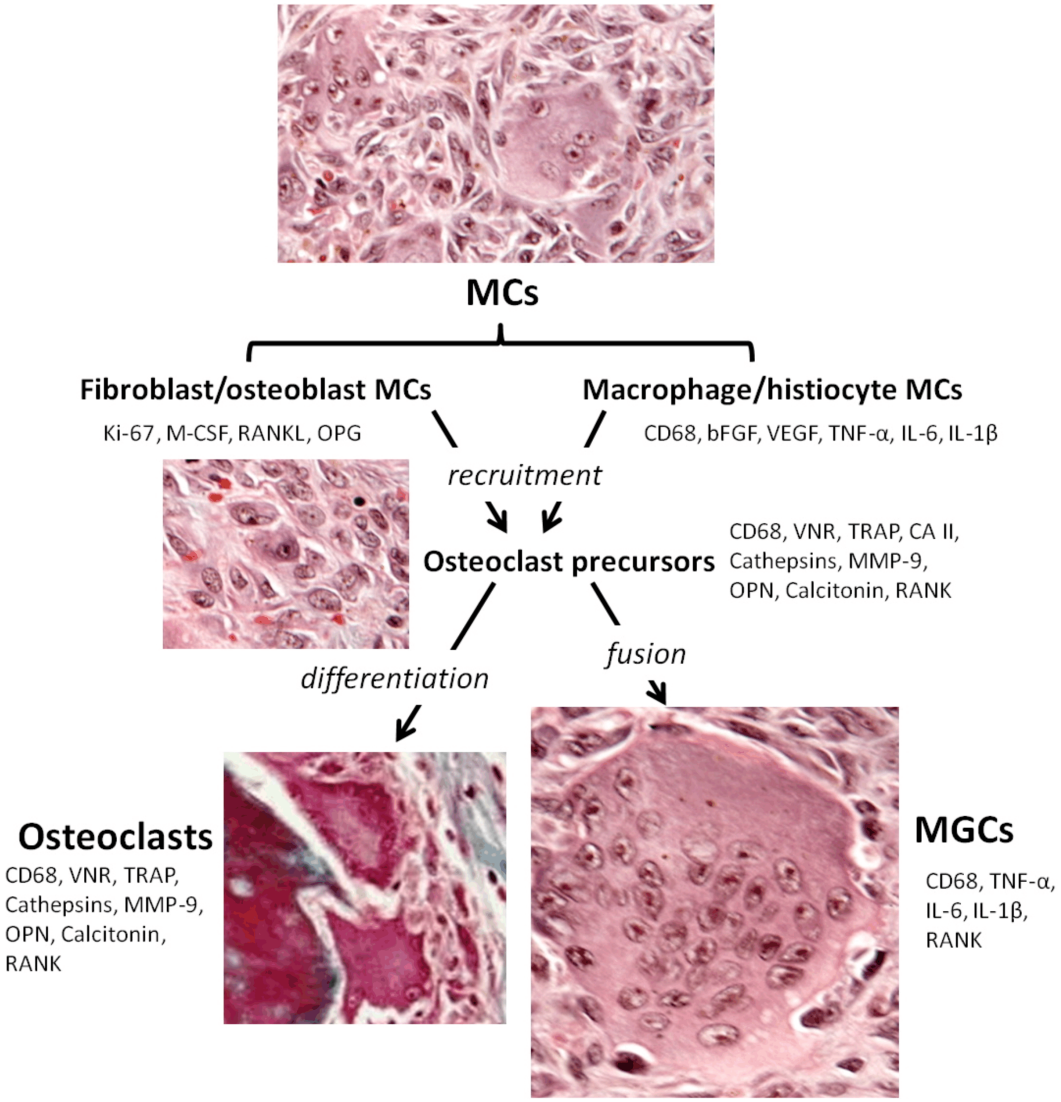

Figure 4 – ***Origin of MGCs: the fibroblast/osteoblast and the macrophage/histiocyte stromal MCs induce the recruitment of the osteoclast precursors and induce their differentiation and fusion to form the osteoclasts and the MGCs. The shared markers expressed by the macrophage/histiocyte MCs, osteoclast precursors and the MGCs suggest the common progenitor (original diagram). bFGF: Basic fibroblast growth factor; CD: Cluster of differentiation; IL: Interleukin; M-CSF: Macrophage colony-stimulating factor; MCs: Mononuclear cells; MGCs: Multinucleated giant cells; MMP-9: Matrix metalloproteinase-9; RANK: Receptor activator of nuclear factor kappa-B; RANKL: RANK ligand; OPG: Osteoprotegerin; OPN: Osteopontin; TNF-α: Tumor necrosis factor-alpha; TRAP: Tartrate-resistant acid phosphatase; VEGF: Vascular endothelial growth factor; VNR: Vitronectin receptor*** $\alpha_v\beta_3$.

In both peripheral and central granulomas, MGCs and a fraction of MCs express CD68, a marker of cells in the monocyte lineage, including histiocytes, tissue macrophages and osteoclasts. CD68 is a transmembrane glycoprotein, but it is also associated with the lysosomal and endosomal membrane. Functionally, CD68 protein binds to the lectins and selectins in the tissues and organs and enables macrophages to home in on particular targets [3, 19]. Therefore, the CD68-positive stromal MCs could be macrophage-like cells [2].

Moreover, the macrophage-like MCs in the stroma and MGCs showed immunopositivity for mononuclear-phagocyte system markers, such as non-specific esterase, acid phosphatase, lysozyme, α1-antitrypsin and α1-anti-chymotrypsin and muramidase [2].

Only a fraction of MCs express osteoclast markers, such as: vitronectin receptor $\alpha_v\beta_3$ (VNR), tartrate-resistant acid phosphatase (TRAP), vacuolar-type H^+-adenosine-triphosphatase (V-ATPase), amino-peptidase, carbonic anhydrase II (CA II), cathepsin K, matrix metallo-proteinase-9 (MMP-9), proliferating cell nuclear antigen (PCNA), osteopontin (OPN) and calcitonin [5, 20]. These MCs are osteoclast-like cells and could be the progenitors for MGCs, since they are capable to fuse under the influence of specific inducting factors. Moreover, the expression of the same markers on MGCs suggests their osteoclastic origin [2, 3, 9, 20].

VNR is an integrin that enables the osteoclast to adhere to the bone surface, and to subsequently undergo differentiation and polarization to initiate the resorption of the bone matrix [21]. Cathepsin K, TRAP and MMP-9 are bone-degrading enzymes released by osteoclasts, which induce the resorption of the bone matrix, after the demi-neralization [22]. TRAP is synthesized as an inactive proenzyme, which is activated by proteolytic cleavage performed by the proteinases such as cathepsins. Active

TRAP degrades the bone matrix by OPN dephosphorylation and reactive oxygen species (ROS) generation [3, 20, 23].

Papanicolau *et al.* reported that MGCs in both PGCG and CGCG were immunopositive for TNF-α, interleukin-6 (IL-6) and interleukin-1β (IL-1β), which can be related to giant cells differentiation from osteoclasts. These osteoclastogenic cytokines seem to play a role in the formation of MGCs and the initiation of bone resorption [4].

Since they have both macrophagic/histiocytic and osteoclastic features, MGCs cannot be precisely divided along these two lines.

The other component of the MCs population is represented by spindle-shaped proliferating cells, which are capable to differentiate into fibroblast/osteoblast-like cells. The fibroblast/osteoblast-like cells induce the differentiation of monocyte/macrophages into osteoclast-like MCs, since they express the receptor activator of nuclear factor (NF)-κB ligand (RANKL), which is responsible for the activation and survival of osteoclasts [4, 9, 15, 24]. Liu *et al.* also reported that RANKL was mainly expressed in spindle-shaped MCs and in some round MCs, whereas osteoprotegerin (OPG) was expressed in both MGCs and MCs. The similar characteristics and mechanisms underlying the formation of MGCs suggest common pathogenic pathways in the development of PGCG and CGCG [20].

Factors that control the differentiation, activation and function of osteoclasts, including macrophage colony-stimulating factor (M-CSF), receptor activator of NF-κB (RANK), RANKL and OPG could play important roles in the development of MGCs from MCs in both PGCG and CGCG (Figure 5) [20, 21].

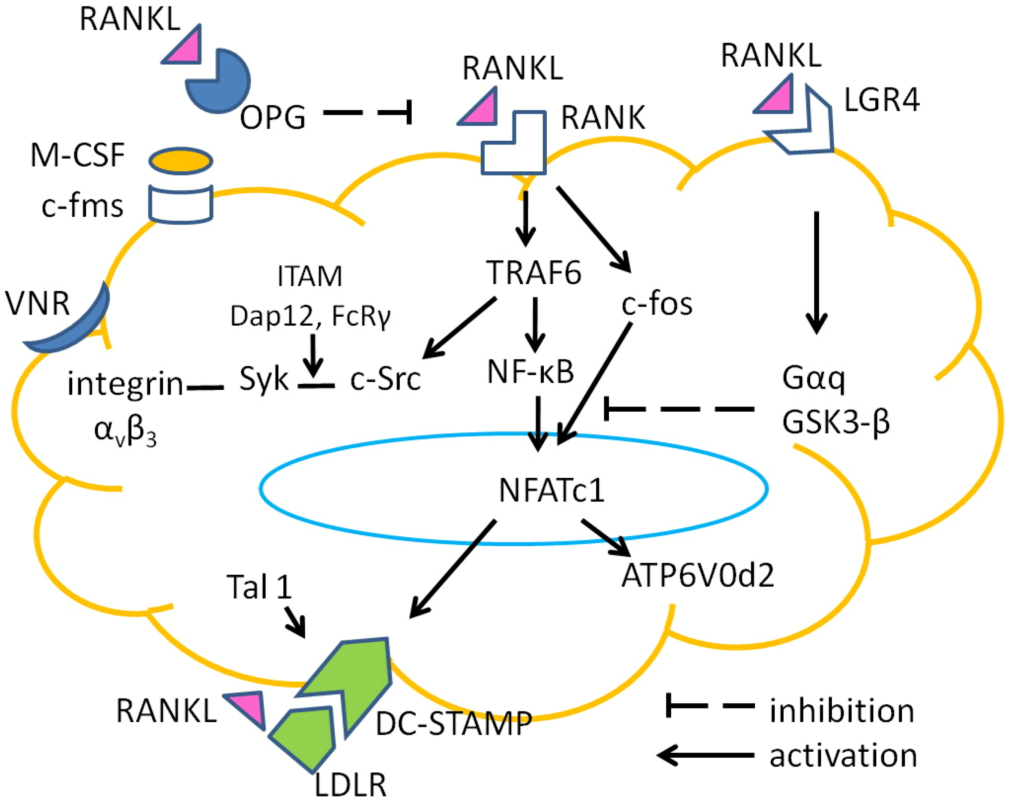

Figure 5 – ***The activation pathways of the osteoclast precursors; the interaction RANK/RANKL induces the activation of NF-κB, c-fos and the related factors, leading to the differentiation, fusion, activation and polarization of the osteoclast precursors. The OPG and the LGR4 competitively bind RANKL and thus inhibit the RANKL/RANKL signaling (original diagram). ATP6V0d2: Adenosine-triphosphatase (ATPase), H^+ transporting, lysosomal 38 kDa, V0 subunit d2; c-fms: Colony-stimulating factor-1 receptor; c-Src: Proto-oncogene tyrosine-protein kinase Src; Dap12: DNAX-activating protein of 12 kDa; DC-STAMP: Dendritic cell-specific transmembrane protein; DNAX: DNA polymerase III (gamma and tau subunits); FcRγ: Fc receptor common gamma chain; Gαq: Alpha subunit of a heterotrimeric guanosine-5'-triphosphate (GTP)-binding protein; GSK3-β: Glycogen synthase kinase 3-beta; ITAM: Immunoreceptor tyrosine-based activation motif; LDLR: Low-density lipoprotein receptor; LGR4: Leucine-rich repeat-containing G-protein-coupled receptor 4; M-CSF: Macrophage colony-stimulating factor; NFATc1: Nuclear factor of activated T-cells, cytoplasmic 1; NF-κB: Nuclear factor kappa-B; OPG: Osteoprotegerin; RANK: Receptor activator of NF-κB; RANKL: RANK ligand; Syk: Spleen tyrosine kinase; Tal 1: T-cell acute lymphocytic leukemia protein 1; TRAF6: Tumor necrosis factor receptor (TNFR)-associated factor 6; VNR: Vitronectin receptor $\alpha_v\beta_3$.***

M-CSF interacts with c-fms receptor found on the surface of monocyte-macrophage precursors and regulates the number of osteoclasts. M-CSF activates RANKL and is also implicated in the organization of the osteoclast cytoskeleton. Immunopositivity for M-CSF strongly suggests that MGCs derive from the differentiation and cytoplasmic fusion of multiple MCs [13].

RANKL, a transmembrane molecule produced by the fibroblast/osteoblast cells binds to the RANK expressed on the surface of stromal monocyte-derived macrophage-like cells and induces the differentiation of cells [9]. Osteoclast-like MCs subsequently fuse to form MGCs, under the influence of RANKL, c-fos and related factors [14, 25]. C-fos is a member of transcriptional activating

protein complex 1 and induces the differentiation of osteoclasts and macrophages from a common progenitor [16].

The interplay between RANK expressed by MGCs and RANKL and OPG expressed by stromal MCs is crucial for promoting the differentiation and activation of osteoclasts, and the subsequent osteolysis [24].

RANKL binds to RANK on the osteoclast precursors and leads to the expression of the mitogen-activated protein kinases, and activates NF-κB *via* tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6). RANKL induces the expression c-fos in osteoclast precursors and subsequently activates positive regulators, an essential mechanism for osteoclast formation [16].

However, Luo *et al.* demonstrated that leucine-rich repeat-containing G-protein-coupled receptor 4 (LGR4), a competitive receptor for binding RANKL, could have an opposing effect, by suppressing the osteoclast formation [26]. The interaction between RANKL and LGR4 circumvents the RANK signaling, but instead leads to the activation of Gαq and glycogen synthase kinase 3-beta (GSK3-β) pathway, and subsequently blocks the expression of nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1), the master regulator of osteoclastogensis and transcriptionally activated by c-fos and NF-κB. Therefore, LGR4 has been considered as a key element of the negative-feedback mechanism that limits the osteoclast formation and function *in vivo* [26].

OPG is a decoy receptor for RANKL secreted by stromal MCs and osteoclasts. OPG binds to RANKL and blocks the interaction RANKL–RANK, thus inhibiting osteoclastogenesis [24].

The adhesion of osteoclasts to the substrate is intermediated by integrins, such as VNR; the contact between the osteoclast and the bone matrix further stimulates the differentiation and the polarization of the osteoclasts, with the reorganization of the cytoskeleton [21].

Two non-receptor tyrosine kinases, c-Src and Syk, are major NF-κB regulators and play a role in the activation of phagocyte cells, rearrangement of cytoskeleton and bone matrix resorption performed by osteoclast-like cells [22, 27, 28].

The Src protein, activated downstream from RANK/TRAF6 pathway, controls the osteoclast activation and survival and promotes the actin rearrangement and the osteoclast motility [29]. The c-Src regulates the organization of the osteoclasts cytoskeleton, by forming a signaling complex with tyrosine kinase Syk and the activated integrin $\alpha_v\beta_3$. The c-Src induces the Syk phosphorylation under the influence of integrin $\alpha_v\beta_3$ only in the presence of the immunoreceptor tyrosine-based activation motif (ITAM) proteins Dap12 and FcRγ [30]. The Syk–c-Src complex promotes the formation of the sealing zone, enables the adherence of the osteoclast to the substrate and initiates the bone resorptive activity [28]. The formation of integrin $\alpha_v\beta_3$–c-Src complexes is essential for cytoskeleton organization in activated osteoclasts [28, 31].

Mechanisms underlying the MGCs biological activity

Even though the MGCs are the characteristic feature of the granulomas associated with the oral cavity, their implication in the pathogenic mechanisms of the lesions are not fully understood [11].

More and more evidence suggests that MGCs might not be the main functional cells, but they are reactive elements of the lesions. MGCs seem to be the secondary cells in the granulomas, formed by the fusion of the monocyte/macrophage MCs differentiated into osteoclasts precursors (osteoclast-like cells) under the influence of cytokines [4, 20]. Instead, stromal MCs in the granulomas are the lesional cells [9, 32]. On one hand, MCs compose the proliferative compartment of the granulomas and by their differentiation give rise to the MGCs; on the other hand, MCs are responsible for the biological activity of the lesions [9].

Itonaga *et al.* demonstrated that the main proliferative activity occurred in the MCs population, based on the expression of Ki-67 cell cycle protein; contrarily, MGCs did not express Ki-67 and showed no proliferating capacity. Therefore, the granulomas growth could be the consequence of a deregulation in MCs proliferation [9].

Souza *et al.* reported the expression of murine double minute 2 (MDM2), PCNA and argyrophilic nucleolar organizer region (AgNOR) in both PGCG and CGCG; contrarily, p53 expression was absent [10]. Nucleolar organizer regions (NORs) are loops of deoxyribonucleic acid (DNA) that are actively transcribed for ribosomal ribonucleic acid (RNA), then to ribosomes and finally to proteins. The NORs can be identified by a silver staining technique, AgNOR, which can be used for assessing the proliferative potential of various neoplastic and non-neoplastic lesions. PCNA and Ki-67 are both expressed during cell division, but in different phases of the cell cycle: PCNA is increased in the G_1 and S phases, whereas Ki-67 is expressed in the active phases. The *p53* gene is a tumor suppressor, which arrests the cell cycle and promotes apoptosis in mammalian cells; *p53* inactivation was seen in numerous tumors. *MDM2* is a proto-oncogene of which transcription is induced by the wild-type *p53*; the product of *MDM2* gene binds to p53 protein and inhibits the regulatory function of *p53* [20].

The mechanisms essentially implicated in the pathogenesis of the PGCG and CGCG could explain the similarities regarding the development and progression of these lesions [4, 33].

Development and function of osteoclasts are directly and indirectly promoted or inhibited by several osteotropic hormones. Calcitonin and steroids influence mainly the MGCs, but not the stromal proliferating cells. Prostaglandins (PGs), parathyroid hormone (PTH) and the parathyroid hormone-related protein (PTHrP) promote the bone resorption [34–36].

Additionally, osteoclastogenesis is locally controlled by several cytokines produced by the stromal MCs (Figure 6). Papanicolau *et al.* reported that spindle-shaped fibroblast/osteoblast MCs expressed TNF-α, IL-6 and IL-1β; these proinflammatory cytokines synergistically induce osteoclastogenesis and contribute to the bone resorption [4].

Cytokines including VEGF, bFGF, TNF-α, TGF-β, IL-1, IL-6, and IL-11 are released by the macrophage-like stromal MCs and play an important role in the recruitment, differentiation and activation of the osteoclasts [2, 34]. The bFGF and VEGF are growth factors

with important roles in angiogenesis by promoting the proliferation and migration of endothelial cells. The bFGF is mainly found in macrophages, but also in other cell types, such as: monocytes, mast cells and endothelial cells. VEGF is found in the cytoplasm of macrophages and fibroblasts [37]. Angiogenesis enables the afflux of circulating monocytes, which are further recruited and stimulated to differentiate into osteoclast precursors, which fuse and form MGCs under the influence of cytokines secreted locally, by the stromal cells: monocyte chemoattractant protein-1 (MCP-1) and TGF-β [37–41]. The expression of VEGF is upregulated by inflammatory cytokines, such as IL-1 and TNF-α [42].

Proteolytic enzymes secreted by osteoclasts, such as MMP-9 and cathepsins, are implicated in bone resorption and remodeling [37, 43]. MMP-9 exerts lytic activity on the organic component of the bone matrix, mainly on type I and II collagen [38, 41, 44]. Moreover, MMP-9 increases the VEGF levels because the lysis of the decalcified bone matrix leads to the release of matrix-bound VEGF and the subsequent effect on the osteoclasts. Thus, MMP-9 acts synergistically with VEGF in recruiting the osteoclast precursors [37]. Cathepsin D indirectly promotes the bone matrix destruction by the activation of cathepsins B and L [43, 45, 46] and was associated with the local invasion of the giant cell granulomas [47]. Zargaran *et al.* demonstrated the expression of cathepsin D in the MGCs in both PGCG and CGCG, which supports the osteoclastic nature of these cells [48]. Proinflammatory mediators, such as TNF-α, in association with IL-1β, induce the production of cathepsin D and exert an osteoclastogenic effect [49]. Moles *et al.* demonstrated that increased levels of cathepsin D induced the synthesis of TGF-β [50]. Furthermore, cathepsin D and TGF-β activate Src, a factor implicated in osteoclastogenesis, which also controls the polarization of the osteoclasts, the formation of the ruffled border and osteolysis [51]. Cathepsin D is also capable to convert the PTH into its active form, PTHrP, which promotes the osteolytic activity of osteoclasts and prevents osteoblast maturation [35, 36].

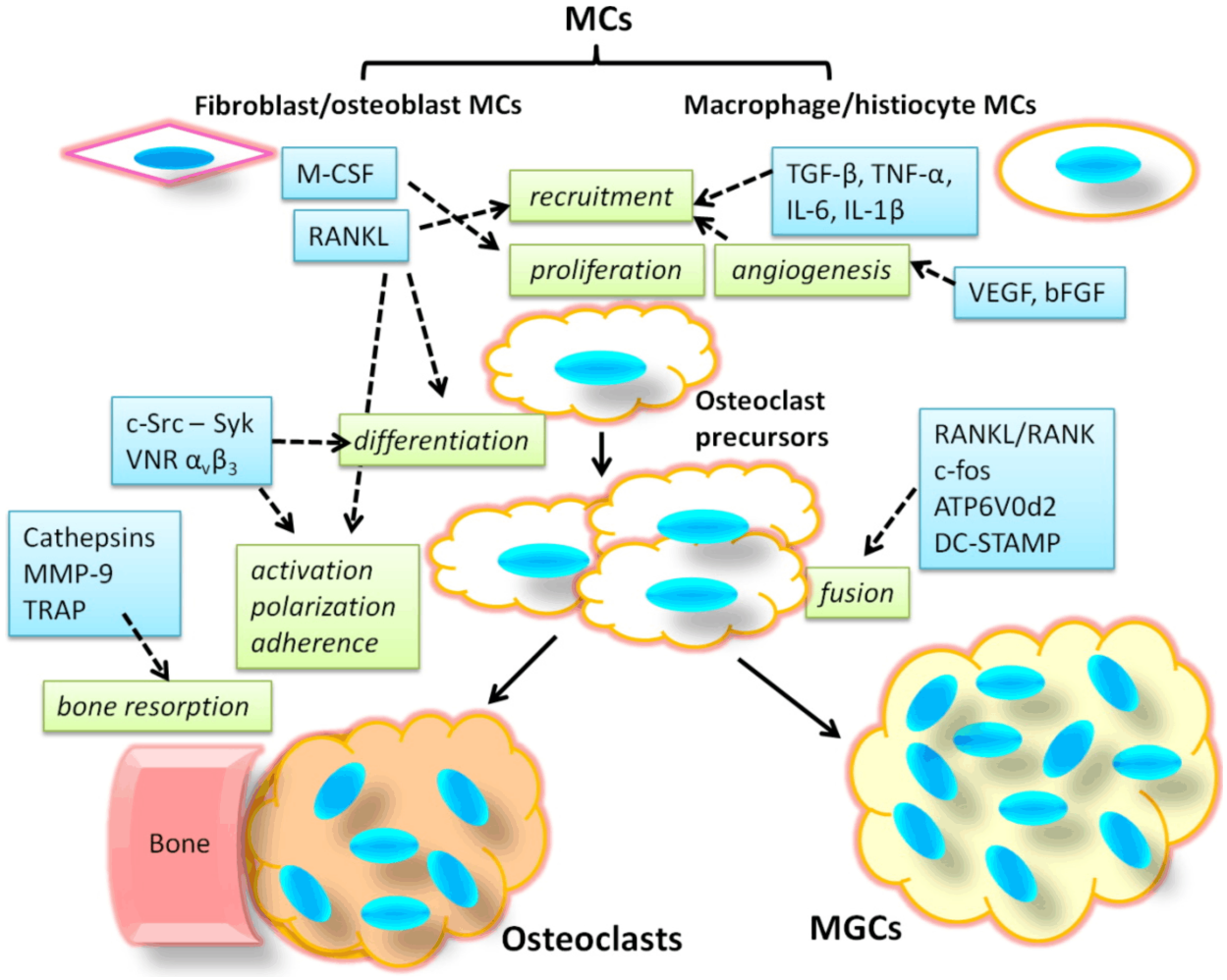

Figure 6 – ***Mechanisms underlying the biological activity of the MGCs; the cytokines and growth factors produced by the fibroblast/osteoblast and macrophage/histiocyte MCs are implicated in the recruitment, proliferation, differentiation, fusion and activation of the osteoclasts; the proteolytic enzymes secreted by the osteoclasts induce the bone resorption (original diagram). ATP6V0d2: Adenosine-triphosphatase (ATPase), H^+ transporting, lysosomal 38 kDa, V0 subunit d2; bFGF: Basic fibroblast growth factor; c-Src: Proto-oncogene tyrosine-protein kinase Src; DC-STAMP: Dendritic cell-specific transmembrane protein; IL: Interleukin; M-CSF: Macrophage colony-stimulating factor; MCs: Mononuclear cells; MGCs: Multinucleated giant cells; MMP-9: Matrix metalloproteinase-9; RANK: Receptor activator of nuclear factor kappa-B; RANKL: RANK ligand; Syk: Spleen tyrosine kinase; TGF-β: Transforming growth factor-beta; TNF-α: Tumor necrosis factor-alpha; TRAP: Tartrate-resistant acid phosphatase; VEGF: Vascular endothelial growth factor; VNR $\alpha_v\beta_3$: Vitronectin receptor $\alpha_v\beta_3$.***

Glucose transporters (GLUTs) are structural proteins that facilitate the glucose transport across the plasma membrane of mammalian cells. GLUTs localization and affinity for glucose is different in the various tissues. GLUT-1 and -3 are highly expressed in the malignant tumors but their role in PGCG and CGCG are not completely clarified. The glucose consumption could be correlated with the energy demanding activities, including proliferation [13]. Vasconcelos *et al.* reported that GLUT-3 was overexpressed in the MCs, but MGCs showed a low reactivity, which was consistent with the high metabolic and proliferating activities demonstrated by previous studies. GLUT-1 had a lower staining intensity, which could suggest the benign clinical behavior of the lesions [13]. Based on the GLUTs expression, in the oral granulomas there seem to be two types of MGCs: active cells and degenerated or apoptotic cells [52]. The expression of the glucose transporters in the MCs and MGCs might be the consequence of the hypoxic and acidic micro-environment in the oral granulomas [13].

WNK1 is a protein kinase which controls the expression of GLUT-1 on the cell surface. WNK1 phosphorylates TBC1D4 and increases the binding of this phosphoprotein to the 14-3-3 protein, while inactivating the exocytic guanosine triphosphatase (GTPase) Rab8A. Upon GTPase inactivation, Rab8A protein becomes activated and initiates the glycolitic activity in the cells [13].

Cell fusion is important for the biological activity of the cells, mainly by increasing the cell function (*e.g.*, the multinucleated osteoclasts are more efficient in resorbing bone compared with the mononuclear cells) [14]. In order to promote cell fusion, several events are involved: chemo-taxis, migration, cell-to-cell recognition and interaction, in order to reach the status that enables the cell fusion [14]. M-CSF promotes the survival and proliferation of macrophage-like MCs, to ensure the required number of fusing cells [21]. Furthermore, RANKL initiates the differentiation into TRAP+ mononuclear osteoclast precursors, which will fuse to form the mature TRAP+ multinucleated osteoclasts. Since the osteoclast precursors are not capable of bone resorption, their fusion is essential for becoming active osteolytic cells [14]. The osteoclast-like MCs fusion is controlled by several cytokines that regulate the dendritic cell-specific transmembrane protein (DC-STAMP). The direct DC-STAMP up-regulation is mediated by the low-density lipoprotein receptor (LDLR) and the Tal1, or by RANK/RANKL pathway by the activation of the NF-κB, c-fos, and NFATc1. The indirect mechanism implicates the non-osteoclastic lineage cells which produce soluble factors, such as RANKL, connective tissue growth factor 2 (CCN2), vitamin E, and integrin-32. Another fusion regulator is ATP6V0d2, which is also up-regulated by the RANKL–NFATc1 pathway [14, 53].

As previously discussed, the osteoclast differentiation and the consequent bone resorption are the result of the interaction between the RANK expressed in the osteoclast-like MCs and the RANKL expressed by the fibroblast/ osteoblast stromal MCs. Both osteoclast-like MCs and MGCs express RANK, suggesting the common progenitor. Won *et al.* reported a positive correlation between the RANK expressed by the MGCs and OPG in stromal MCs, as well as between OPG and RANKL, which are both expressed in the stromal MCs. The authors postulated that the activation of the RANK–RANKL pathway triggers the OPG expression by stromal MCs; this could be inter-preted as a reactive defense mechanism for preventing the excessive osteolysis in oral granulomas [24].

Factors predictable for the clinical behavior of oral granulomas

There is a major debate if PGCG and CGCG are separate entities or varieties of the same type of lesion [1, 54]. It is still hypothesized that PGCG could be a discrete entity or a peripheral variant of CGCG. Both PGCG and CGCG are reactive lesions, share the same pathogenic mechanisms and have common histological features [10].

The main differences between these lesions are related to the localization and the clinical evolution. Commonly, PGCG has a slow progression and limited osteolytic activity. Contrarily, Chuong *et al.* classified the CGCG into non-aggressive lesions – which are slowly growing and asymptomatic – and aggressive, which are associated with increased bone destruction and more severe symptoms [55]. The aggressive forms of CGCG were characterized by larger diameters and local complications, including: root resorption, cortical plate perforation as well as higher recurrence rate [6]. Moreover, based on the clinical behavior and the proliferating activity, some researchers also suggested that CGCG and malignant lesions, such as giant cell tumor could have the same pathogenesis [4, 56].

Numerous recent studies focused on the IHC characterization of the lesions and the identification of proliferation markers and cell cycle associated proteins expressed in the MGCs, in order to establish the differences between PGCG and CGCG and to explain the distinct clinical behavior [3, 10, 20, 57].

Moreover, since both types of oral granulomas, and mostly the CGCG, are accompanied by osteolysis, the factors associated with bone resorption could be used as markers with prognostic value. These markers could indicate the potential aggressive clinical evolution, as well as the risk of recurrence [37].

In PGCG, the regulatory cytokines such as TNF-α, IL-6 and IL-1β control the function of the various types of cells, including MGCs, monocytes/macrophages and fibroblasts/osteoblasts and control the tumor biological activity: growth and osteolysis. The bone resorption in the proximity of the lesion may occur due to the expression of osteolytic enzymes and osteoclasts-activating cytokines in the MGCs [4]. In CGCG, MCs spindle-shaped cells showed higher levels of TNF-α and IL-6 but lower IL-1β expression, compared with PGCG. These findings suggest that, by the synthesis of regulatory cytokines, spindle-shaped MCs could regulate the differentiation of the osteoclast progenitors and promote bone resorption with different intensity in the two lesions [4]. Papanicolau *et al.* reported that in CGCG there was a significantly higher expression of TNF-α and IL-6 in spindle cells, but not in MGCs. In CGCG, MGCs had a higher expression of IL-1β compared with stromal MCs spindle-shaped cells [4].

In CGCG, the increased expression of TNF-α, IL-6 and IL-1β suggests an intense stimulation of osteoclast

progenitor cells, leading to enhanced osteolysis. By contrast, in PGCG, the interrelation between the three osteoclastogenic cytokines seem to control the function of other cell types, including MGCs, stromal MCs of both monocyte/macrophage and fibroblast/osteoblast lineages. This could be the explanation for the granuloma growth and the reduced osteolytic activity [4].

The activity of proteases implicated in bone resorption, such as cathepsin K expressed by the MGCs seem to be related to the bone microenvironment. This explains why the high levels of cathepsin K in the PGCG were not associated with significant bone resorption. It is also possible that the osteoclasts distant from the bone surface to contain the inactive form (procathepsin K), instead of the active enzyme [58].

Souza *et al.* reported that the AgNOR counts were not different in PGCG compared with CGCG, but were significantly increased in the nuclei of MCs and MGCs in recurrent and aggressive CGCG. ***Since AgNOR quantification is correlated with the clinical behavior, this examination could be used for identifying the lesions which have a higher recurrence potential*** [10].

The wide distribution of MDM2 protein not only in the granulomas, but also in the normal oral mucosa suggested that it was not related to the biological activity of the lesions. Moreover, the absence of the negative regulator of cell division p53 immunopositivity in both aggressive and non-aggressive CGCG was consistent with the benign nature of the oral granulomas [6, 10]. Interestingly, only the MCs were positive for Ki-67, not the MGCs, with increased percentage of Ki-67 positive cells in PGCG compared with CGCG and in aggressive CGCG compared with non-aggressive ones [5, 6, 10]. These data prove that PGCG has higher proliferative activity compared with CGCG [10]. ***The correlation between Ki-67 positivity and the growth potential of aggressive CGCG could be useful for the therapeutic approach***.

In bone osteolytic lesions, the increased vascularity is a prerequisite for the tumor growth and bone resorption, which explains the increased VEGF levels in CGCG compared with PGCG [38, 39, 40, 41]. CGCG have more pronounced osteoclastic activity compared with PGCG, since they express higher levels of IL-1β, leading to the synthesis of cathepsin D [43, 45, 46]. Moreover, higher expression of TGF-β in the CGCG compared with PGCG is consistent with the increased bone resorption, since TGF-β maintains the survival of osteoclasts and activates MMP-9 [59, 60]. MMP-9 is also produced under the influence of cathepsin D in MGCs in both lesions, but at higher rate in CGCG [38, 41, 44]. ***Therefore, the high VEGF and MMP-9 levels could be correlated with the extent of bone destruction and the recurrence tendency, features that are common for CGCG, the giant cell tumor of the bone and the aneurismal bone cyst*** [37].

The glucose transport and the glycolitic metabolism supply the energy necessary for the cells function. Therefore, the increased metabolism demands of an aggressive lesion which is associated with bone resorption demands more energy and justifies the higher GLUT-1 expression [13].

The morphological differences regarding the number of nuclei and the immunopositivity for CD68 could be factors responsible for the distinct clinical behavior. Several studies described in both CGCG and PGCG, irregular-shaped MGCs, which were unevenly distributed in the fibro-cellular stroma. This aspect is considered to be characteristic for reactive lesions, since the neoplastic proliferations exhibit a more even distribution of the giant cells. Non-aggressive CGCG contained clusters of small MGCs surrounded by spindle-shaped stromal cells. The aggressive CGCG contained numerous large MGCs uniformly distributed. The number of nuclei in the MGCs ranged between 3 and more than 100 [2, 6].

VK *et al.* reported higher expression of CD68 in MGCs in CGCG compared with PGCG, but they attributed this aspect to the different proportion and the distribution of MGCs in the fibro-cellular stroma [2]. The CD68-positive MGCs contained different numbers of nuclei: in the CGCG, most MGCs had more than 20 nuclei, whereas in PGCG all the MGCs had less than 20 nuclei; these findings suggest a higher metabolic activity of MGCs, and also an increased tendency of MCs to fuse and to form MGCs in the CGCG, which is consistent with the more aggressive evolution. The ratio between CD68-positive macrophages and MGCs was higher in CGCG. The immunopositivity for CD68 in MGCs and a fraction of MCs proved that the positive mononuclear cells are macrophages and the MGCs derive from monocyte/ macrophage lineage [2].

Aksakalli reported identical expression of OPN and integrin α_v in MGCs in both PGCG and CGCG and suggested that these cells might not play essential role in the evolution of the granulomas. Interestingly, MCs showed a higher expression of OPN and integrin α_v in CGCG, compared with PGCG [5]. Binding of OPN to the membrane receptor integrin α_v activates the osteoclasts and increases the osteolytic activity, which is consistent with the clinical behavior of CGCG [18].

De Souza *et al.* reported that patients with CGCG showed increased expression of TNF-α in circulating CD4+ T-lymphocytes and lower expression in CD68+ circulating monocytes, which suggests systemic functional changes in circulating leukocytes [61].

The functional alterations of osteotropic hormones could also be prognostic factors for the oral granulomas. Houpis *et al.* reported the expression of PTHrP and its receptor (PTHR) in MGCs in both lesions, but at a higher rate in CGCG [62].

Therapeutic approaches

Taking into consideration the localization, extension and the risk of recurrence, different therapeutic approaches must be taken into consideration for PGCG and CGCG.

The standard treatment of PGCG consists in surgical excision of the entire lesion, the curettage of the underlying periosteum (Figure 7A), especially when during surgery it is confirmed that the periosteum has been infiltrated and the superficial bone resorption is present. When the granuloma extends profoundly and infiltrates the periodontium, the adjacent teeth are also indicated for extraction (Figure 7C). Since the lesion removal results in a deficient mucosal covering, the suture is recommended (Figure 7, B and D). The removal of the cause: occlusal trauma or the iatrogenic factors, such as inappropriate

dental restorations or prostheses should be considered; follow-up of the patient is also recommended, to identify the possible recurrences. When complete resection is performed, the PGCG has a low recurrence rate: 5% to 11% [1, 5].

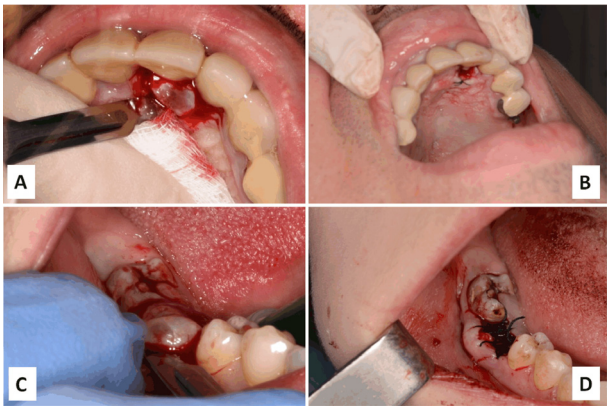

Figure 7 – ***The surgical treatment of the PGCGs: (A) The excision of the lesion and curettage of the underlying periosteum; (B) Suture of the palatal mucosa; (C) Excision of the lesion, followed by the extraction of the remaining roots and the curettage of the alveolus; (D) The suture of the alveolar mucosa (authors collection). PGCG: Peripheral giant cell granuloma.***

In CGCG, the correct diagnosis must be established prior to surgery. Based on the histological and clinical features, and due to the unpredictable behavior, it was hypothesized that CGCG could be a true neoplasm. Therefore, the differential diagnosis of CGCG with malignant tumors should be taken into consideration, for an efficient therapeutic approach. The common treatment involves resection or enucleation, including the curettage of the infiltrated bone at the periphery of the lesion. Conservative peripheral ostectomy is recommended, in order to preserve the bone tissue and to ensure the optimal conditions for healing after the intervention. Moreover, the follow-up is very important, since CGCG has a high recurrence rate: 13 to 49% [5, 55, 63].

In CGCG, recurrence is the main shortcoming of the surgical therapy; however, it seems that the recurrence rate could be associated with the surgical approach and the biological behavior of the lesion. For the aggressive lesions, radical surgical methods are more efficient. When the conservative surgery, such as curettage is used for aggressive lesions in order to prevent patient's morbidity and facial disfigurement, additional therapy is essential [64].

The different clinical behavior and post-therapeutic outcome could be associated with the molecular heterogeneity of the lesions. Therefore, additional and/or adjuvant non-surgical approaches have been developed for an efficient prevention of recurrences and a better outcome. The pharmacotherapy prior to surgery could be beneficial by reducing the size of the lesion and also by promoting the bone formation at the periphery of the lesion; thus, the surgery could be more conservative [64].

In order to limit the bone resorption, adjuvant therapeutic options using drugs, such as corticosteroids, calcitonin, interferon, bisphosphonates, have been administrated [6, 64]. Intralesional injected corticosteroids inhibited the osteolytic activity of the local mature osteoclasts and proved to be efficient in non-aggressive granulomas, but could also be associated with the surgical treatment in aggressive lesions. Calcitonin inhibits the bone resorption by its effect on osteoclasts and proved to be efficient in both aggressive and non-aggressive lesions, leading to a partial or complete regression. The interferon has been proposed for inhibiting angiogenesis, but it was not efficient for the complete remission of the lesions; moreover, this therapy is not indicated due to the toxicity and negative side effects, especially in children [64].

Other adjuvant non-surgical therapies for limiting the osteolysis could be targeted to control the levels of VEGF and MMP-9 expression [37], or to block the RANK signaling pathway [24, 29]. Several studies reported that administration of recombinant OPG, human anti-RANKL antibodies, and RANK-Fc efficiently controlled the bone resorption [24, 65].

These novel therapeutic strategies are promising, but, at this moment, there are no relevant indications regarding their use, since aggressive and non-aggressive lesions have variable responses to the surgical and pharmacological treatment. Moreover, the treatment needs to be individualized according to the clinical, microscopic and molecular markers, which are related to the variations in recurrence and aggressiveness of the oral osteolytic lesions.

Therefore, further research into the interrelation between the pathogenic mechanisms and the clinical behavior are essential in order to develop effective combined therapeutic protocols. Advances in understanding the pathogenesis and the molecular profile of the aggressive and non-aggressive lesions could be the cornerstone in developing the best protocol to manage the oral granulomas.

Conclusions

There still are unknown aspects regarding the histogenesis and the mechanisms underlying the differentiation and activation of the MCs and MGCs. The IHC characterization in a large panel and the continuous search for new markers expressed in the MCs and MGCs are the future directions for the correct diagnosis. In case of CGCG, any histological specimen should be investigated for the proliferation markers, especially Ki-67 and VEGF, in order to differentiate the non-aggressive from the aggressive lesions. Whenever possible, the quantification of the nuclear AgNOR could be useful for the differential diagnosis. However, in order to control and to manage the oral cavity granulomas, the possible origin and the potential biological activity must be taken into consideration as essential elements for an efficient therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

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