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Review

The histogenesis of giant cell tumour of bone: a model of interaction between neoplastic cells and osteoclasts

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Summary. Giant cell tumour of bone (GCT) is a benign primary neoplasm of a bone characterised by distinctive clinical, radiological and pathological features. Females are slightly more often affected than males, and the majority of patients present between the ages of 20 and 50. GCT is locally aggressive and produces expansive and lytic lesions, most commonly in the epiphyses of long tubular bones. Histologically, it is composed of oval and spindle mononuclear cells, uniformly distributed amongst which are large multinucleated osteoclast-like giant cells. Although the term "Giant Cell Tumour" (and the erroneous historical term 'osteoclastoma') may imply that it is the multinucleated giant cells which are responsible for the proliferative capacity of the tumour, there is evidence that the stromal-like cells, the major component of the mononuclear cell population, represent the true neoplastic component of the neoplasm. The diagnosis and management of conventional GCT are often challenging and there is considerable current interest in its pathobiology. The precise histogenesis of GCT and the nature of its varying cellular constituents have remained a matter of some controversy. Factors influencing the clinical course and biological aggression of GCT are also unclear. In this selective review, the clinicopathological characteristics of GCT are summarised and current areas of interest in the study of the neoplasm are presented and discussed. Lastly, a hypothetical model of the mechanism of histogenesis and the biological behaviour of GCT is presented.

Key words: Giant cell tumour of bone, Osteoclast, TGF-ß1, MCP-1, VEGF, RANKL

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This review is dedicated to Professor Liu Tze-Chun on the occasion of his 70th birthday. Professor Liu has spent his entire life in bone histopathology and in particular the study of giant cell tumour of bone in China.

Introduction

Giant cell tumour of bone (GCT), which has also been referred to as 'conventional giant cell tumour of bone' or 'osteoclastoma' (Schajowicz, 1994), is classified according to the WHO as "an aggressive tumour, characterised by richly vascularised tissue consisting of rather plump spindle-shaped or ovoid cells of osteoclast type, which are uniformly distributed throughout the tumour tissue" (Schajowicz, 1994). Although the term "Giant Cell Tumour" and "osteoclastoma" may imply that it is the multinucleated giant cells which are responsible for the proliferative capacity of the neoplasm, there is evidence that the stromal-like cells, the major component of the mononuclear cell population, represent the true neoplastic component. The diagnosis and management of conventional GCT are often challenging and there is considerable current interest in the pathobiology of GCT.

Although bone destruction is a characteristic feature of GCT, there is, as yet, no evidence that the neoplastic cells are capable of bone resorption. Instead, it appears that the neoplastic cells of GCT act by promoting bone resorption. In fact, this interplay between the neoplastic cells and the multinucleate osteoclast-like giant cells found in GCT has been considered as a model of the cellular interactions that occur during bone resorption in both primary and metastatic neoplasms (Grano et al., 1994; Robinson et al., 1994; Zambonin-Zallone et al., 1995).

In this selective review, the clinicopathological characteristics of GCT will be briefly summarised and current areas of interest in the study of the neoplasm will be presented and discussed. Theories of the histogenesis of GCT, the role of cytokines and steroid hormones in its development and behaviour and its karyotypic profile will be reviewed. The role of the neoplastic cells of GCT in promoting osteoclastogenesis and osteoclastic bone absorption will also be discussed, as this is an area of particular current interest.

Clinicopathological characteristics of GCT

GCTs occur most often in skeletally mature individuals (Huvos, 1991). Over 80% of GCTs are diagnosed in patients over the age of 20, with the third to fifth decades of life being the most commonly affected (Robbins et al., 1994). The incidence of GCT is slightly higher in women than men (Leu et al., 1986; Huvos, 1991; Robbins et al., 1994; Schajowicz, 1994; Unni, 1996), the ratio being 3:2 (Huvos, 1991; Schajowicz, 1994). The vast majority of GCTs arise 'de novo', and are solitary and unifocal. 'Multifocal giant cell tumour of bone' is extremely rare, usually occurs in the epiphyses of long bones at two to three anatomic sites and must be distinguished from the osseus lesions of hyperparathyroidism and malignancies rich in giant cells (Huvos, 1991; Robbins et al., 1994; Schajowicz, 1994). Giant cell tumour of bone has been reported in association with Paget's disease of the skeleton; however, its incidence in this setting is much less common than forms of Pagetic sarcoma such as osteosarcoma or malignant fibrous histocytoma (Rock et al., 1986; Huvos, 1991; Schajowicz, 1994).

The incidence of GCT varies geographically and between races. As shown in Table 1, GCT accounts for 5% of primary bone tumours in North America. The incidence of GCT is somewhat lower in Australia as documented by the Western Australian Bone Tumour Registry. The incidence of GCT is higher in Asian populations, in particular Chinese and Japanese, in whom it accounts for almost 15% of all primary bone neoplasms.

GCT may involve virtually any bone of the skeleton, however, in more than 75% of cases, the tumour is found in the epiphyseal ends of long tubular bones (Schajowicz, 1994; Unni, 1996). The most frequently involved sites are the distal femur, proximal tibia, distal radius and proximal humerus (Schajowicz, 1994). Approximately 50% of GCTs arise around the knee (Robbins et al., 1994; Unni, 1996). Less common, but well documented sites of involvement include the sacrum, pelvis, spine, patella, carpal and tarsal bones (Unni, 1996). Origin in the craniofacial bones is exceptional, but GCTs have been detected in the mandible, maxilla, sphenoid and the petrous temporal bone sometimes in association with Paget's disease (Huvos, 1991; Schajowicz, 1994). Origin in the small bones of the hands and feet is also rare. The clinical signs and symptoms of GCT at presentation usually relate to pain and/or local swelling; pathological fracture occurs but is a much less common form of presentation.

The radiological findings in conventional GCT are often quite characteristic. Typically GCT presents as a lucency, which may or may not be well defined, classically epiphyseal, and is subchondral and eccentrically located. Perilesional or intralesional sclerosis is uncommon and reactive new bone formation at the periphery of the tumour can not usually be appreciated radiologically. Cortical expansion and focal destruction may be observed, sometimes with extension into overlying soft tissues. These latter features form the basis of some radiological grading schemes, but these have not been shown to correlate with clinical outcome (Campanacci et al., 1987). Typically epiphyseal in origin, GCT's can extend to involve the metaphysis, but on rare occasion can be solely metaphyseal in location and not involve the epiphysis (Unni, 1996). The articular cartilage is, however rarely penetrated the neoplasm, even in advanced cases. Joint space involvement is perhaps somewhat more frequent, occurring via lateral cortical erosion (Huvos, 1991; Schajowicz, 1994).

Although there is a significant degree of variability in the macroscopic findings of GCT, most exhibit extensive soft and fleshy appearances, and range in colour from grey to light red to dark, reddish-brown (Fig. 1). The grey areas are generally firmer but are friable (Schajowicz, 1994). Haemorrhagic or cystic areas and focal fibrous septa formation are relatively common (Huvos, 1991). Haemorrhage occurs frequently in some cases, the tumour is partly replaced by large, blood-filled cystic cavities, traversed by thin septa, similar to those seen in Aneurysmal Bone Cyst (ABC). A shell of thin bone often surrounds the neoplasm, but this is not a

Table 1. Incidence of GCT in various countries.

COUNTRY	INCIDENCE	SOURCE
USA	5.12%	Mayo Clinic
Australia	2.5%	Bone Tumour Registry of WA
China	14.6%	Sun Yat-Sen University of Medical Sciences
Japan	8%	Japanese Orthopaedic Association

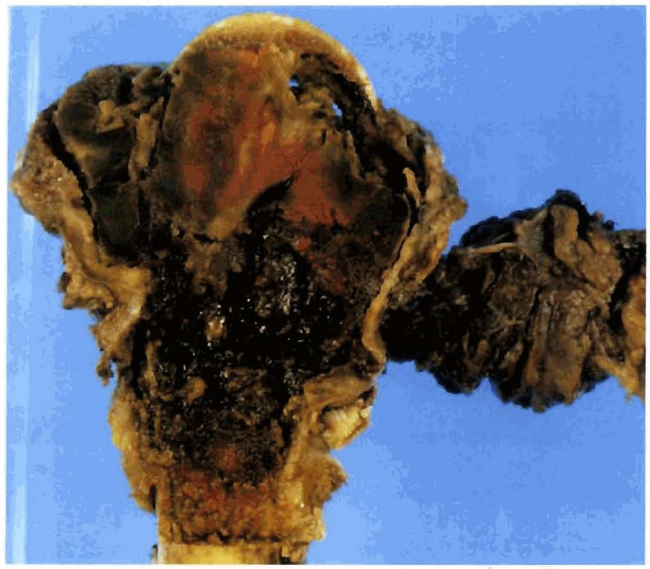

Fig. 1. Gross morphologic features of giant cell tumour of bone. Bisected proximal segment of humerus with extensive soft and fleshy tumour tissue in colour of grey, light red and dark, reddish-brown.

manifestation of the expanded cortex, but is new, displaced cortex formed by osteoclastic resorption of the endosteal surface and persistent deposition of periosteal new bone (Huvos, 1991; Schajowicz, 1994; Unni, 1996). The margins or boundaries of GCT are generally not well defined, which may account for the frequency of local recurrence after curettage.

The classical histological findings of GCT consist of a population of mononuclear cells, plump or spindle in appearance, interspersed amongst which are multinucleate osteoclast-like giant cells. The nuclei of the stromal and multinucleate giant cells are similar in appearance. The stromal cell population may be mitotically active, but no abnormal or atypical mitotic figures are found. Reactive bone may be formed focally, often at the periphery of the lesion, however no chondroid matrix production by the neoplastic population is evident. Secondary reactive changes often observed in GCT include haemorrhage, necrosis, secondary ABC formation, reactive fibrosis and xanthogranulomatous inflammation.

GCT is characterised by the presence of a great number of large, multinucleated giant cells (Huvos, 1991; Robbins et al., 1994; Schajowicz, 1994), uniformly distributed throughout the tumour. Commonly referred to as 'osteoclast-like giant cells', these cells are well defined, with prolongations of their abundant cytoplasm, which is eosinophilic and homogenous, or sometimes, mildly granular (Schajowicz, 1994).

Osteoclast-like giant cells, which resemble osteoclasts morphologically and immunohistochemically, represent a reactive and non-neoplastic population of cells. They are capable of *in vitro* bone resorption and differ from multinucleate histiocytic syncytia of the type found in foreign body type reactions. The nuclei of the multinucleate giant cells in GCT vary in size and number, but usually they possess more than 20 nuclei, sometimes even hundreds (Schajowicz, 1994). The multinucleate giant cells are scattered among a cellular background composed of uniform ovoid or spindle-shaped mononuclear cells that appear to grow in a network or 'syncytial-like' pattern (Robbins et al., 1994; Schajowicz, 1994). They are often found to be related to vascular beds and occasionally, stromal-like tumour cells and giant cells are present within blood vessels. Multinucleated giant cells seen within blood vessels are probably not the result of vascular invasion, but due to in situ formation of osteoclasts by the fusion of blood monocytes that have been activated by tumour cells (Zheng et al., 2000). There is little collagenous stroma in the zones of active tumour growth, but a dense network of reticulin fibrils surrounds individual cells. The vascular pattern of GCTs comprises an abundance of newly developed blood vessels lined by endothelial cells, as well as vascular channels lined by tumour cells. This pattern explains the frequent presence of abundant haemorrhage observed in GCT, with endocytosis of blood pigments in the mononuclear cells (Schajowicz, 1994).

Although multinucleated giant cells are a prominent feature of GCT, their presence is not a sufficient criterion for diagnosing the neoplasm (Huvos, 1991). GCT may be confused with a number of osseous lesions, both reactive and neoplastic, which contain large numbers of multinucleate giant cells. There are a number of reactive processes which may contain considerable numbers of multinucleate giant cells, and equally, a number of benign and malignant osseous neoplasms may be giant cell rich. The differential diagnosis of GCT in different settings may therefore include such diverse processes as giant cell reparative granuloma (Unni, 1996), hyperparathyroidism (Unni, 1996; Huvos, 1991), non-ossifying fibroma, chondroblastoma (Huvos, 1991; Apley and Solomon, 1993; Schajowicz, 1994; Unni, 1996), solid areas of ABC (Roessner, 1989; Huvos, 1991; Apley and Solomon, 1993; Schajowicz, 1994; Unni, 1996), and malignant lesions such as Malignant Fibrous Histiocytoma (MFH) and osteogenic sarcoma (Roessner, 1989; Apley and Solomon, 1993; Schajowicz, 1994). Careful correlation of the microscopic appearances with the clinical and radiological findings is essential to avoid errors in diagnosis.

Biological behaviour of GCT

Although GCT is often considered to represent a "benign" primary osseous neoplasm, it may follow an aggressive and unpredictable clinical course and be difficult to treat effectively (Huvos, 1991; Unni, 1996). A considerable proportion of GCTs are locally aggressive at the time of presentation as judged on clinical and radiological grounds. Radiological evidence of local aggression is manifest by extensive bone destruction, cortical expansion and extension into surrounding soft tissue. Local recurrence is also a significant management problem, with approximately 25 to 35 % of GCTs recurring after simple curettage.

Malignant or sarcomatous transformation is a rare complication of GCT. Sarcomatous change is usually believed to occur in a pre-existing GCT, often following previous irradiation. Examples of primary "*de novo*" malignant giant cell tumour of bone are recognised, but are rare and much less common than secondary malignant change. Histologically, sarcomatous change in GCT is characterised by the identification of sarcomatous tissue associated with conventional GCT. The identification of areas of conventional GCT is critical to this diagnosis, to avoid confusion with other malignancies rich in giant cells. The sarcoma is identified on the basis of the presence of atypical mitotic figures and cytological anaplasia in the spindle cell mononuclear component.

The development of pulmonary metastases is a rare but well documented phenomenon associated with conventional GCT of benign histology. Such pulmonary metastases or implants probably occur in no more than 2% of patients, and must be distinguished from metastases arising from malignant giant cell tumours

(Vanel et al., 1983). The pulmonary metastases of histologically benign conventional GCT demonstrate the same morphology as the primary osseous lesion, and tend to have a relatively favourable prognosis, though this is not invariable and patients may succumb as a result of progressive growth of pulmonary lesions. Although some pulmonary deposits develop after surgical intervention, and may represent seeding of vessels as a result of surgical handling and curettage, others have been identified at the time of presentation and before any form of surgical intervention. In these circumstances, the metastases are possibly related to vascular invasion, a finding evident in a significant proportion of GCTs (Vanel et al., 1983; Wray et al., 1990).

In recognition that GCT may behave in a locally aggressive fashion or recur after surgical treatment, several authors (Jaffe, 1940; Dahlin et al., 1970; Sanerkin, 1980; Fornasier et al., 1996) have attempted to identify histological features which might be predictive of local aggression and/or recurrence. Histological grading schemes however are generally regarded to be of limited value, as the presence of local aggression or recurrence does not appear to be related to any specific histological feature in conventional GCT. Although certain cellular characteristics were identified by histomorphometric study in GCT with more aggressive biological behaviour, this cumbersome tool has not yet applied to the point where it is clinically useful in predicting the biological behaviour of GCT (Fornasier et al., 1996).

Several biological factors have also been evaluated with respect to their prognostic significance. Some investigators have proposed that overexpression of c-myc (Gamberi et al., 1998) and urokinase plasminogen activator (Zheng et al., 1995) may be associated with the behaviour of GCT, but generally attempts to predict the neoplasm's aggressiveness using such parameters have largely been unsuccessful. In search of markers for biological behaviour of GCT, we analysed the gene expression of vascular endothelial growth factor (VEGF) and determined the prognostic value of its expression using Enneking's clinical stage at presentation as a parameter of "clinical/biological aggressiveness". We demonstrated that three isoforms of VEGF (121, 165 and 189) were expressed in GCT but isoform 121 was the most abundant gene transcript (Zheng et al., 2000). Moreover, we have shown that the levels of VEGF gene expression correlated with Enneking's clinical staging. A relatively high level of VEGF was gene expression, as detected by semi-quantitative RT-PCR, was found in stage III GCT than in the stage I/II. VEGF is expressed in spindle-shaped stromal-like tumour cells, round-shaped macrophage-like cells and osteoclast-like multinucleate giant cells. It is possible that the production of VEGF by tumour cells and the induction of neoangiogenesis is part of tumour progression during the evolution of the neoplasm. The frequently incomplete, fenestrated endothelial barrier, in the newly formed tumour blood vessels, not only enables neoplastic cells to enter the circulation, but also assists blood monocytes migrating into the lesion. In fact, it was well appreciated by histological evaluation that GCT tumour cells are often situated within blood vessels (Fig. 2).

Histogenesis of GCT

The precise histogenesis of GCT and the nature of its various cellular constituents have remained a matter of some controversy. Issues related to the histogenesis of GCT include questions such as: 1) do the multinucleate giant cells share a common ancestry with the mononuclear cells? 2) what biological significance can be attached to the co-existence of the various cell types in GCT? 3) what are the determinants of its biological behaviour? 4) does GCT originate from osteoclast lineage or stromal cell lineage?

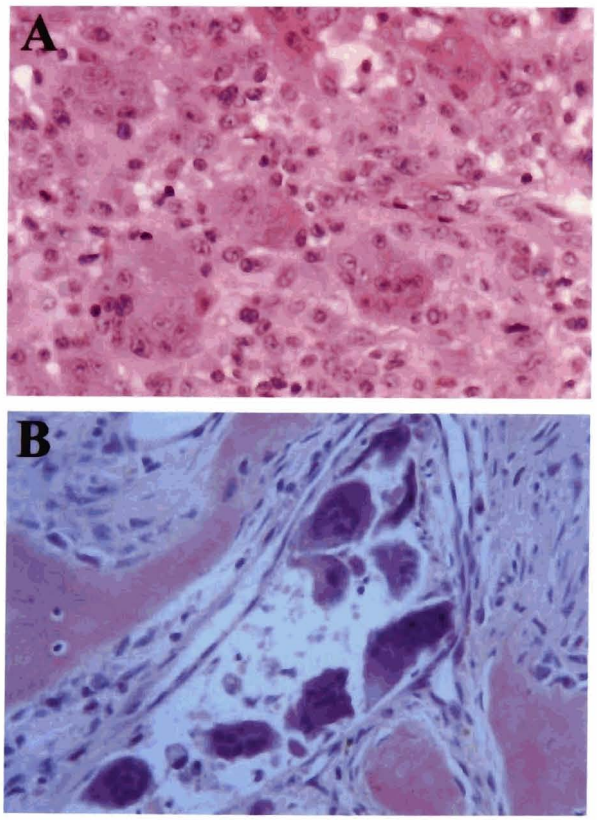

Fig. 2. Photomicrographs of giant cell tumour of bone. **A.** Typical histological features of GCT. Multinuclear giant cells are uniformly distributed throughout mononuclear cells. **B.** A blood vessel area in giant cell tumour of bone. Both stromal-like tumour cells (arrow) and osteoclast-like giant cells (arrow head) are present within blood vessels. Note that the multinucleate giant cells are probably present as the result of in situ formation of osteoclasts by the fusion of blood monocytes which have been activated by tumour cells, rather than the result of vascular invasion. x 400

Identification of cell types in GCT

Although the terms "Giant Cell Tumour" and 'osteoclastoma' may imply that it is the multinucleated giant cells which are responsible for the proliferative capacity of the tumour, there is evidence that the fibroblast-like stromal cells, the major component of the mononuclear cell population, represent the true neoplastic component (Goldring et al., 1987; Doussis et al., 1992; Zheng et al., 1994, 1999). We, and others (Athanasou et al., 1985; Goldring et al., 1987; Feng, 1990; Zheng et al., 1993, 1994), have previously shown that GCT consists of three major cell types. The first population, the spindle-shaped stromal mononuclear cells, proliferate well in culture and are the most likely candidate for the tumour's true neoplastic population. These cells phenotypically resemble connective tissue stromal derivatives, express no macrophage surface antigens, produce collagen types I and III and possess parathyroid hormone receptors (Goldring et al., 1987; Schajowicz, 1994; Zheng et al., 1994). In addition, these cells display cytogenetic aberrations including telomeric fusion, aneuploidy and chromosome deletion (Zheng et al., 1999). The second population of mononuclear cells, so called round-shaped macrophage-like cells, do not persist in long term culture and express monocyte-macrophage markers, such as CD68 antigen (Goldring et al., 1987; Schajowicz, 1994; Zheng et al., 1994). These macrophage-like cells account for 30% of the total cell population of GCT (Zheng et al., 1998). The third population of cells, the multinucleate giant cells, possess features of osteoclasts. These cells express calcitonin receptors, carbonic anhydrase II, tartrate-resistant acid phosphatase and CD 68 antigen (Nicholson et al., 1987; Zheng et al., 1993, 1995, 1998) and are capable of resorbing bone *in vitro* (Zheng et al., 1993). These multinucleate cells, although detectable in early cultures, disappear after the first passage (Zheng et al., 1994).

Ultrastructural studies have also confirmed that three distinct cell types exist in GCT (Steiner et al., 1972; Aparisi et al., 1977; Zheng et al., 1995). The mononuclear spindle-shaped tumour cells resemble fibroblastic cells at the ultrastructural level. They possess accentuated nuclear chromatin clumps along the nuclear envelope whereas the cytoplasm contains prominent endoplasmic reticulum, free ribosomes, and irregularly shaped mitochondria. Collagen fibres are often seen in the vicinity of these cells (Fig. 3). The ultrastructural features of the round mononuclear cells are similar to those of macrophages. The nuclei are oval with low

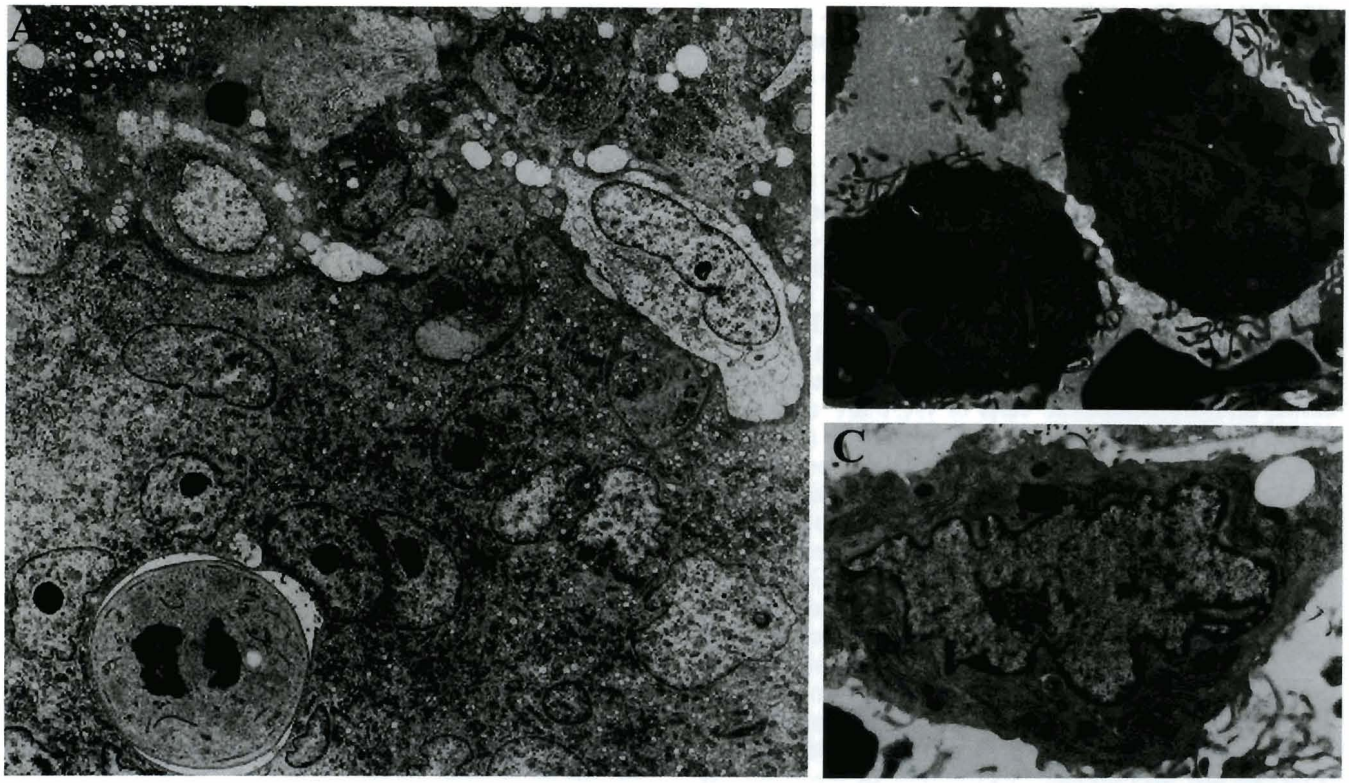

Fig. 3. Identification of cell types in giant cell tumour of bone. **A.** Ultrastructure of osteoclast-like giant cells. There are abundant mitochondria, vacuoles and lysosome-like bodies in the cytoplasm. x 6,500. **B.** Ultrastructure of round macrophage-like cells. The nucleus contains low density chromatin. There are numerous lysosomes, endoplasmic reticulums and free ribosomes. x 5,000. **C.** Ultrastructure of spindle-shaped stromal-like tumour cells. There is much heterochromatin along the nuclear membrane. The cytoplasm has membranes of granular endoplasmic reticulum and abundant free ribosomes. Collagen fibers are seen in the vicinity of cells. x 4,800

chromatin density. The cytoplasm is rich in electron dense lysosomes, vesicles, well developed endoplasmic reticulum, Golgi apparatus, and free ribosomes (Fig. 3). The multinucleate giant cells appear morphologically similar to osteoclasts. They contain numerous nuclei of mostly oval shape. There are abundant mitochondria, distinct lysosome-like bodies, and large vacuoles in the cytoplasm (Fig. 3). In some instances, ruffled borders formed by finger-like projections of the cytoplasm have been seen.

Multiple lines of evidence indicate that the fusion of round shaped macrophage-like cells (or their precursors) result in the formation of multinucleate osteoclast-like giant cells (Athanasou et al., 1985; Goldring et al., 1987; Zheng et al., 1993, 1994, 1998; Huang et al., 2000). In spite of the fact that the multinucleate giant cells are considered to be reactive osteoclasts, their morphological features, in particular their degree of multinucleation, differ somewhat from those of osteoclasts in normal bone. One possible explanation for this is that these syncytia are not found or formed in a normal bone microenvironment. Instead, as a result of sustained signalling of the rich cytokine environment generated by tumour cells, processes of cell fusion are enhanced resulting in the formation of large multinucleate giant cells. In fact, large multinucleate giant osteoclasts could always be observed in the case of normal osteoclast culture systems such as cultures of bone marrow cells (Suda et al., 1996), spleen cells or macrophage cell line RAW cells (Huang et al., 2000).

The role of cytokines in GCT

Osteolysis of cortical and trabecular bone within the lesion is undeniable evidence of a resorptive process that is mediated by the multinucleate osteoclast-like giant cells of GCT, but the mechanism by which this is achieved is not understood. It appears that the neoplastic cells (spindle-shaped stromal cells) of GCT produce cytokines that promote osteoclastogenesis and osteoclastic bone resorption. Our previous studies have shown that the neoplastic cells of GCT are capable of producing transforming growth factor-ß1 (TGF-ß1) which stimulates recruitment of reactive osteoclasts (Zheng et al., 1994). The osteoclast-like multinucleated giant cells express TGF-ß type II receptor, further supporting a paracrine mechanism. Conditioned medium from tumour cell cultures established from GCTs have been shown to chemoattract osteoclasts and tartrate-resistant acid phosphatase-positive mononuclear cells (Zheng et al., 1994). Interestingly, administration of monoclonal antibodies against TGF-ß1 only partly abolishes the chemotactic activity of the conditioned medium (Zheng et al., 1994). In search of other relevant cytokines that may enhance recruitment of reactive cells (especially those of $CD68^+$ macrophage/osteoclast lineage) in GCT, we evaluated the presence of MCP-1 gene transcript and protein in GCT and demonstrated that both MCP-1 gene transcript and MCP-1 protein were present in the cytoplasm of the stromal-like tumour cells of GCT (Zheng et al., 1998). Conditioned media from GCT cultures promoted the chemotactic migration of $CD68^+$-peripheral monocytes, an activity which was abolished by the addition of MCP-1 antibody to the medium. Studies by others have also demonstrated that tumour cells of GCT produce TNF, interleukins-1, -6, -11, -17, and -18, M-CSF and PTHrP (Chambers et al., 1985; Sasaguri et al., 1992; Mills et al., 1997; Atkins et al., 2000). In short, neoplastic cells of GCT are capable of recruiting monocytes, osteoclast precursor cells and even osteoclasts through the generation of various cytokines.

It is noteworthy that although individual cytokines have direct and/or indirect role in the recruitment of osteoclasts and in osteoclast formation and bone resorption, none of these factors have been shown to directly induce macrophage-like cells to differentiate into osteoclasts. In fact, cells in other bone neoplasms such as osteosarcoma also express a similar cytokine profile (Andersen et al., 1998; Franchi et al., 1998; Mariani et al., 1998) as do those in GCT, but in these tumours much reduced degrees of osteoclast formation are observed. In search of other factors produced by the GCT neoplastic cells which may have the ability to generate osteoclast-like giant cells, possibly from recruited macrophage-like cells, we have identified that the ligand for receptor activator of NF-κB (RANKL), also known as osteoclast differentiation factor (ODF) (Yasuda et al., 1998), osteoprotegerin ligand (OPGL) (Lacey et al., 1998), and TNF-related activation-induced cytokine (TRANCE) (Anderson et al., 1997; Fuller et al., 1998; Yasuda et al., 1998), as the factor necessary for the formation of the multinuclear osteoclast-like giant cells (Huang et al., 2000).

RANKL is a member of the membrane-associated TNF-ligand family which directly regulates osteoclast differentiation and bone resorption (Anderson et al., 1997; Simonet et al., 1997; Tsuda et al., 1997; Lacey et al., 1998; Yasuda et al., 1998). It has been shown, using *in vitro* culture system, that RANKL can both induce osteoclastogenesis and activate mature osteoclasts (Lacey et al., 1998; Yasuda et al., 1998). The expression of RANKL in osteoblast/stromal cells coincides with the formation of osteoclasts in co-cultures with bone marrow or spleen cell population. However, recombinant RANKL can replace the requirements for stromal cells in any *in vitro* model of osteoclastogenesis (Lacey et al., 1998). Mice with a disrupted *opgl* gene completely lack osteoclasts as a result of the inability of osteoblasts to support osteoclastogenesis (Kong et al., 1999). The cell surface receptor that interacts with RANKL has been shown to be the ligand for the TNFR-related protein receptor activator of NF-κB (RANK) (Hsu et al., 1999). On the other hand, the decoy receptor of RANKL, OPG, also known as osteoclastogenesis inhibitory factor (OCIF) (Tsuda et al., 1997), has been shown to neutralize and interrupt stromal cell-derived RANKL-signals resulting in reduction of osteoclastogenesis

(Simonet et al., 1997; Tsuda et al., 1997). Interestingly, OPG is a soluble member of the TNF-receptor family.

We have shown that RANKL is abundantly expressed in stromal-like neoplastic cells of GCT whereas RANK, the receptor for RANKL, is expressed in macrophage-like cells and multinucleate osteoclast-like giant cells. Furthermore, the neoplastic cells of GCT are able to induce differentiation of RANK expressed myeloid $RAW_{264.7}$ cells into osteoclast-like cells in the presence of $1,25(OH)_2D_3$ and dexamethasone (Huang et al., 2000). Thus, these findings strongly suggest that RANKL acts as an osteoclastogenesis-inducing factor linked to interaction between stromal like tumour cells and osteoclast progenitors in GCT. To summarise, GCT is a neoplasm of connective tissue stromal cells, which have the ability to recruit macrophage and multinucleate osteoclast-like giant cells.

The role of steroid hormones in giant cell tumour of bone

Given that steroid hormones play an important role in the maintenance of mineral homeostasis in bone through direct and indirect actions on osteoblasts and osteoclasts, it is reasonable to assume that these hormones have some effect on the development and progression of GCT. Indeed, several lines of clinical evidence suggest that the growth of GCT may be associated with steroid hormones. First, GCT has a marked female predilection in spite of the fact that most of the primary bone tumours are more common in males. Second, GCT has been reported to grow dramatically during pregnancy (Goldring et al., 1986). In particular, GCT in the pelvic bones may demonstrate unexpected and rapid growth during pregnancy (Goldring et al., 1986; Komiya et al., 1999). Thirdly, GCTs have been shown to have a good therapeutic response to the administration of dexamethasone (Ziambaras et al., 1997). It is noteworthy that the majority of these GCT patients who received dexamethasone therapy also had Paget's disease of bone (Jacobs et al., 1979; Potter et al., 1991; Kim et al., 1997; Ziambaras et al., 1997). In a total of six cases reported in the literatures, administration of short-term, high-dose dexamethasone rapidly reduced the size of these tumours. However, discontinuation of the steroid treatment led to re-growth of the tumours in five of the six cases reported.

In recognition of the role steroid hormones may play in the histogenesis of GCT, we and others have conducted a series of studies for the detection of receptors for oestrogen, glucocorticoid and vitamin D_3 using *in-situ* hybridisation and RT-PCR techniques (Collier et al., 1998; Huang et al., 1999). It was found that both ERα and ERß mRNA are expressed in GCT and primary cultures of the neoplasm. ERß mRNA expression in GCT was negligible compared with osteosarcoma, suggesting that oestrogen receptor α (ER-α) is predominantly expressed. *In-situ* hybridisation further confirmed that ER-α gene transcript is expressed in stromal-like tumour cells and macrophage-like cells but not in osteoclast-like giant cells. However, other investigators have indicated that it is the osteoclasts but not the neoplasmic cells of GCT that express ER receptors (Oursler et al., 1994). The inconsistency may be due to the purification of cells for RT-PCR analysis. The latter has used 90-95% pure preparation of osteoclast-like giant cells which may contain some tumour cells in the assay. In addition, nuclear binding

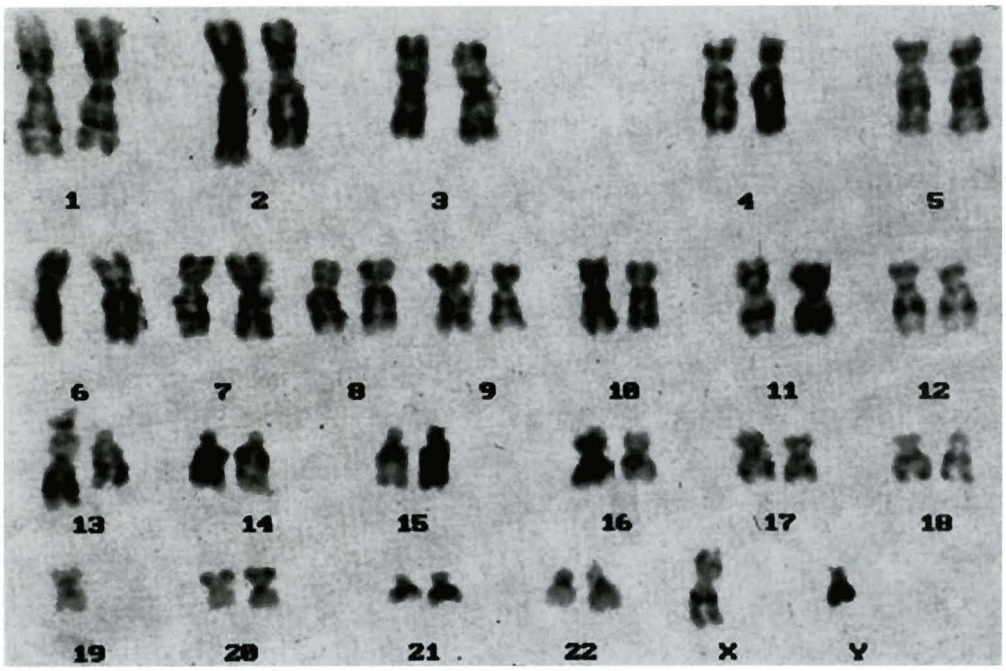

Fig. 4. Representative karyotype of spindle-shaped mononuclear cells in giant cell tumour of bone. Telomeric fusion is observed between chromosomes 13p and 19q. (From: Zheng et al. Telomeric fusion is a major cytogenetic aberration of giant cell tumors of bone. Pathology 1999, 31, 373-378)

assay for oestrogen receptor showed that only one out of eleven cases of GCT have detectable oestrogen receptors in the solid tumour tissue (Wold et al., 1989). On the other hand, progesterone receptor proteins have been detected in GCT (Malawer et al., 1984). Taken together, GCT expresses both ER-α and progesterone receptors. Both clinical and laboratory evidence suggests that the tumour cells but not osteoclast-like giant cells are oestrogen target cells.

We have also demonstrated that glucocorticoid receptor (GR) α and ß, and Vitamin D_3 receptor are expressed in GCT (Huang et al., unpublished data; Huang et al., 1999). The three major cell types seen in GCT i.e. stromal-like tumour cells, macrophage-like cell types and osteoclast-like giant cells all expressed both GRα and GRß as evidenced by *in-situ* hybridisation. Administration of dexamethasone stimulated the formation of osteoclast-like cells in GCT in the presence of 1,25 $(OH)_2D_3$. Furthermore, dexamethasone induced the production of RANKL but suppressed OPG in stromal-like neoplasmic cells of GCT (Huang et al., 2000).

Cytogenetics of GCT

Various chromosomal aberrations have been reported in GCTs, including telomeric fusion, ring formation, translocations, marker chromosomes, deletions, double minutes, aneuploidy, polyploidy, telomeric reduction, chromosome and chromatid breaks, acentric chromosomes, dysenteric chromosomes, inversions, and premature chromatid separations (Noguera et al., 1989; Bridge et al., 1990, 1992; Schwartz et al., 1993, 1995; McComb et al., 1996; Zheng et al., 1999); however, the most consistently observed chromosome aberration in GCT is telomeric fusion (Fig. 4) (Bridge et al., 1992; Zheng et al., 1999).

Telomeric fusion of specific chromosomes is a rare event involving the end-to-end joining of the ends of 2 or more chromosomes, without evidence or visible loss of genetic material from either of the chromosomes involved (Schwartz et al., 1995; McComb et al., 1996; Bridge et al., 1993; Harrison et al., 1994; Sawyer et al., 1993; Pandita et al., 1996; Vagner-Capodano et al., 1992; Kuwano et al., 1992; Meltzer et al., 1993; Schmitt et al., 1994). Telomeres are specialised structures, comprised of DNA and protein, capping the ends of eukaryotic chromosomes (Strachan and Read, 1996). Telomeres serve several important functions. Telomeres maintain the structural integrity of a chromosome, preventing fusions, recombinations and degradations. Telomeres, through the activity of telomerase, also ensure the complete replication of the extreme ends of chromosome termini during cell division.

Cytogenetics analyses of GCT (Bridge et al., 1992) have demonstrated that random chromosomal abnormalities were detected in 6 of the 10 tumours shown to be locally aggressive, recurrent or metastatic, and all but 1 of the 20 analysed yielded chromosomal abnormalities. Telomeric fusion, an unusual event, was the most striking random chromosomal aberration observed (Bridge et al., 1990). Telomeric fusion was observed in 14 of the specimens analysed, i.e. 70%, suggestive of a strong relationship between GCT and telomeric fusion. The most frequently involved telomeres were those on 11p, 15p, 19q, 21p, 18p, 13p and 20q, in decreasing order of frequency (Bridge et al., 1990, 1992). Our studies, however showed that the acrocentric chromosomes, especially chromosomes 13, 14, and 21, were most commonly involved in telomeric fusion. Acrocentric chromosomes consist of very short p arms of indiscriminate length, comprised mainly of heterochromatin and possess satellites at the tips of the p arms (Rooney and Czepulkowski, 1992). The short arm almost appears to be an extension of the centromeric region and contains many short sequences of repetitive DNA (Rooney and Czepulkowski, 1992), which are susceptible to cytogenetic damage. It was the p arms of these acrocentric chromosomes which were most frequently fused (as was the case for all chromosomes). The one exception was chromosome 19, which was also commonly fused (Bridge et al., 1992), especially with chromosome 14. Interestingly, acrocentric chromosomes were often fused with other acrocentric chromosomes. Fusion of chromosomes 13 and 21 at the p arms was a frequent event, as was fusion of chromosomes 14 at the p arms, and chromosomes 15 and 21, also at the p arms (Bridge et al., 1992). The p arms of acrocentric chromosomes are generally more unstable and vulnerable to aberration than other chromosomes. This may be due to the short p arm having relatively small telomeres but possessing of satellites, which are commonly found to associate with the satellites of acrocentrics. Thus, these chromosomes may be more

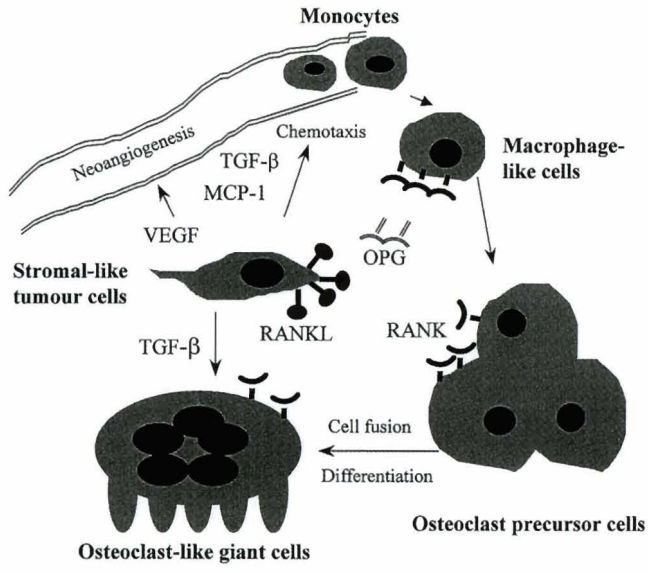

Fig. 5. Hypothetical model of the mechanism of histogenesis and the biological behaviour of GCT.

susceptible to damage, such as telomeric fusion.

Given telomeric fusion would imply a degree of genetic instability, such as that witnessed in many malignant neoplasms in which non-random telomeric fusion has been reported (Apley and Solomon, 1993), it may then serve as a potential precursor lesion to other abnormalities, such as translocations, aneuploidy and ring formation (Bridge et al., 1990; McComb et al., 1996). Thus, telomeric fusion is considered to be the major cytogenetic alteration of GCT (Zheng et al., 1999). It is not yet clear why telomeric fusions occur, or why some chromosomes are selectively affected in certain neoplasms. The prevalence of telomeric fusion in GCT suggests they may be a consequence of genomic instability induced during the neoplastic process (Apley and Solomon, 1993; Schwartz et al., 1995; Pandita et al., 1996).

Summary and Conclusion

In conclusion, GCT is a primary benign neoplasm that produces expansile well delineated lytic lesions. The evidence indicates that it is a neoplasm of connective tissue stromal cells, which have the ability to recruit macrophage and multinucleate osteoclast-like giant cells. The neoplastic cells of GCT have various chromosomal aberrations. The most consistently observed chromosome aberration was shown to be telomeric fusion. Perhaps dysfunctional telomeres may be crucial in GCT oncogenesis. A hypothetical model on the mechanism of histogenesis and the biological behaviour of GCT is presented in Fig. 5. As illustrated in the diagram, stromal-like tumour cells produce VEGF during progression of the neoplasm. This leads to the formation of incomplete vascular barriers (during angiogenesis) which provide a source of monocytes from the circulation. The latter are then recruited into the lesion by the local production of MCP-1 and TGF-ß from the neoplastic cells. The tumour cells further stimulate the monocyte/macrophage toward the formation of osteoclast-like giant cells by the production of RANKL. Thus, three major cell types, the stromal-like neoplasmic cells, the macrophage-like cells and the multinuclear osteoclast-like giant cells all interact to form the lesion that characterises GCT of bone.

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