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Parathyroid hormone–related peptide (PTHrP), parathyroid hormone/parathyroid hormone–related peptide receptor 1 (PTHR1), and MSX1 protein are expressed in central and peripheral giant cell granulomas of the jaws

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Objective. Parathyroid hormone–related peptide (PTHrP) binds to the parathyroid hormone receptor type 1 (PTHR1), which results in the activation of pathways in osteoblasts that promote osteoclastogenesis through the RANK/RANKL system. RANK/RANKL expression has been shown in central giant cell granuloma of the jaws but PTHrP/PTHR1 has not. MSX1 protein is a classical transcription regulator which promotes cell proliferation and inhibits cell differentiation by inhibiting master genes in tissues such as bone and muscle. It has been implicated in the pathogenesis of cherubism, and its expression has been reported in a single central giant cell granuloma (CGCG) case. We aimed, therefore, to study the expression of those proteins by the different cellular populations of central and peripheral giant cell granulomas (PGCGs) of the jaws.

Study design. Twenty cases of CGCG and 20 cases of PGCG of the jaws were retrospectively examined by immunohistochemistry for the percentage of positively staining cells to antibodies for PTHrP, PTHR1, and MSX1, using a semiquantitative method.

Results. In both CGCG and PGCG of the jaws, PTHrP and PTHR1 were abundantly expressed by type I multinucleated giant cells (MGC) and mononucleated stromal cells (MSC) with vesicular nuclei, whereas type II MGC and MSC with pyknotic nuclei expressed those proteins to a lesser extent. In both CGCG and PGCG of the jaws, MSX1 was abundantly expressed by type I MGC and MSC but type II MGC did not express it. A statistically significant difference ($P < .05$) was observed between CGCG and PGCG in the expression of PTHrP in type II MGC and MSC with pyknotic nuclei and in the expression of PTHR1 in type II MGC.

Conclusions. We suggest that in CGCG and PGCG of the jaws, PTHrP-positive immature osteoblasts activate PTHR1-positive mature osteoblasts to produce RANKL which interacts with RANK on the PTHrP/PTHR1-positive osteoclast-precursor cells found in abundance in the stroma of giant cell lesions and induces osteoclastogenesis through the classic pathway. Cells of the jawbones, the periodontal ligament, or the dental follicle, originating from the neural crest, may be involved in the pathogenesis of giant cell lesions of the jaws. Further study is required for these suggestions to be proved. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:415-424)**

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The giant cell granuloma of the jaws is a nonneoplastic lesion characterized by the presence of few to many multinucleated giant cells (MGC) in a cellular background composed of mononucleated stromal cells (MSC) with ovoid to spindle-shaped nuclei.[1] Those cell populations have further been subclassified into: 1) type I MGC, showing slightly basophilic cytoplasm and large vesicular nuclei with discrete nucleoli which correspond to metabolically active cells; 2) type II MGC, which are smaller with eosinophilic cytoplasm and pyknotic nuclei that correspond to degenerating cells; 3) MSC with ovoid vesicular nuclei; and 4) MSC with spindle-shaped pyknotic nuclei.[2] The correlation between phenotype and function of the 2 latter cell populations has not been completely clarified yet. Areas of hemorrhage, hemosiderin deposition, and osteoid or new bone formation are also seen. Giant cell granulomas may occur within the bone (central giant cell granuloma [CGCG]) or on the gingivae or edentulous alveolar processes (peripheral giant cell granuloma [PGCG]). The CGCG accounts for approximately 7% of benign lesions of the jaws, is locally destructive, and occasionally shows an aggressive biologic behavior, especially in younger patients.[3,4] In contrast, the PGCG is a common lesion thought to arise as a reaction to local stimulating factors and runs an indolent course.[2,5]

Parathyroid hormone–related peptide (PTHrP), initially identified as the cause of malignant hypercalcemia, is genetically, structurally, and functionally very similar to parathyroid hormone (PTH), but it differs in that it exerts its effects locally, in an autocrine or paracrine mode.[6] PTH and PTHrP bind with the same specificity to the parathyroid hormone receptor type 1 (PTHR1),[7,8] which belongs to a group of transmembrane receptors bound with G proteins.[9] PTH/PTHrP binding to PTHR1 results in the activation of pathways in osteoblasts that promote osteoclastogenesis, the formation of osteoclasts from prodromal cells, by increasing receptor activator of NF-kappa B ligand (RANKL) cytokine and inhibiting osteoprotegerin (OPG, osteoclastogenesis inhibitory factor) expression.[10,11] RANKL is the "master" cytokine necessary and sufficient for osteoclastogenesis.[12] Its binding on the receptor activator of nuclear factor kB (RANK) receptor expressed by osteoclast precursor mononuclear cells from the peripheral blood activates the NF-kappaB and c-jun *N* terminal kinase pathways and promotes their differentiation.[12] Similarly, its binding on the RANK receptor expressed on mature osteoclasts activates the protein kinase B (PKB)–Akt pathway promoting their activation and survival.[12] In contrast, OPG binds RANKL and blocks osteoclastogenesis by inhibiting the formation of RANK/RANKL complex.[13] RANK/RANKL expression has been shown in CGCG of the jaws.[14,15] However, PTHrP/PTHR1 has not been studied in giant cell lesions of the jaws, although a role for PTHrP/PTHR1 in the pathogenesis of CGCG and giant cell tumors of the tendon sheath[16] and bones,[17] 2 lesions with microscopic similarities to giant cell granulomas, has been suggested.

MSX1 protein is a classic transcription regulator acting through DNA binding and is encoded by *MSX1* homeotic gene located in chromosome 4p16.3.[18,19] MSX1 promotes cell proliferation and inhibits cell differentiation by inhibiting master genes in tissues such as bone and muscle,[20,21] and its down-regulation promotes final differentiation of those tissues.[22,23] MSX1 has been implicated in the pathogenesis of cherubism, a disease that shows microscopic similarities to CGCG.[24] In this disease, it is thought that the mutations in *SH3BP2* gene affect both the PTHrP-PTHR1 pathway and the function of *MSX1* gene, leading to activation of the osteoclasts,[24] especially during the intense osseous metabolism accompanying tooth eruption.[25] MSX1 overexpression has been reported in a single CGCG case.[24]

In the present study, the immunohistochemical distribution of PTHrP, PTHR1, and MSX1 protein were recorded in the cell populations of CGCG and PGCG, and differences between the 2 lesions were evaluated.

MATERIALS AND METHODS

This was a retrospective study in archival tissue material drawn from the files of the Department of Oral Pathology, Dental School, University of Athens, and the Division of Oral Pathology, Dental School, University of Minnesota. The material consisted of 40 cases of giant cell lesions of the jaws diagnosed on clinical, radiologic, and histopathologic grounds as either CGCG or PGCG. In each case, gender and age of the patient were recorded. Blood tests for all cases of CGCG were negative for hyperparathyroidism (serum Ca and P, alkaline phosphatase, and PTH immunoassay). According to the patients' referring histories, none had received any form of pharmacologic treatment for the lesion before its surgical excision. Owing to the retrospective nature of the study, information on the clinical behavior of CGCG was not available for all specimens.

Five-micrometer-thick formalin-fixed and paraffin-embedded tissue sections were immunohistochemically stained with a standard streptavidin-biotin-peroxidase technique in the Ventana BenchMark XT fully automated slide preparation system (Ventana Medical Systems, Tucson, AZ), using the iView DAB detection kit (Ventana). Rinses were performed with Tris-buffered saline. For antigen retrieval, the sections were treated with Cell Conditioning CC1 (Ventana) at 98°C for 40

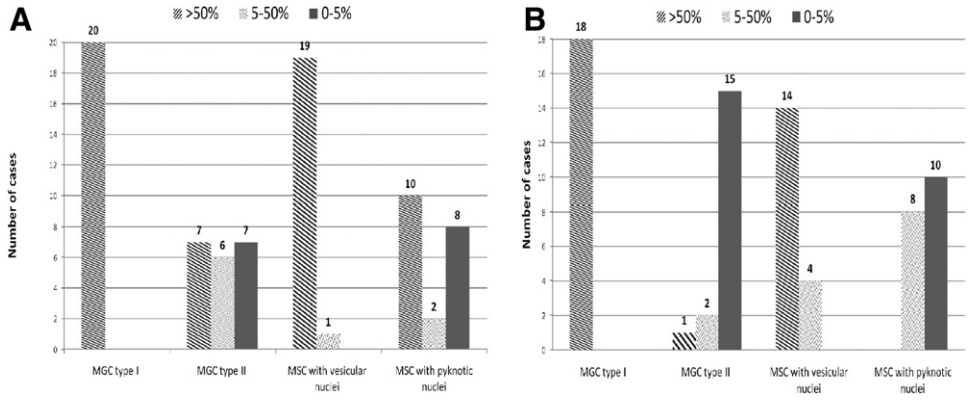

Fig. 1. Immunohistochemical expression of parathyroid hormone–related peptide (PTHrP) in central giant cell granuloma (CGCG) (**A**) and peripheral giant cell granuloma (PGCG) (**B**). *MGC*, Multinucleated giant cells; *MSC*, multinucleated stromal cells.

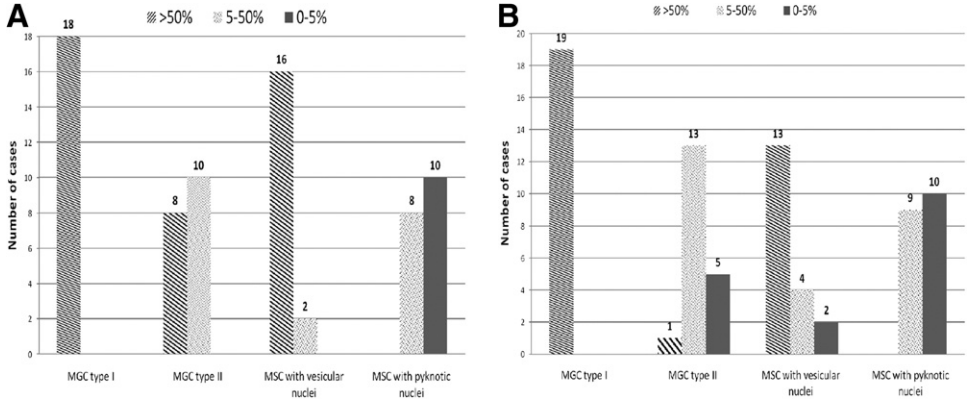

Fig. 2. Immunohistochemical expression of parathyroid hormone receptor type 1 (PTHR1) in CGCG (**A**) and PGCG (**B**). Abbreviations as in Fig. 1.

minutes. Antibodies used were polyclonal rabbit anti-PTHrP (dilution 1:10; Phoenix Pharmaceuticals, Belmont, CA), polyclonal rabbit anti-PTHR1 (dilution 1:50; Acris Antibodies, Hiddenhausen, Germany), and monoclonal mouse anti-MSX1 (dilution 1:500, clone 4F11; Covance, Emeryville, CA). Bound peroxidase was visualized by 3,3′-diaminobenzidine hydrochloride, and counter-stain was developed with Gill haematoxylin.

Staining was blindly and independently evaluated by 2 observers using a 3-scale semiquantitative system modified from Vered et al.[26,27]: absent/limited (<5% of cells stained), intermediate (5%-50% of cells stained) and abundant (>50% of cells stained). Owing to differences in the histologic technique followed by the 2 cooperating laboratories (in tissue fixation and processing and in decalcification method), staining intensity was not evaluated. Positive external control samples were a kidney adenocarcinoma for PTHrP, a breast adenocarcinoma for PTHR1, and skin, liver, and 2 dental follicles from impacted teeth for MSX-1. Substitution of the primary antibody with phosphate-buffered saline served as the negative control.

The staining reaction was evaluated in each of the cell populations described by Katsikeris et al.[2] For statistical analysis of staining reaction of each cell population between GCGC and PGCG, chi-squared was applied ($P < .05$).

RESULTS

In CGCG, 3 patients were male and 17 female, with an average age of 33.15 (±23.93) years. In PCGC, 12 patients were male and 8 female, with an average age of 49.2 (±19.6) years.

Cytoplasmic reaction for PTHrP antibody was seen in endothelial cells and vascular smooth muscle cells,[28-30] epithelial basal cells in the oral mucosa overlying the lesions,[31,32] and normal osteoblasts and oste-

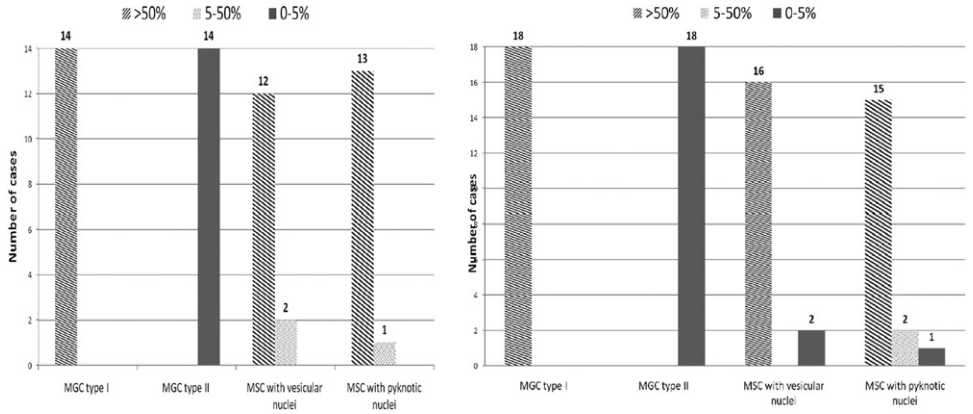

Fig. 3. Immunohistochemical expression of MSX1 in CGCG (**A**) and PGCG (**B**). Abbreviations as in Fig. 1.

oclasts[33-35] from bone trabeculae adjacent to the tumors. PTHR1 was located in the cytoplasm of endothelial cells and vascular smooth muscle cells[30,36] in fibroblasts[37,38] and normal osteoclasts.[34] Finally, basal cells of the oral epithelium showed perinuclear reaction for MSX-1.[39]

Results for CGCG for each molecule are shown in Figs. 1*A*, 2*A*, and 3*A*, respectively, and results for PGCG for each molecule are shown in Figs. 1*B*, 2*B*, and 3*B*, respectively.

CGCG

In all cases of CGCG (20 out of 20), PTHrP was abundantly expressed by type I MGC (Fig. 4*A*), whereas type II MGC showed limited, medium, and abundant staining in equal numbers of cases (Fig. 4*A*). MSC with vesicular nuclei unanimously (19 out of 20 cases) expressed PTHrP (Fig. 4*B*), whereas MSC with pyknotic showed absent/limited (10 out of 20 cases) or abundant (10 out of 20 cases) staining (Fig. 4*C*).

For PTHR1, 2 cases were excluded from the study because the internal control samples were negative. In the rest of the cases (18 out of 18), PTHR1 was abundantly expressed by type I MGC (Fig. 5*A*), and type II MGC showed abundant expression in less than one-half of the cases (8 out of 18; Fig. 5*A*). MSC with vesicular nuclei stained abundantly in almost all specimens (16 out of 18; Fig. 5*B*), whereas staining was absent/limited and intermediate in MSC with pyknotic nuclei in 8 out of 18 and 10 out of 18 cases, respectively (Fig. 5*C*).

For MSX1, lack of staining of internal control samples was seen in 6 cases which were therefore excluded from our study. Staining was located in the periphery of the nucleus. Abundant staining was seen in type I MGC (Fig. 6*A*) and MSC (Fig. 6*B*) in almost all cases of CGCG evaluated (14 out of 14, 12 out of 14, and 13 out of 14 respectively), whereas type II MGC showed absent/limited staining in all cases (Fig. 6*C*).

PGCG

For PTHrP, in 2 cases staining of internal control samples was negative and they were excluded from the study. PTHrP was abundantly expressed by all MGC, but type II MGC usually showed limited staining (15 out of 18 cases). Regarding MSC, PTHrP was abundantly expressed by all cells with vesicular nuclei (14 out of 18 cases), and in more than one-half of the specimens (10 out of 18 cases) staining of MSC with pyknotic nuclei was absent/limited.

One case was excluded from the study because the internal control samples did not react for PTHR1. PTHR1 was abundantly expressed by type I MGC in all cases of PGCG (19 out of 19), whereas type II MGC showed either absent/limited or intermediate expression. PTHR1 was abundantly expressed by all MSC with vesicular nuclei in most cases (13 out of 19), but was absent/limited from MSC with pyknotic nuclei in 10 out of 19 and intermediate in 9 out of 19 cases.

For MSX1, lack of staining of internal control samples was seen in 2 cases that were therefore excluded from evaluation. MSX1 was expressed by all type I MGC and MSC in almost all cases evaluated (18 out of 18, 16 out of 18, and 15 out of 18, respectively). In contrast, MSX1 was not expressed in type II MGC.

Statistical analysis with chi-squared test showed that type II MGC expressed more abundantly PTHrP (χ^2 = 9.33; df = 2; P = .009) and PTHR1 (χ^2 = 10,817; df = 2; P = .0045), and MSC with spindle-shaped pyknotic nuclei expressed more abundantly PTHrP (χ^2 = 13.8; df = 2; P = .001) in CGCG compared with PGCG. No statistically significant difference was found

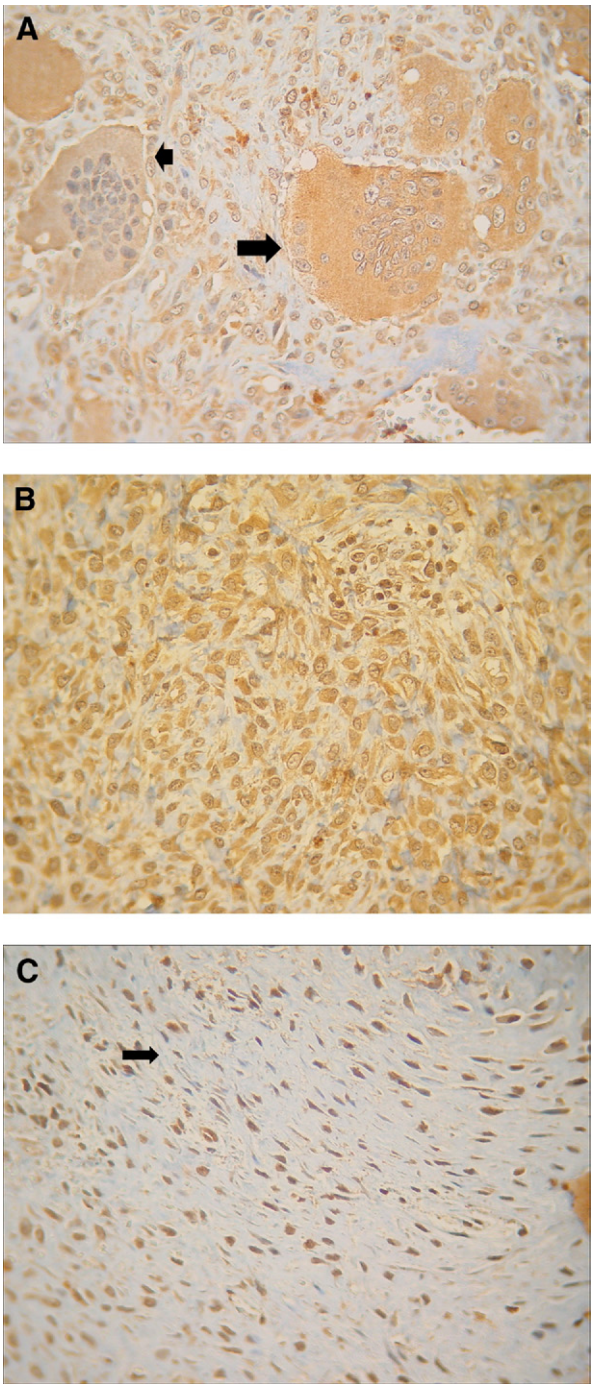

Fig. 4. (**A**) Immunohistochemical expression of PTHrP in a CGCG by MGC type I (*long arrow*) and MGC type II (*short arrow*). ABC staining, original magnification ×400. (**B**) Immunohistochemical expression of PTHrP in a CGCG byMSC with vesicular nuclei: abundant expression. ABC staining, original magnification ×400. (**C**) Immunohistochemical expression of PTHrP in a CGCG by MSC with pyknotic nuclei: intermediate expression (*arrow*). ABC staining, original magnification ×400. Abbreviations as in Fig. 1.

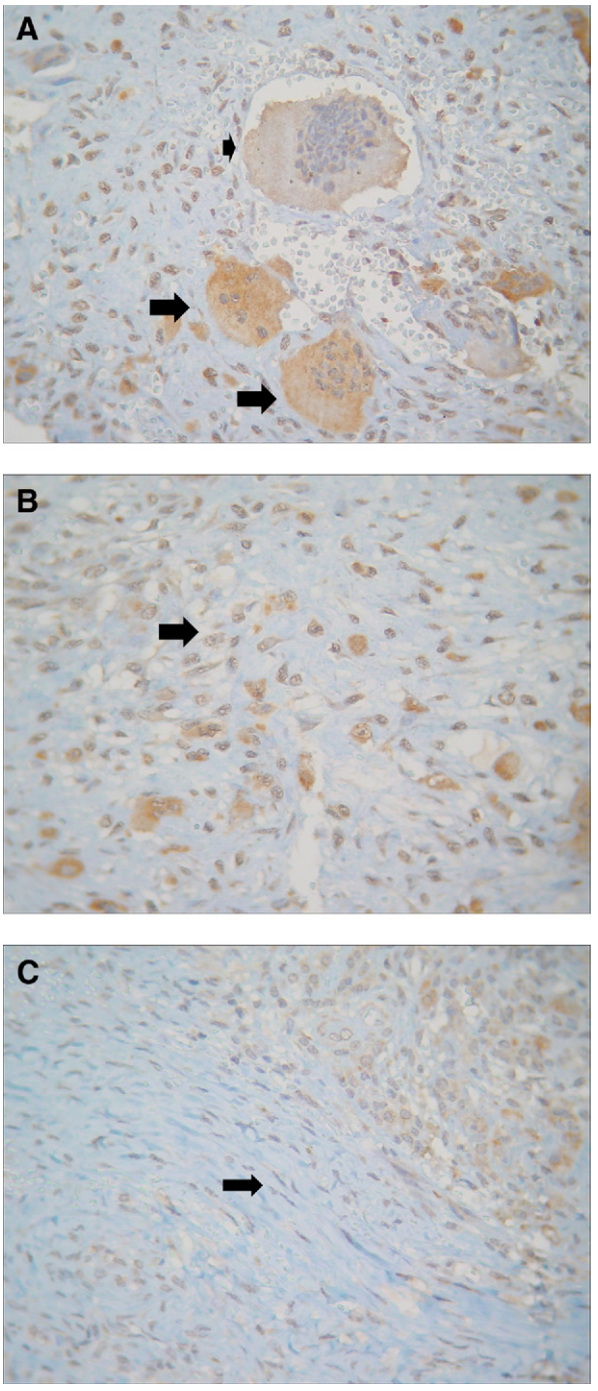

Fig. 5. (**A**) Immunohistochemical expression of PTHR1 in a CGCG by MGC type I (*long arrows*) and MGC type II (*short arrow*). ABC staining, original magnification ×400. (**B**) Immunohistochemical expression of PTHR1 in a CGCG by MSC with vesicular nuclei: abundant expression (*arrow*). ABC staining, original magnification ×400. (**C**) Immunohistochemical expression of PTHR1 in a CGCG by MSC with pyknotic nuclei: limited expression (*arrow*). ABC staining, original magnification ×400. Abbreviations as in Figs. 1 and 2.

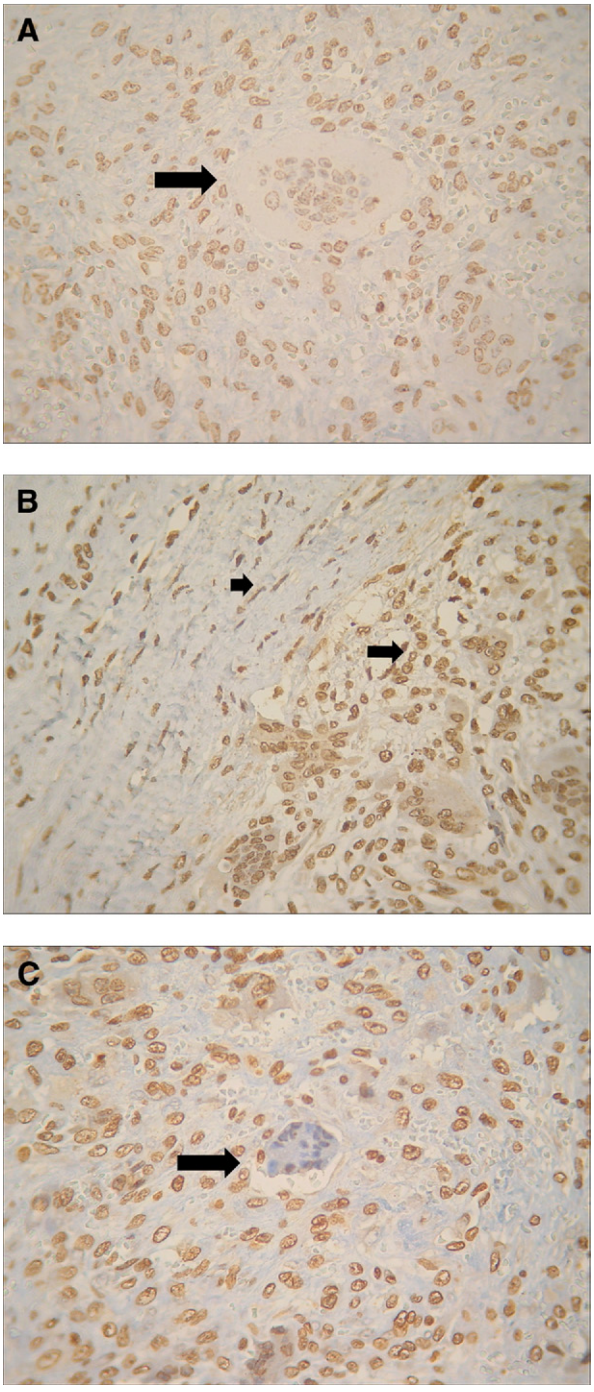

Fig. 6. (**A**) Immunohistochemical expression of MSX1 in a CGCG by MGC type I (*arrow*). ABC staining, original magnification ×400. (**B**) Immunohistochemical expression of MSX1 in a CGCG by MSC with vesicular nuclei: positive (*long arrow*); and by MSC with pyknotic nuclei: positive (*short arrow*). ABC staining, original magnification ×400. (**C**) Lack of immunohistochemical expression of MSX1 in a CGCG by MGC type II (*arrow*). ABC staining, original magnification ×400. Abbreviations as in Fig. 1.

between any cell type of CGCG and PGCG regarding the expression of MSX1.

DISCUSSION

In 1993, Harris[40] suggested that the critical event in the pathogenesis of CGCG is the formation of osteoblasts or osteoblast precursors that secondarily induce osteoclastogenesis and pointed to PTHrP as the primary stimulus for this process. In the present study, it was shown that PTHrP and PTHR1 are expressed in CGCG and PGCG of the jaws and therefore may be the stimulating event for the activation of the RANKL/RANK pathway.[14,15] Although the biologic behaviors of CGCG and PGCG are not the same, there was no statistically significant difference, over all, in the expression of PTHrP and PTHR1 by the different cellular populations between those lesions. Therefore, it could be assumed that the formation of MGC via the PTHrP/PTHR1 mechanism does not have any obvious impact on the biologic behavior of the lesion. Owing to the lack of information on the clinical behavior of CGCG included in the present study, this point needs further research.

PTHrP expression has been shown in normal osteoclasts,[17,33] osteoclasts from Paget disease of the bone[33] and chronic inflammatory diseases of the bones and joints,[17,33] osteoclast-like giant cells of central giant cell tumors of the bones[16,17,33] and anaplastic thyroid cancer,[17] as well as various other forms of multinucleated giant cells.[17] Results on PTHR1 expression are inconsistent. PTHR1 has been shown in normal animal[34,35,41-43] and human[44,45] osteoclasts, as well as blood mononuclear cells and MGC with an osteoclastic phenotype formed from the latter under the influence of RANKL and macrophage-specific colony-stimulating factor.[45] In another study,[17] however, normal osteoclasts and osteoclasts associated with chronic inflammatory diseases were negative for PTHR1, and osteoclast-like giant cells from giant cell tumors of bones and giant cells of malignant tumors expressed PTHR1 in approximately 50% and 100%, respectively. The synchronous presence of PTHrP and PTHR1 in normal osteoclasts has led to the suggestion that osteoclasts may be influenced by PTHrP both indirectly, activated from the osteoblasts,[46] and directly, through an autocrine-paracrine mechanism.[45] In the present study, both osteoclasts from normal bone found in the periphery of some lesions and type I MGC unanimously expressed PTHrP and PTHR1. This finding is consistent with the osteoclastic phenotype of MGC and does not need to be explained by the presence of an alternative mechanism of osteoclastogenesis, independent of the classic mechanism, as has been hypothesized in giant cell tumors of bones and in malignant neoplasms.[17]

The more restricted and variable expression of PTHrP and PTHR1 by type II MGC found in the present study is consistent with the assumption that they represent MGC cells in various stages of degeneration.[2] Another reasonable explanation could be that in PGCG there is a considerable subpopulation of non-osteoclastic MGC. Several studies show that most MGC are consistent with osteoclasts, because they express tartarate-resistant acid phosphatase protein (TRAP), vacuolar H+-ATPase, carbonic anhydrase II, cathepsin K, matrix metalloproteinase 9, RANK,[15] and the calcitonin receptor,[27] react with osteoclast-specific monoclonal antibodies (13C2,23C), and resorb bone in vitro.[47] Other MGC, however, manifest a monocyte/macrophage lineage, e.g., they are positive for CD-68 antigen, glucocorticoid receptor, HLA-DR, and alpha-1-antichymotrypsin,[48,49] whereas reactive giant cells (Langerhans/foreign body type) are positive for PTHrP but not for PTHR1.[17] In the present study, statistically significant differences were seen between CGCG and PGCG regarding the expression of PTHrP and PTHR1 by type II MGC. In particular, in CGCG, type II MGC showed intermediate to abundant expression of PTHrP and PTHR1, whereas in PGCG those cells showed limited expression of PTHrP and intermediate expression of PTHR1. Thus, in CGCG most type II MGC showed an osteoclastic phenotype, but in PGCG those cells could be reactive giant cells. Differences in the immunophenotype of type I and type II MGC have not been previously reported between GGCG and PGCG. However, we cannot exclude that the different tissue-processing techniques applied on the present material, particularly decalcification in tumors with bone, may account for those differences.

Mononucleated stromal cells are also phenotypically heterogeneous, because they may express alkaline phosphatase[14] and RANKL[15] (features consistent with osteoblasts), the osteoclastic lineage marker calcitonin receptor,[27] the monocyte/macrophage lineage markers CD-68 antigen, glucocorticoid receptor, HLA-DR, and alpha-1-antichymotrypsin,[48-50] or the myofibroblastic lineage marker α–smooth muscle actin.[49] There are also osteoclast-precursor cells, whose fusion leads to the generation of MGC.[15] Regezi[51] proposed that MSC originate from the mesenchymal spindle cells of bone marrow that according to Vered et al.[49] undergo a dynamic process of differentiation into various cell types. Furthermore, MSC show high PCNA (proliferating cell nuclear antigen)[15,52,53] and Ki67[4,53] proliferation indices and increased expression of MDM2 protein[53] and cyclin D1.[54]

In the present study, MSC with vesicular nuclei[2] expressed both PTHrP and PTHR1 in CGCG and PCGC. PTHrP-positive or PTHR1-positive MSC could be immature[55] or mature[46,56] osteoblasts, respectively, and PTHrP-positive and PTHR1-positive cells could be osteoclast-precursors.[45] Double staining for PTHrP and PTHR1 was not performed, so we cannot estimate the relative proportions of each population. However, because most MSC with vesicular nuclei were PTHrP positive or PTHR1 positive, it seems reasonable to hypothesize that they were mostly positive for both antibodies and represent osteoclast-precursor cells. Furthermore, PTHR1 has been considered to be an osteoblastic differentiation marker[57,58] whose expression increases in parallel to the differentiation of osteoblasts.[57-59] This is associated with lack of proliferation[17] and could be expected to result in new bone formation.[14] However, MSC show intense proliferation, and osteoid or new bone formation is limited; therefore, the proportion of PTHR-positive differentiated osteoblasts must be limited.

Mononucleated stromal cells with pyknotic nuclei[2] did not express PTHrP, and expression of PTHR1 was variable and usually more limited than that in MSC with vesicular nuclei. Those cells usually formed strands surrounding areas composed of MSC with vesicular nuclei and MGC. This population could be composed of PTHrP-positive mature osteoblasts, undifferentiated stromal cells, cells of monocytoid/macrophagic lineage, or myofibroblasts.[48]

Biphosphonates inhibit PTHrP expression in human osteosarcoma cells,[60] and estrogens suppress the PTH-stimulated formation of osteoclast-like cells by blocking intracellular pathways.[61] Because the PTHrP/PTHR1 pathway seems to be activated in CGCG, the role of those pharmacologic substances in the treatment of large or aggressive variants of CGCG must be further investigated.

Type I MGC and both types of MSC in the present study showed perinuclear staining for MSX1. In vitro, intense nuclear expression of MSX1 has been shown in TRAP-positive osteoclasts of rat jaw periosteum[62] and preosteoblasts and osteoblasts of the periosteum and endosteum of mice jaws,[62,63] as well as cells of the periodontal ligament of mice.[62] Although in vitro findings may not always be applicable to humans, the presence of MSX1 in type I MGC is consistent with their osteoclastic phenotype and in MSC with their proliferative capacity.[20,21,64,65] It may, also, be suggestive of the origin of giant cell lesions of the jaws from the periosteum or the endosteum of the jaw bones or the periodontal ligament, as has been proposed for PGCG.[2] Expression of MSX1 by pro-osteoblasts or osteoblasts in the bone callus of long bones has been associated with the reactivation of *MSX1* gene that is involved in embryonic osteogenesis.[66]

MSX1 has been found in areas where cells of neural crest origin migrate and differentiate or epithelial-mes-

enchymal interactions occur, such as the dental follicle of the tooth buds and the craniofacial skeleton.[63] Cells of the dental follicles express MSX1 and play a critical role in localized osteoclastogenesis associated with tooth eruption, through stimulation of their PTHR1 receptor by PTHrP produced by the cells of dental (enamel) organ.[67-69] Remnants of the dental follicle may persist even after tooth eruption in the tooth-bearing areas of the jaws, where CGCG develop. Harris hypothesized the involvement of "some dental change, such as tooth development and/or eruption," in the development of CGCG based on their occurrence in early puberty. Deliberating on the pathogenesis of cherubism, Hykel et al.[24] proposed that germline mutations in exon 9 of the gene encoding Src homology 3 binding protein 2 (SH3BP2) affect both the PHTrP-PTHR1 interaction and the *MSX1* gene. Overexpression of *MSX1* induces proliferation of ectomesenchymal cells of bone[62] and dental follicular cells that do not have the capacity to differentiate to osteoblasts and form the "granulation tissue" and osteoclast-like giant cells.[70] This mutation has not been reported in CGCG or PGCG but MSX1 may be activated in a different way.[71,72] The possible connection of CGCG with remnants of dental follicular cells through MSX1 could possibly explain their almost unique presentation in the dental-bearing areas of the jaws and in ages close to the period of odontogenesis.

In conclusion, we suggest that in CGCG and PGCG of the jaws, PTHrP-positive immature osteoblasts activate PTHR1-positive mature osteoblasts to produce RANKL, which interacts with RANK on the PTHrP/PTHR1-positive osteoclast-precursor cells found in abundance in the stroma of giant cell lesions and induces osteoclastogenesis through the classic pathway. Furthermore, cells originating from the neural crest, particularly cells of the jawbones, the periodontal ligament, or the dental follicle, may be involved in the pathogenesis of giant cell lesions of the jaws.

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