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Review

Central giant cell granuloma of the jawbones – new insights into molecular biology with clinical implications on treatment approaches

Marilena Vered, Amos Buchner and Dan Dayan

Department of Oral Pathology and Oral Medicine, School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel

Summary. Central giant cell granulomas (CGCG) constitute about 10% of benign jawbone lesions. Approximately one-third of CGCG exhibit local aggressive behavior with bone destruction and a tendency to recur. Cure of patients with aggressive CGCG can be achieved by en bloc resection with clear margins at the possible cost of esthetic, functional and psychological problems, mainly in young patients. It is in these cases where pharmacologic agents are most needed as an alternative treatment approach. Until now, pharmacologic agents for CGCG have been used empirically and, in a small number of cases, with various degrees of success. The purpose of this review is to present the recent findings on the phenotypic profile of the constituent cells in CGCG at the molecular level and discuss the inter-relations among them; to analyze the osteolytic potential concealed in the lesional cells; to provide an evidence-based rationale for the use of pharmacologic agents, and, consequently, to suggest a revised approach for their use.

Key words: Central giant cell granuloma, Molecular markers, Pharmacological agents

Introduction

Jaffe (1953) was the first to distinguish central giant cell granulomas (CGCG) of the jawbones from other giant cell lesions of bones and originally called them "central giant cell reparative granulomas" since they were believed to be a reactive-reparative process that might heal spontaneously (Pogrel et al., 1999). However, further case documentation has shown that most lesions are not reparative in nature but rather neoplastic, and that approximately 70% have the biological behavior of a non-aggressive, asymptomatic, slow-growing lesion, whereas the remaining 30% show an aggressive, progressively destructive behavior. The latter lesions are frequently associated with pain, paresthesia, expansion, rapid growth, tooth displacement, root resorption and remarkable cortical bone destruction (Chuong et al., 1986). This aggressive biological behavior of some CGCG is reminiscent of that of giant cell tumor of bones (GCTB), and it was proposed that CGCG and GCTB belong to the same spectrum of lesions (Whitaker and Waldron, 1993). In addition, further studies have failed to identify any histological or biochemical differences between non-aggressive and aggressive types of CGCG (Vered et al., 2007a).

CGCG account for about 10% of all benign lesions of the jawbones (Waldron, 1995). They are found in all age groups, but most cases are diagnosed in patients under the age of 30 years. Women are more affected than men. Radiologically, CGCG are expansile, radiolucent lesions, often multilocular and generally with well-defined uncorticated borders (Kaffe et al., 1996). Lesions are more common in the anterior portions of the jaws, while the mandibular lesions frequently cross the midline (Kaffe et al., 1996).

Histologically, CGCG consist of spindle-shaped stromal cells (fibroblasts or myofibroblasts) loosely arranged in a fibrous stroma, sometimes of a fibromyxoid type with hemorrhagic areas, hemosiderin deposits, macrophages and varying amounts of inflammatory cells (Waldron, 1995). The hallmark of CGCG is the multinucleated giant cells that are located especially in the hemorrhagic areas and display clusters or a diffuse distribution. Metaplastic bone formation is also seen, and mitoses might be abundant. Other similar giant cell lesions of the jawbones include the "brown tumor" of hyperparathyroidism, aneurysmal bone cyst

Offprint requests to: Dr. Marilena Vered, Department of Oral Pathology and Oral Medicine, School of Dental Medicine, Tel Aviv University, Tel Aviv 69978, Israel. e-mail: mvered@post.tau.ac.il; lmy@netvision.net.il

and cherubism (Pogrel et al., 1999).

The conventional therapy for CGCG of the jawbones is local curettage, but a recurrence rate of up to 70% has been reported, mainly for lesions that display an aggressive biological behavior (Chuong et al., 1986; Kaban et al., 2007; Vered et al., 2007a). For them, extensive surgical procedures are needed that often result in serious mutilation of the jaws and face as well as loss of teeth and dental germs in young patients. It is for this reason that new therapeutic modalities and operative strategies have become a necessity.

The purpose of this review is to present the various phenotypes of the constituent cells in CGCG and the inter-relations among them; to analyze the osteolytic potential concealed in the constituent cells due to their being suppliers for angiogenic- and osteoclastogenesis-associated factors; to provide an evidence-based rationale for the use of pharmacologic agents (indicated for the aggressive CGCG lesions), and, consequently, to suggest a revised approach for the use of these agents.

CGCG are dynamic lesions with multi-phenotypic cells

The nature of the giant cells, which are the histological hallmark of CGCG, is still uncertain, and they have been considered as being phagocytes, foreign body cells, or osteoclasts (Li et al., 2003). Most of the investigatory efforts were directed toward demonstrating that multinucleated giant cells possess osteoclast-specific characteristics, including lacunar resorption of bone, responsiveness to calcitonin, binding of osteoclast-specific monoclonal antibodies (Flanagan et al., 1988), and expression of tartrate-resistance acid phosphatase (TRAP) (Tiffee and Aufdemorte, 1997; Li et al., 2003). However, the best marker for osteoclast cells is the calcitonin receptor (CTR), which had initially been identified in isolated CGCG lesions (Pogrel et al., 1999) and more recently in several large series (Tobon-Arroyave et al., 2005; Vered et al., 2006a). The most recent of these studies showed that both giant cells and mononuclear cells were CTR-positive, but staining was not uniform in extent and intensity. Rather, almost every lesion presented its own individual staining pattern with a different proportion of lesional cell types that were positive for CTR (Vered et al., 2006a).

Along with the CTR-positive cells, which are considered to be of an osteoclast lineage, another group of cells in CGCG (both giant cells and mononuclear cells) was shown to be positive for glucocorticoid receptor (GCR) (Tobon-Arroyave et al., 2005; Vered et al., 2006a), CD-68 (Tiffee and Aufdemorte, 1997; Vered et al., 2004), HLA-DR (Vered et al., 2004) and alpha-1-antichymotrypsin (Tiffee and Aufdemorte, 1997), all of which are markers of macrophage cells. In fact, some investigators maintain that CGCG are lesions composed of a mixed population of cells, where some are of osteoclast lineage and some are of macrophage lineage (Tiffee and Aufdemorte, 1997; Vered et al., 2006a). To make things more complicated, it was also shown that some of the cells were simultaneously positive for both types of markers, i.e., osteoclast and macrophage cells, leading to the assumption that the lesional cells are a product of the macrophage lineage with features intermediate between osteoclasts and macrophages (Tiffee and Aufdemorte, 1997; Vered et al., 2006a).

Yet another type of cell has been identified among the mononuclear cell population, consisting of a myofibroblastic phenotype, based on ultrastructural and immunohistochemical studies (Vered et al., 2007a). For the latter, myofibroblast identification was performed by using an antibody for alpha smooth muscle actin, which is the classic marker for the contractile actin fibers characteristic of these cells. Myofibroblasts are considered an integral part of the lesional cells since their presence is closely associated with the core of the CGCG lesions, while they are usually very rare in the uninvolved adjacent tissue. Myofibroblasts are usually abundant within the lesional tissue, although their density varies among lesions. Two possible sources have been suggested for the emergence of myofibroblasts in CGCG: the first relates to the undifferentiated mesenchymal spindle cells of bone marrow origin, which are able to further differentiate into osteoblasts, fibroblasts, histiocytes as well as into myofibroblasts (Vered et al., 2007a). The second source relates to the fully differentiated macrophages, which were shown to have the potential to undergo a process of trans-differentiation and acquire spindle-shaped morphology and alpha smooth muscle actin (smooth muscle marker) expression, consistent with a myofibroblastic phenotype (Vered et al., 2007a). However, there is evidence that cells with a myofibroblastic phenotype do not always remain in a constant state and that they are able to acquire an osteoblast-like phenotype under adequate stimuli [provided by inflammatory cytokines and activation of their receptors, i.e., tumor necrosis factor alpha (TNF-α) and receptor activator of nuclear factor kappa B ligand (RANKL), respectively] (Kaden et al., 2005; Rajamannan et al., 2005). The importance of the presence of myofibroblasts within CGCG lesions lies in the fact it was shown to be directly related to increased aggressiveness in the biological behavior of different lesions (both benign and malignant) (Vered et al., 2005). No direct relationship between increased density of myofibroblasts and their aggressiveness could be proven in CGCG (Vered et al., 2007a), however, and it is not clear whether the source of these cells (either bone marrow or differentiated macrophages) could play a role in determining the extent of aggressiveness. The interchangeable relations and trans-differentiation processes that take place among the lesional cells in CGCG are illustrated in Fig. 1.

In light of the described observations, it can now be assumed that CGCG lesions are characterized by a population of lesional cells of a heterogeneous phenotypic profile and that these cells are in a dynamic trans-differentiation state. This creates a unique setting

in which each CGCG lesion at any given time point is marked by an individual phenotypic profile of cells and that under certain circumstances, which are yet to be defined, this profile might change and create altered proportions among the various lesional cell phenotypes. This bears significance in regard to treatment decision making in cases of extensive lesions, where pharmacological rather than surgical options are considered. This aspect will be further discussed later on in this review.

Angiogenesis in CGCG lesions

Based on the light microscopic findings in CGCG that are comprised of many blood vessels, extravasated red blood cells and giant cells adjacent to blood vessel walls, it has been suggested that CGCG is part of the spectrum of mesenchymal, primary vascular jawbone tumors (Kaban et al., 1999, 2002). GCTB, which is assumed to be related to CGCG (Whitaker and Waldron, 1993), is also known to be richly vascular.

The development of new capillaries from pre-existing blood vessels is defined as angiogenesis (Seghezzi et al., 1998; Bocci et al., 2001). It is a highly controlled phenomenon in physiological conditions, such as embryonic development and wound healing. In contrast, angiogenesis becomes uncontrolled in pathological settings, such as tumor growth and progression, osteoporosis, and skeletal inflammatory disorders (e.g., rheumatoid arthritis, periodontal disease), all of which are associated with tissue or bone destruction, respectively. Due to its vital importance, angiogenesis is governed by many factors, of which the most potent that has been recognized thus far are the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (Seghezzi et al., 1998).

Members of the vascular endothelial growth factors are prominent among the extracellular signaling molecules that guide vascular development (Leung et al., 1989; Seghezzi et al., 1998; Bocci et al., 2001; Ferrara et al., 2003). VEGF, a 34-46 kDa homodimeric glycoprotein, also known as vascular permeability factor (VPF), acts in an autocrine and paracrine way as a highly specific mitogen for vascular endothelial cells, promotes angiogenesis in several in-vitro and in-vivo models, markedly induces vascular permeability and acts as a survivor factor for newly formed blood vessels (Bocci et al., 2001; Ferrara et al., 2003). It is a soluble molecule that is produced and released from activated monocytes and macrophages (Tolany et al., 1998; Pakala et al., 2002). More recently, vascular endothelial cells were also shown to be a major source of VEGF (Bocci et al., 2001).

bFGF is a prototype member of a family of 13 structurally related, heparin-binding growth factors that has the ability to modulate cell functions in an autocrine and paracrine way (Seghezzi et al., 1998; Ferrara, 2002; Collin-Osbody et al., 2002). It is ubiquitously expressed in cells of mesodermal and neuroectotermal origin as well as in a variety of tumor cells. In vivo, bFGF, is a potent inducer of angiogenesis and has pleiotropic effects on development and differentiation in various organs (Seghezzi et al., 1998; Collin-Osbody et al., 2002).

Recently, a large series of CGCG lesions was analyzed to determine the extent of angiogenesis using the immunohistochemical expression of VEGF and bFGF as reliable indicators for this process (Vered et al., 2006b). The study results showed that angiogenic activity was low in these lesions, as reflected by the few numbers of blood vessels positive for these angiogenic factors; however, a remarkable percent of the mononuclear cells and giant cells were positive for VEGF and bFGF (Figs. 2, 3, respectively). The significance of this observation probably lies in the fact that, in addition to their being potent angiogenic factors, VEGF and bFGF have been shown to play a pivotal role in the process of formation of osteoclasts (Collin-Osbody et al., 2002).

Osteoclastogenesis and its relation to angiogenesis

Osteoclasts are multinucleated giant cells that are capable of removing both the mineral and organic components of bone (Okada et al., 2003). Osteoclasts, granulocytes and macrophages are believed to be derived from a common hematopoietic progenitor cell. Studies have shown that production of mature osteoclasts from progenitor cells, a process termed osteoclastogenesis, is regulated by growth factors, cytokines and hormones (Okada et al., 2003). Osteoclast differentiation is enhanced by interactions between marrow progenitor cells and either mesenchymal cells or osteoblast cells. bFGF, produced by stromal cells, is essential in osteoclast cell formation and differentiation in response to hormones and cytokines (Collin-Osbody et al., 2002; Okada et al., 2003).

The dual role that bFGF plays as a most potent

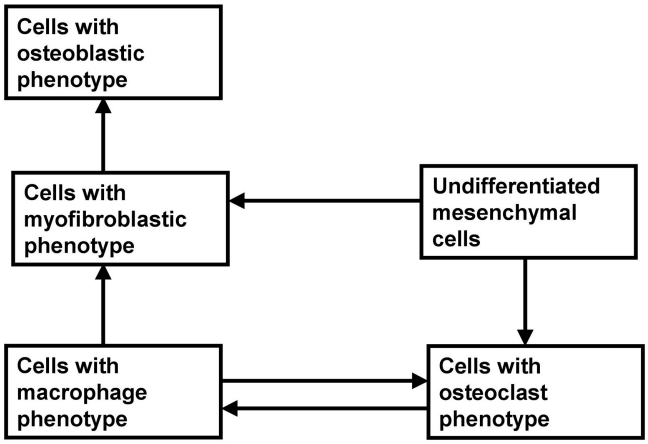

Fig. 1. The various cell phenotypes in CGCG and their inter-relations.

angiogenic factor, along with being a key factor in osteoclastogenesis, was revealed by a large series of in-vitro and in-vivo studies (Zhou et al., 1998; Montero et al., 2000; Collin-Osbody et al., 2002; Yamashita et al., 2002). In a clinical study involving patients with rheumatoid arthritis, bFGF was the only factor that strongly correlated with the extent of osteoclast formation, degree of joint destruction and severity of disease (Manabe et al., 1999). As such, it would appear that bFGF acts as the link between osteoclast formation and function and endothelial cells and their associated factors. It has been shown that in its activated form, bFGF is capable of promoting local recruitment, formation, differentiation and activation of bone resorptive osteoclasts at sites of stimulated angiogenesis, as reflected by an increased number of osteoclasts and increased activity for bone pit resorption (Collin-Osbody et al., 2002).

Among the factors induced by bFGF in the process of angiogenesis is the production of VEGF by the endothelial cells (Seghezzi et al., 1998) which, together with bFGF, trigger these cells to display additional regulatory signals on the surface of their membranes. As a result, VEGF increases vascular permeability and acts

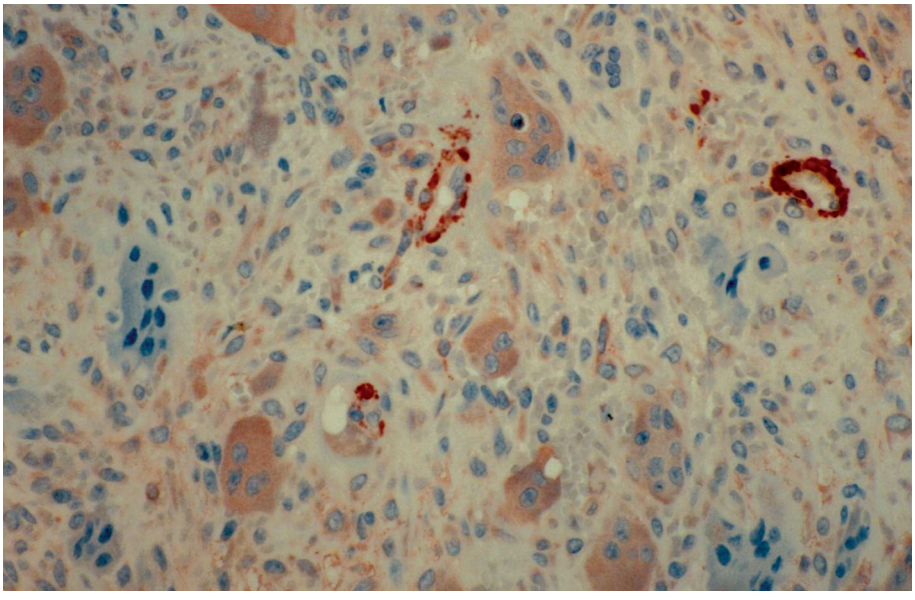

Fig. 2. VEGF-positive giant cells and mononuclear cells are observed as well as a few small blood vessels (anti-VEGF, ABC method, x 200).

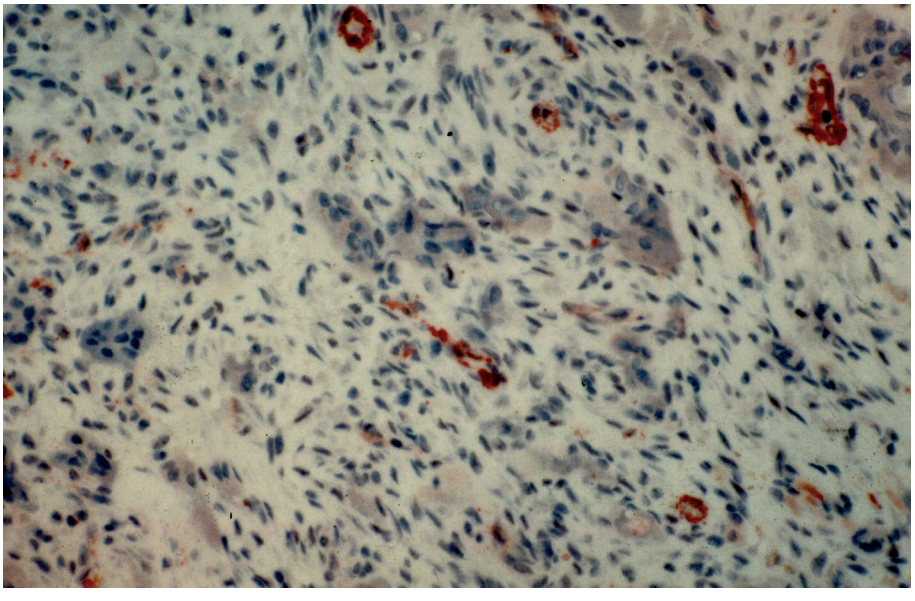

Fig. 3. bFGF immunoreactivity is seen in giant cells and mononuclear cells (weak staining intensity) and in several small blood vessels (strong staining intensity) (anti-bFGF, ABC method, x 200).

as a chemoattractant for osteoclast progenitor cells and as a stimulator for their differentiation (Zheng et al., 2000; Kumta et al., 2003). In this way, angiogenic stimulation enables greater numbers of osteoclast progenitors to emigrate from the peripheral circulation into the bone tissue and develop into resorptive osteoclast cells.

Given that mononuclear cells and giant cells in CGCG are positive for VEGF and bFGF and, most likely, serve as an important local source for these factors, they may play an integral role in the process of osteoclastogenesis and contribute considerably to the progression of the osteolytic CGCG lesions of the jawbones.

The vicious circle of CGCG that involves lesional cells, VEGF, bFGF and osteoclastogenesis

Combining the results of various studies on the angiogenesis-osteoclastogenesis axis, a scheme of events is proposed to explain these inter-related biological processes in CGCG in connection with the expression of bFGF and VEGF by the lesional cells (Fig. 4). The course of action is most probably initiated by parathyroid hormone (PTH) and, in spite of lack of biochemical evidence for parathyroid pathology in CGCG (Harris, 1993), it is assumed that there is an amplified responsiveness to this hormone in the downstream events that ultimately lead to the net formation of an osteolytic lesion. PTH is considered a potent inducer of osteoclast formation, directly exerting its action, in part, through endogenous bFGF synthesis (Okada et al., 2003). Under the influence of PTH, there is local increased production of bFGF-mRNA and its respective protein in cells with an osteoblastic phenotype (Collin-Osbody et al., 2002; Okada et al., 2003). In the particular case of CGCG, either undifferentiated stromal mesenchymal mononuclear cells perform this function or it can be the result of myofibroblast-osteoblast transformation (Vered et al., 2007a). From this point on, bFGF has a double-arm action: the first relates to bone-associated remodeling cells, i.e., with osteoblast- and

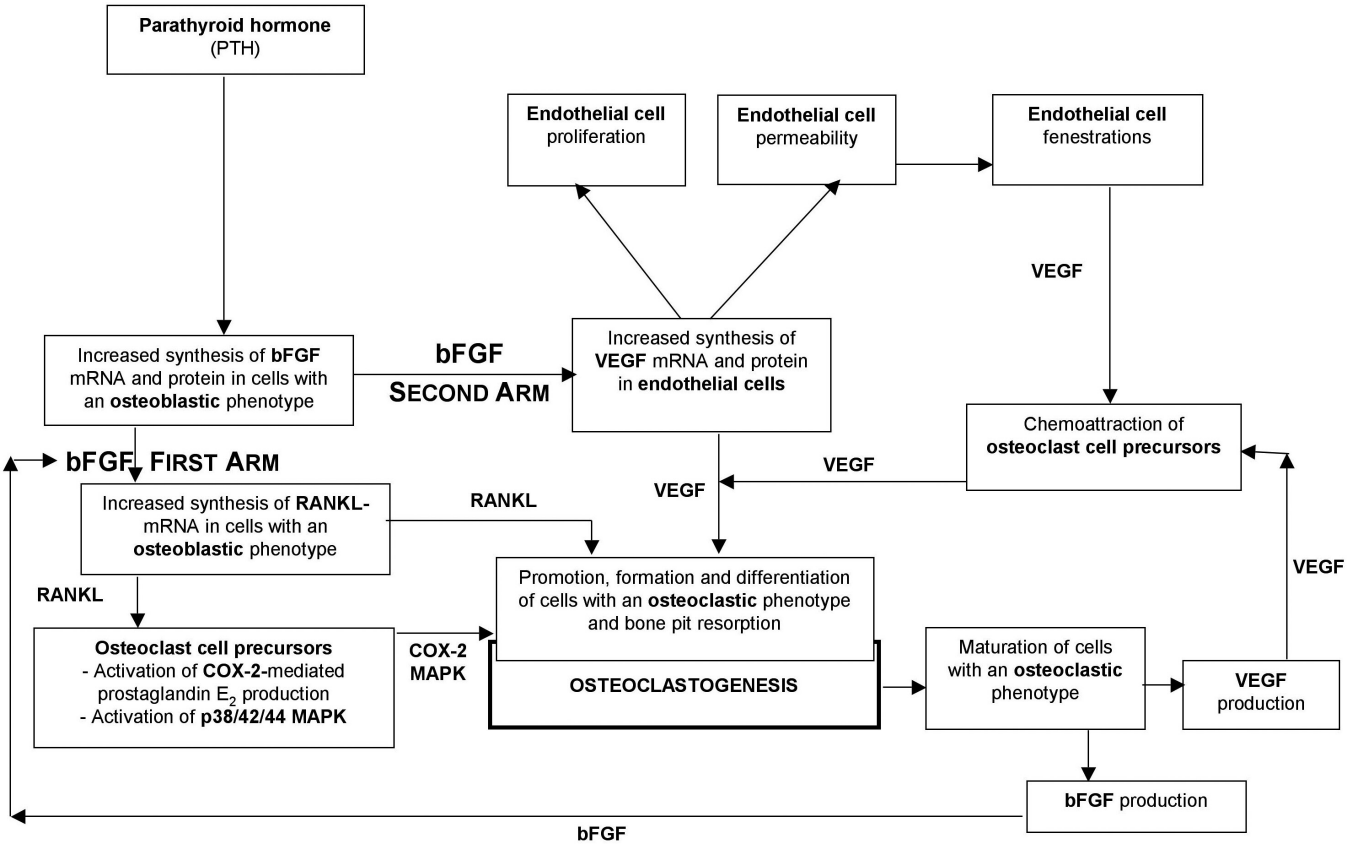

Fig. 4. Proposed mechanism of action of VEGF and bFGF as mediators of angiogenesis and osteoclastogenesis in CGCG of the jawbones. Parathyroid hormone (PTH), the initiator of the process, stimulates bFGF synthesis in osteoblast cells. Secreted bFGF is responsible for activation of two pathways: RANKL-COX-2-p38/42/44 MAPK and VEGF-endothelial cells-osteoclast progenitor cells. These pathways finally converge in the stage of promotion, formation and differentiation of cells with an osteoclastic phenotype and bone pit resorption (e.g., lesional mononuclear and giant cells), a process defined as osteoclastogenesis. These cells, which themselves are a considerable source of VEGF and bFGF, contribute to enhancement of the osteoclastogenetic process in a vicious cycle that are clinically and radiologically reflected by enlargement of the CGCG lesion.

osteoclast-bearing phenotypes, and the second relates to the endothelial cells. Through the first arm, bFGF increases synthesis of RANKL-mRNA and its related protein in cells with an osteoblastic phenotype (Abbas et al., 2003). Expression of RANKL was demonstrated in CGCG lesions (Li et al., 2003) as well as in the CGCG-related lesion, GCTB (Lau et al., 2005). Based on evidence from both animal models and human conditions involving osteoclast activity (Choi et al., 2005; Fukashima et al., 2005), it can be assumed that RANKL goes on to selectively induce cyclo-oxygenase (COX)-2 expression in cells, which are recognized as osteoclast precursors (Boyle et al., 2003; Han et al., 2005). This, in turn, can lead to the production of prostaglandin E2 (PGE2) and the mitogen-activated protein (MAP) kinases (MAPK), especially p38, p42 and p44 MAPK, which have been shown to play a key role in osteoclastogenesis (Ip and Davis, 1998; Herlaar and Brown, 1999; Matsumoto et al., 2000). Altogether, the first arm of bFGF-induced RANKL, COX-2 and MAPK pathways culminate in osteoclastogenesis that encompasses recruitment, formation, differentiation, and activation of bone resorptive cells with an osteoclastic phenotype (Collin-Osbody et al., 2002).

Through the second arm, bFGF induces VEGF synthesis in endothelial cells (Seghezzi et al., 1998; Kumta et al., 2003). VEGF is responsible for endothelial cell proliferation and production of new blood vessels, i.e., angiogenesis, which is considered a requisite for osteoclastogenesis observed in osteolytic bone lesions (Parffit, 1998; Niida et al, 1999; Kumta et al., 2003). Paradoxically, CGCG, which are typical osteolytic lesions, are almost deficient in angiogenesis. This is reflected by the remarkably low frequency of newly formed blood vessels positive for VEGF (Vered et al., 2006b). The structure of the few existing newly formed vascular structures in CGCG seems to be irregular (Lim and Gibbins, 1995; El-Labban, 1997; Vered et al., 2006b). Knowing that VEGF can also act as a factor that increases endothelial cell permeability, these irregularities are interpreted as fenestrations in the vascular network that can serve as the portal of entry for osteoclast precursors from the peripheral circulation to the lesional area. Furthermore, VEGF is able to act as a potent chemoattractant factor that stimulates differentiation of osteoclast precursors and has direct effects on their activation and functioning (Gamberi et al., 1998; Niida et al., 1999).

In contrast to the minor expression of angiogenic factors by the blood vessel endothelial cells in CGCG, it was shown that both types of lesional cells, mononuclear cells and giant cells, were largely immunoreactive for VEGF and, to a lesser extent, for bFGF. This can be a strong indication of an intrinsic production of angiogenic- and osteoclastogenesis-related factors in CGCG by the lesional cells themselves (Vered et al., 2006b). In light of this finding, it is assumed that by acting in an autocrine and paracrine way on the appropriate cells, VEGF and bFGF play a pivotal role in the initiation and propagation of osteoclastogenesis in CGCG, even in the absence of a well-developed vascular network. The moment that mature cells with an osteoclast-like phenotype emerge within CGCG as the final product of osteoclastogenesis, they serve as a source for additional VEGF and bFGF production, all of which, in turn, will further contribute to the process of osteoclastogenesis. Thus, a cycle comprising an intrinsic reservoir of cells with osteoclast-like phenotypes that produce osteoclastogenesis-related factors is created with the net outcome of an enlarging osteolytic lesion.

Commonly used therapeutic agents in CGCG and a revised approach for improving their clinical achievements

The recent molecular findings on the diversity of the cellular phenotypes in CGCG and the possible pathways of trans-differentiation among them merit a revision of the pharmacological agents and their manner of administration. This is particularly important in cases of large CGCG, especially in children, where a curable surgical procedure might result in facial mutilation, functional disorders and severe psychological problems. Nowadays, these pharmacological agents consist mainly of corticosteroids, calcitonin and interferon alpha-2α-(INFα–2α).

The rationale for treating CGCG with corticosteroids was initially based solely on the histological similarity between this lesion and granulomas of sarcoidosis that are composed of a collar of epithelioid macrophages surrounding a central area of multinucleated giant cells of macrophage origin (Terry and Jacoway, 1994). This therapeutic strategy was only later supported by the fact that at least part of the mononuclear cells and giant cells in CGCG are of macrophage origin, as shown by their respective markers, mainly GCR (Tiffee and Aufdemorte, 1997; Li et al., 2003; Vered et al., 2006a). The accepted clinical protocol for corticosteroid administration is weekly intralesional injections for 6 weeks, although a successful outcome was recently reported for a case in which combined systemic and local, intralesional glucocorticoids were used (Comert et al., 2006). There are fewer than 50 cases in the English literature in which corticosteroid therapy was administered. Clinical outcome of this treatment varied from significant reduction in lesion size to complete cure (Carlos and Sedano, 2002).

The rationale for treating CGCG with calcitonin was derived from the histologic similarity to the "brown tumor" of hyperthyroidism (Harris, 1993). It was assumed that in spite of the absence of parathyroid disease, CGCG lesions will be responsive to calcitonin, which is supposed to act against cells with an osteoclastic phenotype. The first report on calcitonin treatment was published by Harris (1993), but definitive evidence-based data for the existence of this type of cells in CGCG lesions did not appear until approximately 6 years later with the identification of mRNA of CTR,

which is the main marker of cells with an osteoclastic phenotype (Pogrel et al., 1999). Additional support was provided more recently, when CTR was identified in several large series of CGCG by routine immunohistochemical stains (Tobon-Arroyave et al., 2005; Vered et al., 2006a). Calcitonin was initially administered as subcutaneous injections of human calcitonin; it has since been replaced by salmon calcitonin administered as a nasal spray. Treatment usually lasts more than 20 months. In all, there are about 50 cases in the English literature of patients who were treated with calcitonin. The degree of success ranged from complete remission (Pogrel 1999, 2003; de Lange et al., 1999) to reduction in tumor size (de Lange et al., 2006a) and to failure (de Lange et al., 2006b; Vered et al., 2007b).

It is not conceivable that so few cases of CGCG had actually been treated pharmacologically. Experience with these agents is likely to be considerably more extensive than what is reported in the literature. One reason for this discrepancy may lie in the fact that clinicians tend to report only cases with a fair degree of success rather than those with less encouraging outcomes. Nevertheless, the new observations on the molecular biology of CGCG lesions, as reviewed herein, could add to our understanding of how to more efficiently treat the aggressive type of lesions by means of medications.

A fundamental issue to be borne in mind when treating CGCG lesions by pharmacological approaches is the fact that these lesions are not composed of a homogeneous population of lesional cells, but rather of cells of various phenotypes that may be in a dynamic state of trans-differentiation at any given time point, leading to continuously changing relative proportions among them. Each type of cell is expected to react to a different pharmacological agent, and this could explain why agents with various acting mechanisms were reported to have a fair degree of success in treating CGCG. At a practical level, treatment should no longer be empirical, but rather based on the molecular characterization of the phenotypic profile of the lesional cells prior to and during treatment, until achieving favorable clinical and/or radiological clearance.

Another important issue in treating CGCG with steroids and calcitonin is the "escape phenomenon" (Pondel, 2000). Continuous and prolonged administration of calcitonin causes a significant decrease in the expression of the CTR-gene. The ultimate result is that calcitonin no longer inhibits osteoclastic activity. This is referred to as the “escape phenomenon”. In terms of molecular biology, we can now explain this as the reflection of the potential of the lesional cells to transiently demonstrate either macrophage- or osteoclast-associated markers, GCR and CTR, respectively. The factors responsible for this dynamic, elusive phenotypic trans-differentiation of the cells at a molecular level have not been well defined and warrant investigation. At a clinical level, however, it is suggested that the immunohistochemical profile of the lesional cells for CTR and GCR should be established prior to initiating pharmacologic treatment. The principle is to start treatment with the therapeutic agent that specifically targets the receptor demonstrating the most intense staining reaction. If lesions do not show satisfactory clinical and/or radiological response during treatment, they should be biopsied to re-evaluate changes in the CTR and GCR staining status (Vered et al., 2006a). This will enable accurate alteration of the therapeutic agent (calcitonin or corticosteroids) to achieve the most rapid and effective clinical results. Furthermore, combination of both corticosteroid and calcitonin agents is feasible as long as lesional cells co-express GCR and CTR at the same time. There is only one case reported in the English literature in which this combination was successfully used on an empirical basis in an aggressive CGCG associated with tertiary hyperparathyroidism (Pinto et al., 2006). Moreover, it has been shown that the lesional cells in CGCG can also demonstrate a myofibroblast phenotype, and this finding can be expected to lead to the enhancement of the pharmacologic armamentarium by the addition of anti-myofibroblastic agents (Vered et al., 2007a).

The belief that CGCG may actually represent a proliferative vascular lesion led clinicians to assume that it would respond to anti-angiogenetic pharmacological agents commonly used for angiogenic lesions (i.e., pulmonary hemangiomatosis and life-threatening hemangiomas) (Kaban et al., 1999, 2002). Thus, anti-angiogenic pharmacological treatment with interferon alpha (INF-α)-2a has been empirically used in a several cases of CGCG, with fair to limited results. Only about 30 cases of aggressive giant cell lesions treated in this way have been reported in the English language literature (Collins, 2000; Goldman, 2005; de Lange et al., 2006a; Kaban et al., 2007). In contrast to the corticosteroids and calcitonin modalities, where patients are spared surgery unless they are unresponsive, in regard to INFα-2a treatment, there is a pre-requisite for initial either conservative (enucleation with preservation of adjacent nerves and teeth) or radical (partial jaw resection) surgery. Forty-eight hours post-surgery, treatment with INFα-2a is commenced as an adjuvant therapy. In the largest series, all patients treated with INFα-2a showed satisfactory results (Kaban et al., 2007). Cure was achieved in approximately 60% of the patients in whom lesions completely disappeared after daily treatment with INFα-2a injections for more than 6 months. However, cases with a limited response were also reported by others (de Lange et al., 2006b). The main disadvantage with this therapeutic approach is the serious side effects that eventually necessitate treatment modification/cessation (Goldman, 2005; Kaban et al., 2007). The anti-angiogenic potential of INFα-2a lies in its ability to inhibit the production of bFGF. Since bFGF is a key factor in osteoclastogenesis, inhibition of its synthesis and subsequent downstream events would reduce the development of cells with an osteoclast

phenotype (Folkman, 2002). The results of a large series of CGCG, however, showed that the newly formed vascular component was negligible (Vered et al., 2006b). In addition, the lesions consisted of a relatively small number of cells positive for bFGF compared to high positivity for VEGF. Since INFα-2a contributed to the final shrinking and disappearance of the residual tumor, it is assumed that it acted not only through the bFGF pathway, but also through alternative mechanisms. Recently, it has been suggested that INFα-2a may also act to stimulate osteoblasts and osteoblast precursors, at least in cell culture (Abukawa et al., 2006). Therefore, the mediators associated with INFα-2a and its full adjuvant potential in CGCG lesions warrant investigation in future studies. Since nearly all the cells in CGCG are VEGF-positive, it makes them an attractive target for anti-VEGF treatment. This could include dexamethasone, which has the potential to reduce VEGF expression (Heiss et al., 1996) and the recently developed humanized anti-VEGF monoclonal antibodies (Ferrara, 2002; Ferrara et al., 2003). Similar to the use of calcitonin and glucocorticoids, the therapeutic agents used against VEGF and bFGF in CGCG should be clinically recommended solely on a selective basis, in particular, for cases in which the immunohistochemical stains confirm strong and diffuse positivity for these markers.

Conclusions

1. CGCG are characterized by multi-phenotypic cell populations that are in a dynamic state of trans-differentiation. This creates a distinguished phenotypic profile for each lesion at different time points, with changing proportions among the types of lesional cells. Therefore, each of these lesions should be considered individually in terms of biological behavior and treatment.

2. Pharmacologic agents are currently pursued by both clinicians and pathologists as an alternative treatment approach, mainly for the aggressive CGCG lesions. Their aim is to accomplish complete resolution or to at least significantly reduce lesion size in order to minimize the extent of the surgical procedure.

3. Up to now, pharmacologic agents in CGCG have been used empirically, based on the mere histopathological similarity of this lesion to other giant cell-, macrophage-, and osteoclast-containing lesions or richly vascular lesions in other sites of the body.

4. Based on this similarity, glucocorticoids were used against cells of macrophage lineage, calcitonin against cells of osteoclastic lineage and INFα-2a as an anti-vascular agent. A fair degree of clinical success was reported in several cases of aggressive CGCG treated with pharmacological agents. However, it can also be said with a high degree of certainty that the number of cases treated in the same way and in which the clinical outcomes showed failure of treatment is very under-reported. Therefore, the currently employed therapies would appear to be inadequate or, alternatively, the way by which they are administered needs to be improved.

5. On a molecular level, it became clear that CGCG contain cells that are in a dynamic state of transformation from one phenotype to another. The phenotypic composition of the lesion comprises cells with macrophage, osteoclast and myofibroblast phenotypes, and the relative portion of each phenotype can change in a spatio-temporal manner. The factors governing these changes remain to be determined.

6. The actively changing phenotypes of CGCG cells are the likely explanation of why one single pharmacologic agent cannot always be clinically efficient for cure. Adjusting the pharmacologic agents to the phenotypic profile of the lesional cells prior to their administration, both at the beginning and during treatment, is recommended. Combinations of more than one therapeutic agent can further enhance the synergistic effect on the progression of the curing process.

7. In light of these recent findings, it is recommended that new therapeutic options be further investigated.

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References

Abbas S., Clohisy J.C. and Abu-Amer Y. (2003). Mitogen-activated protein (MAP) kinases mediate PMMA-induction of osteoclasts. J. Orthop. Res. 21, 1041-1048.

Abukawa H., Kaban L.B., Williams W.B., Terada S., Vacanti J.P. and Troulis M.J. (2006). Effect of interferon alpha-2b on porcine mesenchymal stem cells. J. Maxillofac. Surg. 64, 1214-1220.

Bocci G., Fasciani A., Danesi R., Viacava P., Genazzani A.R. and Del Tacca M. (2001). In-vitro evidence of autocrine secretion of vascular endothelial growth factor by endothelial cells from human placental blood vessels. Mol. Hum. Reprod. 7, 771-777.

Boyle W.J., Simonet W.S. and Lacey D.L. (2003). Osteoclast differentiation and activation. Nature 423, 337-342.

Carlos R. and Sedano H.O. (2002). Intralesional corticosteroids as an alternative treatment for central giant cell granuloma. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 93, 161-166.

Choi B.K., Moon S.Y., Cha J.H., Kim K.W. and Yoo Y.J. (2005). Prostaglandin E(2) is a main mediator in receptor activator of nuclear factor-kappaB ligand-dependent osteoclastogenesis induced by Pophyromonas gingivalis, Treponema denticola, and Treponema socranskii. J. Periodontol. 76, 813-820.

Chuong R., Kaban L.B., Kozakewich H. and Perez-Atayade A. (1986). Central giant cell lesions of the jaws: a clinicopathologic study. J. Oral Maxillofac. Surg. 44, 798-813.

Collin-Osbody P., Rothe L., Bekker S., Anderson F., Huang Y. and Osbody P. (2002). Basic fibroblast growth factor stimulates osteoclast recruitment, development, and bone pit resorption in association with angiogenesis in vivo on the chick chorioallantoic membrane and activates isolated avian osteoclast resorption in vitro. J. Bone Miner. Res. 17, 1859-1871.

Collins A. (2000). Experience with anti-angiogenic therapy of giant cell granuloma of the facial bones. Ann. R. Australas. Coll. Dent. Surg. 15, 170-175.

Comert E., Turanli M. and Ulu S. (2006). Oral and intralesional steroid therapy in giant cell granuloma. Acta Otolaryngol. 126, 664-666.

de Lange J., Rosenberg A.J., van der Akker H.P., Koole R., Wirds J.J. and van der Berg H. (1999). Treatment of a central giant cell granuloma of the jaw with calcitonin. Int. J. Oral Maxillofac. Surg. 28, 372-376.

de Lange J., van den Akker H.P., Veldhuijzen van Zanten G.O., Engelshove H.A., van der Berg H. and Klip H. (2006a). Calcitonin therapy in central giant cell granuloma of the jaw: a randomized double-blind placebo-controlled study. Int. J. Oral Maxillofac. Surg. 35, 791-795.

de Lange J., van den Akker H.P., van den Berg H., Richel D.J. and Gortzak R.A. (2006b). Limited regression of central giant cell granuloma by interferon alpha after failed calcitonin therapy: a report of 2 cases. Int. J. Oral Maxillofac. Surg. 35, 865-869.

El-Labban N.G. (1997). Intravascular fibrin thrombi and endothelial cell damage in central giant cell granuloma. J. Oral Pathol. Med. 26, 1-5.

Ferrara N. (2002). VEGF and the quest for tumour angiogenesis factors. Nat. Rev. Cancer 2, 795-803.

Ferrara N., Gerber H-P. and LeCouter J. (2003). The biology of VEGF and its receptors. Nat. Med. 9, 669-676.

Flanagan A.M., Nui B., Tinkler S.M., Horton M.A., Williams D.M. and Chambers T.J. (1988). The multinucleate cells in giant cell granuloma of the jaws are osteoclasts. Cancer 62, 1139-1145.

Folkman J. (2002). Antiangiogenetic therapy with interferon alpha for giant cell lesions of the jaws (comment). J. Oral Maxillofac. Surg. 60, 1111-1113.

Fukashima H., Jimi E., Okamato F., Motokawa W. and Okabe K. (2005). IL-1-induced receptor activator of NF-kappa B ligand in human periodontal ligament cells involves ERK-dependent PGE2 production. Bone 36, 267-275.

Gamberi G., Benassi M.S., Bohling T., Ragazzini P., Molendini L., Sollazzo M.R., Merli M., Ferrari C., Magagnoli C., Bertoni F. and Picci P. (1998). Prognostic relevance of c-myc gene expression in giant cell tumor of bone. J. Orthop. Res. 16, 1-7.

Goldman K.E. (2005). Complications of alpha-interferon therapy for aggressive central giant cell lesion of the maxilla. Oral Surg. Oral Med. Oral Pathol. Oral Endod. Radiol. 100, 285-291.

Han S.Y., Lee N.K., Kim K.H., Jang I.W., Yim M., Kim J.H., Lee W.J. and Lee S.Y. (2005). Transcriptional induction of cyclooxygenase-2 in osteoclast precursors is involved in RANKL-induced osteoclastogenesis. Blood 106, 1240-1245.

Harris M. (1993). Central giant cell granulomas of the jaws regress with calcitonin therapy. Br. J. Oral Maxillofac. Surg. 31, 89-94.

Heiss J.D., Papavassiliou E., Merrill M.J., Nieman L., Knightly J.J., Walbridge S., Edwards N.A. and Oldfield E.H. (1996). Mechanism of dexamethasone suppression of brain tumor-associated vascular permeability. J. Clin. Invest. 98, 1400-1408.

Herlaar E. and Brown Z. (1999). p38 MAPK signaling cascades in inflammatory disease. Mol. Med. Today 5, 439-447.

Ip Y.T. and Davis R.J. (1998). Signal transduction by the c-jun N-terminal kinase - from inflammation to development. Curr. Opin. Cell Biol. 10, 205-219.

Jaffe H.L. (1953). Giant cell reparative granuloma, traumatic bone cyst and fibrous (fibro-osseous) dysplasia of the jawbones. Oral Surg. Oral Med. Oral Pathol. 6, 159-175.

Kaban L.B., Mulliken J.B., Ezekowitz R.A., Ebb D., Smith P.S. and Folkman J. (1999). Antiangiogenetic therapy of a recurrent giant cell tumor of the mandible with interferon alpha-2a. Pediatrics. 103 (6 Pt 1), 1145-1149.

Kaban L.B., Troulis M.J., Ebb M., August M., Hornicek F.J. and Dodson T.B. (2002). Antiangiogenic therapy with interferon alpha for giant cell lesions of the jaws. J. Oral Maxillofac. Surg. 60, 1103-1111.

Kaban L.B., Troulis M.J., Wilkinson M.J., Ebb D. and Dodson T.B. (2007). Adjuvant antiangiogenic therapy for giant cell tumors of the jaws. J. Oral Maxillofac. Surg. 65, 2018-2023. (Discussion p. 2024).

Kaden J.J., Kilic R., Sarikoc A., Hagl S., Lang S., Hoffman U., Bruekmann M. and Borggrefe M. (2005). Tumor necrosis factor alpha promotes an osteoblast-like phenotype in human aortic valve myofibroblasts: a potential regulatory mechanism of valvular calcification. Int. J. Mol. Med. 16, 869-872.

Kaffe I., Ardekian L., Taicher S., Littner M.M. and Buchner A. (1996). Radiologic features of central giant cell granuloma of the jaws. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 81, 720-726.

Kumta S.M., Huang L., Cheng Y.Y., Chow L.T.C., Lee K.M. and Zheng M.H. (2003). Expression of VEGF and MMP-9 in giant cell tumor of bone and other osteolytic lesions. Life Sci. 73, 1427-1436.

Lau Y.S., Sabokbar A., Gibbons C.L., Giele H. and Athanasou N. (2005). Phenotypic and molecular studies of giant-cell tumors of bone and soft tissue. Hum. Pathol. 36, 945-954.

Leung D.W., Cachianes G., Kuang W-J., Goeddel D.V. and Ferrara N. (1989). Vascular endothelial factor is a secreted angiogenic mitogen. Science 246, 1306-1312.

Li B., Yu S-F. and Li T-J. (2003). Multinucleated giant cells in various forms of giant cell containing lesions of the jaws express features of osteoclasts. J. Oral Pathol. Med. 32, 367-375.

Lim L. and Gibbins J.R. (1995). Immunohistochemical and ultrastructural evidence of a modified microvasculature in the giant cell granuloma of the jaws. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 79, 190-198.

Manabe N., Oda H., Nakamura K., Kuga Y., Uchida S. and Kawaguchi H. (1999). Involvement of fibroblast growth factor-2 in joint destruction of rheumatoid arthritis patients. Rheumatology 38, 714-720.

Matsumoto M., Sudo T., Saito E., Osada H. and Tsujimoto M. (2000). Involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-kappa B ligand (RANKL). J. Biol. Chem. 275, 31155-31161.

Montero A., Okada Y., Tomita M., Ito M., Tsurakami H., Nakamura T., Doetscman T., Coffin J.D. and Hurley M.M. (2000). Disruption of the fibroblast growth factor 2-gene results in decreased bone mass and bone formation. J. Clin. Invest. 105, 1085-1093.

Niida S., Kaku M., Amano H., Yoshida H., Kataola H., Nishikawa S., Tanne K., Maeda N., Nishikawa S. and Kodama H. (1999). Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption. J. Exp. Med. 190, 293-298.

Okada Y., Montero A., Zhang X., Sobue T., Lorenzo J., Doetschman T., Coffin J. and Hurley M.M. (2003). Impaired osteoclast formation in bone marrow cultures of Fgf2 null mice in response to parathyroid hormone. J. Biol. Chem. 23, 21258-21266.

Pakala R., Watanabe T. and Benedict CR. (2002). Induction of endothelial proliferation by angiogenic factors released by activated monocytes. Cardiovasc. Radiat. Med. 3, 95-101.

Parffit A.M. (1998). Osteoclast precursors as leukocytes: importance of

the area code. Bone 23, 491-494.

Pinto L.P., Cherubinim K., Salum F.G., Yurgel L.S. and de Fugueiredo M.A. (2006). Highly aggressive brown tumor in the jaw associated with tertiary hyperparathyroidism. Pediatr. Dent. 28, 543-546.

Pogrel M.A. (2003). Calcitonin therapy for central giant cell granuloma. J. Oral Maxillofac. Surg. 61, 649-653.

Pogrel M.A., Regezi J.A., Harris S.T. and Goldring S.R. (1999). Calcitonin treatment for central giant cell granulomas of the mandible: report of two cases. J. Oral Maxillofac. Surg. 57, 848-853.

Pondel M. (2000). Calcitonin and calcitonin receptors: bone and beyond. Int. J. Exp. Pathol. 81, 405-422.

Rajamannan N.M., Nealis T.B., Subramanian M., Pandya S., Stock S.R., Ignatiev C.I., Bebo T.J., Rosengart T.K., Edwards W.D., McCarthy P.M., Bonow R.O. and Spelberg T.C. (2005). Calcified rheumatic neoangionesis is associated with vascular endothelial growth factor expression and osteoclast-like bone formation. Circulation. 111, 3269-3301.

Seghezzi G., Patel S., Ren C.J., Gualandris A., Pintucci G., Robbins E.S., Shapiro R.L., Galloway A.C., Rifkin D.B. and Mignatti P. (1998). Fibroblast growth factor–2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis. J. Cell Biol.141, 1659-1673.

Terry B.C. and Jacoway J.R. (1994). Management of central giant cell lesions: an alternative to surgical therapy. Oral Maxillofac. Surg. North. Am. 6, 579-600.

Tiffee J.C. and Aufdemorte T.B. (1997). Markers for macrophage and osteoclast lineages in giant cell lesions of the oral cavity. J. Oral Maxillofac. Surg. 55, 1108-1112.

Tobon-Arroyave S.I., Franco-Gonzales L.M., Isaza-Guzman D.M., Florez-Moreno G.A., Bravo-Vasquez T., Castaneda-Palez D.A. and Vieco-Duran B. (2005). Immunohistochemical expression of RANK, GRa and CTR in central giant cell granuloma of the jaws. Oral Oncol. 41, 480-488.

Tolany E., Kuhnen C., Voss B., Wiethege T. and Muller K-M. (1998). Expression and localization of vascular endothelial factor and its receptor flt in pulmonary sarcoidosis. Virchows Arch. 432, 61-65.

Vered M., Buchner A. and Dayan D. (2004). Immunohistochemical profile of glucocorticoid receptor, calcitonin receptor, CD68 and HLA-DR in central giant cell granuloma of the jawbone. J. Appl. Res. Clin. Dent. 1, 47-55.

Vered M., Shohat I., Buchner A., and Dayan D. (2005). Myofibroblasts in stroma of odontogenic cysts and tumors can contribute to variations in the biologic behavior of the lesions. Oral Oncol. 41, 1028-1033.

Vered M., Buchner A. and Dayan D. (2006a). Immunohistochemical expression of glucocorticoid and calcitonin receptors as a tool for selecting therapeutic approach in central giant cell granuloma of the jawbones. Int. J. Oral Maxillofac. Surg. 35, 756-760.

Vered M., Buchner A. and Dayan D. (2006b). Giant cell granuloma of the jawbones – a proliferative vascular lesion? Immunohistochemical study with vascular endothelial growth factor and basic fibroblastic growth factor. J. Oral Pathol. Med. 35, 613-619.

Vered M., Nasrallah W., Buchner A. and Dayan D. (2007a). Stromal myofibroblasts in central giant cell granuloma of the jaws cannot distinguish between non-aggressive and aggressive lesions. J. Oral Pathol. Med. 36, 495-500.

Vered M., Shohat I., Buchner A., Dayan D. and Taicher S. (2007b). Calcitonin nasal spray for treatment of central giant cell granuloma – clinical, radiologic and histologic findings and immunohistochemical expression of calcitonin and glucocorticoid receptors. Oral Surg. Oral Med. Oral Pathol. Oral Endod. Radiol. 104, 226-239.

Waldron C.A. (1995). Bone pathology. In: Oral and maxillofacial pathology. Neville B.W., Damm D.D., Allen C.A. and Bouquot J.E. (eds). Saunders. Philadelphia, PA. pp 453-455.

Whitaker S.B. and Waldron C.A. (1993). Central giant cell lesions of the jaws. A clinical, radiologic, and histopathologic study. Oral Surg. Oral Med. Oral Pathol. 75, 199-208.

Yamashita A., Yonemitsu Y., Okano S., Nakagawa K., Nakashima Y., Irisa T., Iwamoto Y., Nagai Y., Hasegawa M. and Sueishi K. (2002). Fibroblast growth factor-2 determines severity of joint disease in adjuvant-induced arthritis in rats. J. Immunol. 168, 450-457.

Zheng M.H., Xu J., Robbins P., Pavlos N., Wysocki S., Kumta S.M., Wood D.J. and Papadimitriou J.M. (2000). Gene expression of vascular endothelial growth factor in giant cell tumors of bone. Hum. Pathol. 31, 804-812.

Zhou M., Sutliff R.L., Paul R.J., Lorenz J.N., Hoying J.B., Haundenschild C.C., Moying Y., Coffin J.D., Kong L., Kranias E.G., Luo W., Boivin G.P., Duffy J.J., Pawlowski S.A. and Doetschman T. (1998). Fibroblast growth factor 2 control of vascular tone. Nat. Med. 4, 210-207.

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