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Gene Expression of Transforming Growth Factor-β1 and Its Type II Receptor in Giant Cell Tumors of Bone

Possible Involvement in Osteoclast-Like Cell Migration

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Giant cell tumor of bone (GCT) is a relatively rare skeletal neoplasm characterized by multinuclear giant cells (osteoclast-like cells) scattered in a mass of mononuclear cells. The currently favored hypothesis for the origin of cells within GCT is that the multinuclear giant cells are reactive osteoclasts, whereas the truly neoplastic cells are the major component of the mononuclear population. However, the pathological significance and the precise relationship of tumor cells and osteoclast-like cells in GCT have not been fully established. In this study, we evaluated two GCTs for the presence of transforming growth factor-β1 (TGF-β1) and TGF-β type II receptor gene transcripts and attempted to establish a possible role for TGF-β1 in the interaction between tumor cells and osteoclast-like cells. By using in situ hybridization and Northern blot analysis, we have demonstrated that TGF-β1 mRNA transcript is consistently detected in both tumor mononuclear cells and osteoclast-like cells, whereas TGF-β type II receptor gene transcript is only present in osteoclast-like cells. Moreover, isolated rat osteoclasts were tested for their ability to migrate in response to GCT-conditioned medium (GCTCM) in an in vitro chemotactic assay. Our results showed that GCTCM stimulates the migration of osteoclasts in a dose-dependent manner. Interestingly, only osteoclasts containing less than three nuclei can migrate through 12-μ pore filters. Addition of monoclonal antibody against TGF-β significantly reduced but did not abolish the chemotactic activity of GCTCM. Moreover, TGF-β type II receptor mRNA has been demonstrated in the normal rat osteoclasts and may be involved in the chemotactic action of TGF-β1. We concluded that TGF-β1, possibly in concert with other cytokines, is involved in the recruitment of osteoclast-like cells in GCT by acting in an autocrine or paracrine fashion. (Am J Pathol 1994, 145:1095–1104)

Giant cell tumor of bone (GCT) is a relatively uncommon primary skeletal neoplasm occurring in patients who are generally more than 20 years of age. Histologically, GCT is characterized by multinuclear giant cells scattered among a mass of mononuclear cells. The histogenesis of these two cellular components, however, is controversial. The multinuclear giant cells have been thought to arise by fusion of undifferentiated mononuclear mesenchymal (stromal) cells,[1,2] a distinct phenotypic subset of bone marrow-derived histiocytes,[3,4] osteoclast precursor cells,[5–11] or cells of the monocyte-macrophage lineage.[12–14] The mononuclear cells have been considered to arise from osteoblasts,[15,16] histiocytes,[17,18] a monocyte-macrophage lineage,[4,19] or bone marrow mesenchymal (stromal) cells.[6,10,16,20–22] The currently favored hypothesis[5–11,16,20–22] for the cellular origin of GCT is that the majority of mononuclear cells in GCT that phenotypically resemble bone marrow mononuclear

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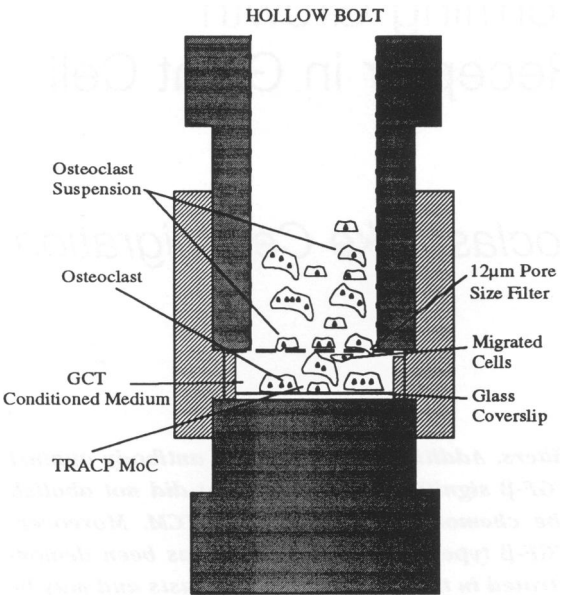

Figure 1. *Model of the microchamber used for the chemotactic assays.*

mesenchymal (stromal) cells are tumor cells, whereas a minor population of mononuclear cells are reactive macrophages and/or osteoclast precursor cells. On the other hand, the multinuclear giant cells in the GCT (the so-called osteoclast-like cells) have been considered to be, indeed, reactive osteoclasts. In support of this hypothesis, it has been shown that these multinuclear giant cells possess calcitonin and vitronectin receptors,[6,23] express osteoclastic bone-resorbing enzymes such as carbonic anhydrase II[24,25] and tartrate-resistant acid phosphatase (TRACP),[24,25] display conspicuous ruffled border and clear zone,[26] and are capable of excavating resorption lacunae on devitalized bone *in vitro*.[24,26,27] However, it remains unclear why there is an abundance of multinuclear giant cells in GCT or if any significance can be attached to the co-existence of these cell types in the neoplasm.

Transforming growth factor-β1 (TGF-β1) is a prominent component in extracellular bone matrix.[28] It has been reported that bone marrow monocytes and stromal cells as well as osteoblasts produce TGF-β1 in abundance.[29] It acts as a potent chemotactic ligand for monocytes,[30] fibroblasts,[31,32] and osteoblasts[33] and stimulates the production of extracellular matrix proteins such as collagen and fibronectin as well as the receptors for these matrix proteins.[34] TGF-β1 has also been implicated in a number of pathological conditions including neoplasms. For example, TGF-β1 and other isoforms have been detected in small round cell tumors (these are of either mesenchymal or neuroectodermal origin and include Ewing's sarcoma).[35] The precise role of TGF-β1 in neoplasms, however, is unknown. It has been suggested that TGF-β1 may be associated with promotion of tumor growth and suppression of differentiation of tumor cells.[35]

In this study, we hypothesized that the mononuclear cells (the neoplastic component) of GCT produce cytokines that attract osteoclasts to the neoplastic lesion. Because TGF-β1 is produced by various components of bone and is chemotactic for several cell types,[30–33] we proceeded to evaluate GCT for the presence of TGF-β1 and its type II receptor gene transcripts and attempted to establish a possible role of TGF-β1 in the interaction between tumor cells and the osteoclast-like cells in GCT by seeking evidence for osteoclast chemotactic activity in GCT-culture fluids.

Materials and Methods

Case Reports

Two cases of GCT were studied. Case 1 was a 37-year-old man who sustained an undisplaced pathological fracture at the proximal end of the right humerus. A roentgenogram showed a lytic lesion located in the area of the humeral tuberosities measuring approximately 4 × 4 cm. An excision biopsy was conducted and the diagnosis of GCT was confirmed. Case 2 was a 20-year-old female with a 10-month history of right upper leg pain and swelling in the posterolateral aspect of the right knee of 1 month's duration. A roentgenogram showed a 4 × 2.7 cm lytic area at the lateral aspect of the right tibia. An excision biopsy was conducted and the diagnosis of GCT confirmed. The tumor specimens were collected at the time of surgery.

Imprint and Culture of GCT

The tumor tissues (8 mm^3) were placed in cold Hanks' balanced salt solution and then imprinted with minimal pressure onto diethyl pyrocarbonate (DEPC)-treated siliconized glass slides. The slides were allowed to dry in air for 30 minutes at 4 C. For culture, the tumor tissues were freshly chopped in Dulbecco's minimum essential medium (DMEM) containing 10% fetal calf serum (FCS) and 100 U/ml penicillin and 100 μg/ml streptomycin. The resultant cell suspension together with the small tissue fragments was transferred to 25-cm^2 flasks for culture at 37 C in 5% CO_2 and 95% air.[16,22] The small tissue fragments contained

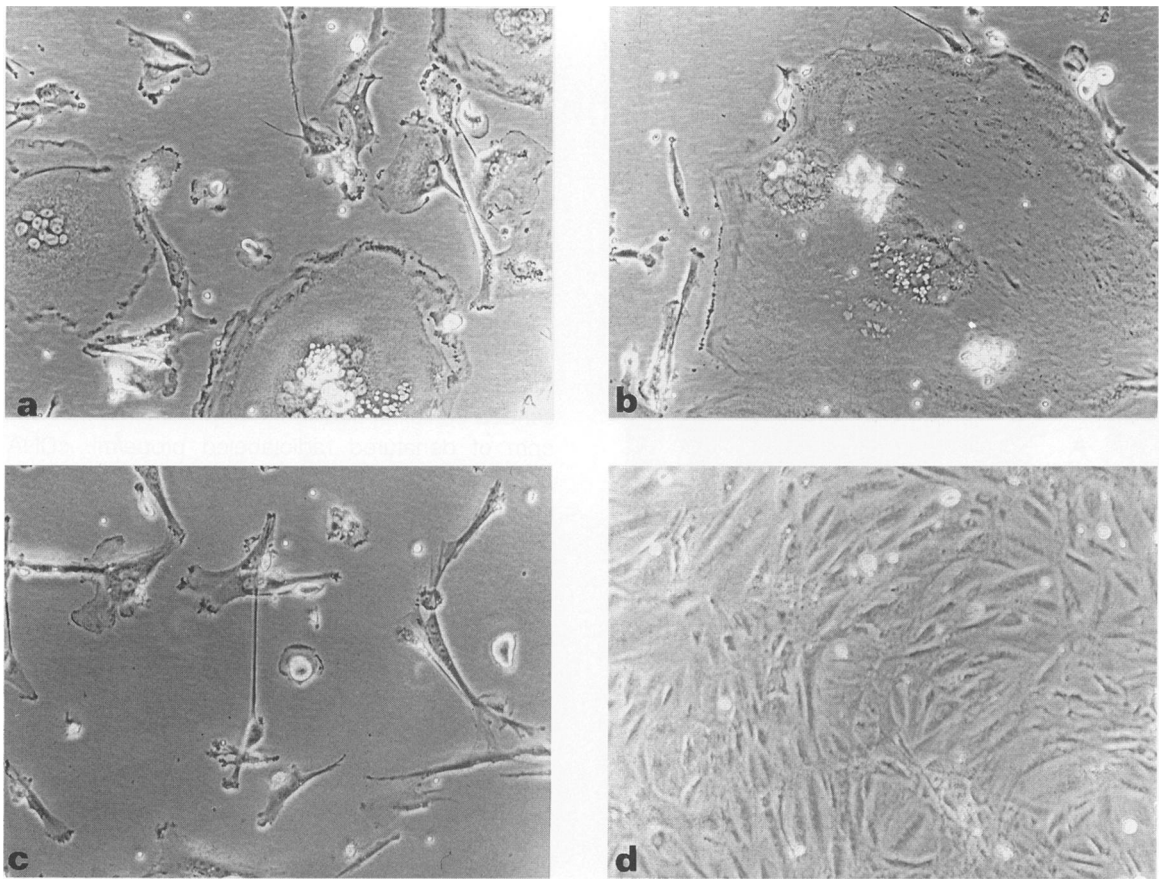

Figure 2. *Morphology of cultured GCT from case 2.* a: *The primary GCT culture composed of multinuclear giant cells, spindle-shaped stromal cells, and macrophage-like cells.* b: *After 5 days in culture, multinuclear giant cells were swollen and their nuclei had aggregated into small groups.* c: *GCT culture after the third passage, spindle-shaped stromal cells, and a few macrophage-like cells are observed but no multinuclear giant cells seen in the culture.* d: *GCT at the eighth passage, only spindle-shaped stromal cells remain.*

many multinuclear giant cells and mononuclear cells that migrated into the culture dishes and contributed to the cell population in cultures and therefore were not removed. Half the culture media was changed every 3 days with fresh DMEM containing 10% FCS and 100 U/ml penicillin and 100 μg/ml streptomycin.

Rat Osteoclasts Preparation

Osteoclasts were isolated from 1-day-old Sprague-Dawley rats as previously described.[36] Long bones were dissected free of soft tissue and chopped (6 bones/ml) in medium 199 (ICN, Sydney, Australia). Cell suspensions were pipetted into the wells of chamber slides (Lab-Tek, Sydney, Australia). After incubation at 37 C for 30 minutes the cultures were rinsed with minimum essential medium (MEM) to remove nonadherent cells. Osteoclast cultures were then incubated for a further 30 minutes in 95% air/5% CO_2 with MEM before experimentation.

Bone Resorption Pit Assay

Devitalized bovine femoral bones were sliced with a low-speed saw. The slices (3 × 3 × 0.2 mm) were washed with distilled water, dried, and sterilized by overnight exposure to ultraviolet light. Multinuclear giant cell-rich cell suspensions were prepared by using the method previously described,[24] placed on bovine bone slices in a 96-well plate (100 μl suspension in each well), and incubated for 60 minutes at 37 C. The cultures were washed to remove less adherent cells and then incubated in MEM containing 10% FCS for 24 hours. The cells on the bone slices were reacted with TRACP to detect and visualize osteoclast-like cells,[24] which were then removed by using an ultrasonifer to enable examination of the bone slice sur-

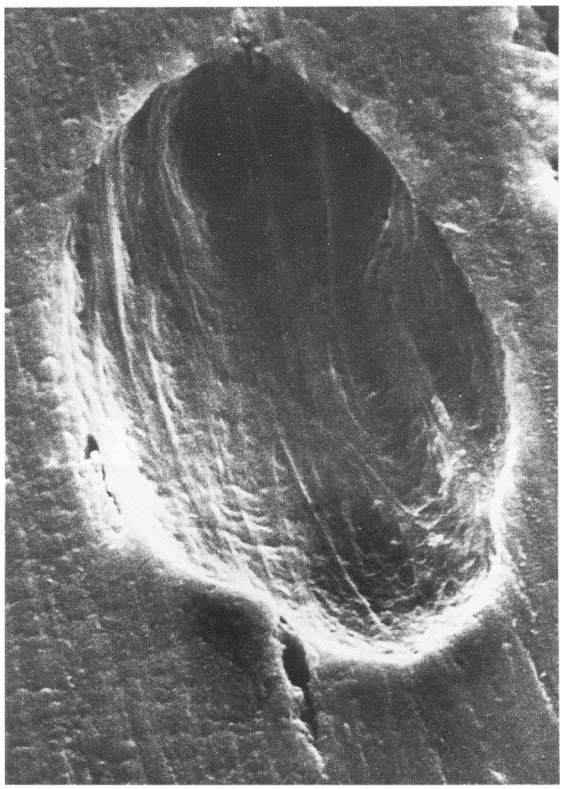

Figure 3. *Resorbing pits excavated by multinuclear giant cells (osteoclast-like cells). The cells prepared from GCT were incubated on a bovine bone slice in DMEM containing 10% FCS for 24 hours. The surfaces of the bone slices were viewed by a scanning electron microscopy and excavations detected (×6400).*

face. The latter was air-dried, sputter coated with gold, and examined on a scanning electron microscope (Philips). Evidence of osteoclastic bone resorption was accepted if distinct excavations in the bone slice surface were observed.

In Situ *Hybridization*

The preparation of TGF-β1 (ATCC, Rockville, MD) and TGF-β type II receptor (Dr. Weinberg, The Whitehead Institute, Cambridge, MA) cDNA probes was performed by the standard procedures described by Sambrook et al.[37] Inserts of TGF-β1 (2.1 kb) and TGF-β type II receptor (4.5 kb) were labeled with digoxigenin using a DNA labeling and detection kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. *In situ* hybridization was performed using a previously described method.[24] The hybridization solution consisted of 45% deionized formamide, 10% dextran sulfate, fivefold standard saline citrate (SSC), and 1 mg/ml of denatured and sonicated salmon sperm DNA.

Northern Blot Analysis

Total cellular RNA was isolated by the RNAzol method (Biotecx, Houston TX) from solid tumors and from the tumor cultures prepared from the second patient's GCT (9th passage). Twenty-five micrograms of total cellular RNA from each extraction was fractionated on 1% agarose gels and transferred to nylon filters (Hybond N+; Amersham, Arlington Heights, IL) by capillary blotting. Filters were prehybridized for 2 hours at 42 C in a buffer containing 50% formamide and fivefold Denhardt's solution. Hybridization was conducted in a buffer containing all the ingredients described earlier, in addition to approximately 1×10^6 cpm of denatured radiolabeled probe/ml. cDNA probes for TGF-β1 and TGF-β type II receptor were labeled with [α-^{32}P]dCTP (3000 ci/mmol; Amersham) using a random primer kit (Amersham). Filters were washed with 2× SSPE/0.1% sodium dodecyl sulfate (SDS) at room temperature, 1× SSPE/0.1% SDS, and 0.1× SSPE/0.1% SDS at 65 C in sequence. The filters were then exposed to X-ray films at −70 C in cassettes using intensifying screens.

Osteoclast Chemotactic Assay

The conditioned medium for osteoclast chemotactic assay was collected from cultured GCT during the eighth passage and filtered through 2.5-μ filters. The cells at a density of 10^4 cells/ml were cultured in DMEM containing 5% FCS for 3 days before the collection of GCT-conditioned medium (GCTCM). The conditioned medium was stored at −70 C and used for the chemotactic assay within 1 week. Osteoclast suspensions containing both osteoclasts and TRACP-positive mononuclear cells were so adjusted that each suspension contained the osteoclasts that could be extracted from 6 long bones/ml of 199 medium with 5% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Assay of chemotaxis was conducted by using the special microchambers as previous described.[38] Briefly, osteoclasts were allowed to settle for 40 minutes at 37 C onto a polycarbonate filter with 12-μ pores within the upper well of the microchamber. The GCTCM was diluted in medium 199 (1:10, 1:50, and 1:100) and placed in the lower well of the microchamber, which was separated from the upper well by a 12-μ filter. Inside the lower well a glass coverslip (13 mm) was placed on the bottom (Figure 1).

For negative controls, medium 199 with DMEM (50:1) in the presence of 5% FCS was placed in the lower well. TGF-β1 monoclonal antibody (Genzyme, Boston, MA) in dilutions of 30 and 60 μg/ml was

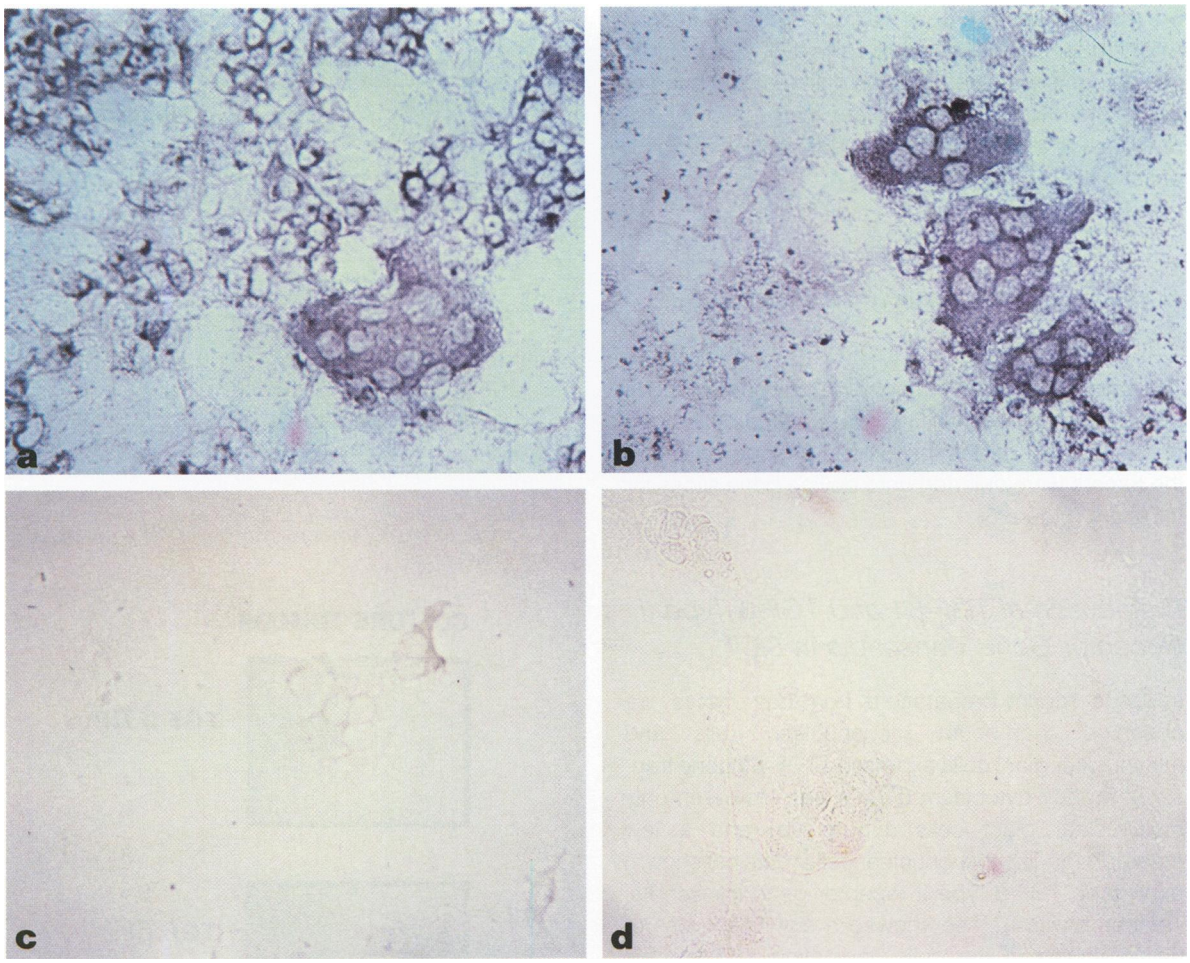

Figure 4. *Location of TGF-β1 and TGF-β type II receptor gene transcripts in tissue imprint preparation of case 1 GCT.* **a:** *TGF-β1 mRNA are observed in the cytoplasm of both mononuclear cells and multinuclear giant cells.* **b:** *TGF-β type II receptor mRNA is seen in multinuclear giant cells.* **c:** *TGF-β1 mRNA disappeared when the cells were incubated with 100 μg/ml of RNase before hybridization.* **d:** *TGF-β type II receptor mRNA disappeared when the cells were incubated with 100 μg/ml of RNase before hybridization* (×250).

added into the lower wells of microchambers. Approximately 20 μg/ml of the antibody neutralized 0.5 ng/ml of total TGF-β as indicated in the kit. All tests were performed in triplicate. After incubation for 40 minutes at 37 C the coverslips were removed from the bottom wells and stained with TRACP as previously described.[24] TRACP-positive multinuclear osteoclasts that had migrated through the filters and attached to the coverslips were then enumerated under light microscopy. Only very few osteoclasts were found on the bottom side of the filter. Chemotactic activity for osteoclasts was defined as the total number of these cells per coverslip. Chemotactic activity for TRACP-positive mononuclear cells was defined as the number of these cells per 25 × objective field. Student's *t*-test was used to determine the significant differences.

Results

Morphological Observation of Cultures of GCT

Adherent cells in primary culture of GCT included mononuclear cells and multinuclear giant cells. The majority of mononuclear cells were spindle shaped but a small portion displayed a rounded epithelioid appearance and were considered to be macrophages. Most multinuclear giant cells displayed a round "fried-egg" appearance with centrally located nuclei and a wide, thin peripheral rim of cytoplasm (Figure 2a). After 3 to 5 days' culture, multinuclear giant cells were swollen, whereas their nuclei had aggregated into small groups (Figure 2b). Subsequently, multinuclear giant cells could not be found

but macrophage-like cells still remained (Figure 2c). After the third passage, only spindle-shaped mononuclear cells remained and the cultures continued to proliferate. Figure 2d shows the morphological appearance of these spindle-shaped cells at the eighth passage.

Bone-Resorbing Activity of Multinuclear Giant Cells of GCT

The bone resorption capacity of multinuclear giant cells from both cases of GCT was verified by the production of distinct resorption pits when the cells from the tumors were cultured onto bovine bone slices for 24 hours (Figure 3).

Distribution of TGF-β1 and TGF-β Type II Receptor Gene Transcripts in GCT

In tissue imprint preparations from both cases, we observed that both mononuclear cells and multinuclear giant cells expressed TGF-β1 gene transcript in their cytoplasm (Figure 4a). However, only multinuclear giant cells (Figure 4b) and a few macrophage-like mononuclear cells (data not shown) expressed TGF-β type II receptor gene transcripts. Treatment with RNAse showed a significant loss of these signals in the cells, indicating that labeled probes were specifically recognizing the mRNA sequences (Figure 4, c and d). Northern blot analyses demonstrating that both GCTs contained various levels of mRNAs for TGF-β1 and TGF-β type II receptor (approximately 2.3 and 5.2 kb, respectively) are shown in Figure 5. However, when ninth passaged cultures that contained only the mononuclear tumor cells were compared with the solid tumor of their origin (case 2 of GCT), it was clear that the cultured mononuclear tumor cells expressed only TGF-β1 but not TGF-β type II receptor gene transcripts (Figure 6). This result confirmed the *in situ* hybridization findings.

Chemotactic Activity of GCTCM

To determine whether GCTCM had chemotactic properties, rat osteoclast suspensions were tested for their ability to migrate in response to addition of GCTCM using an *in vitro* chemotaxis assay. Our results showed that GCTCM stimulates the migration of osteoclasts and TRACP-positive mononuclear cells (putative osteoclast precursor cells) in a dose-dependent manner (Figure 7a). There was no difference in chemotactic properties between heat-treated GCTCM (60 C for 20 minutes) and nonheat-treated GCTCM. In the osteoclast chemotactic assay, only osteoclasts with less than three nuclei had migrated through the 12-μ pore filters to attach onto the coverslips in the lower chamber (data not shown). Moreover, administration of monoclonal antibody against TGF-β significantly reduced but did not abolish the chemotactic activity of GCTCM on both TRACP-positive mononuclear cells and osteoclasts (Figure 7b).

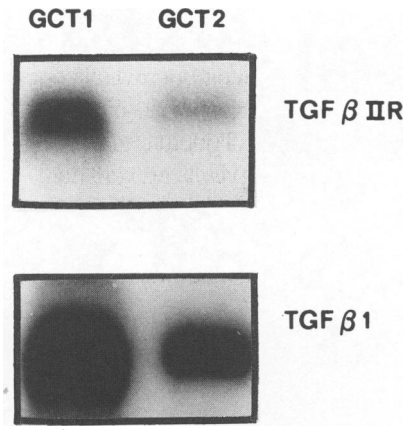

Figure 5. *Northern blot analyses of TGF-β1 and TGF-β type II receptor gene transcripts in both cases of GCT. Twenty micrograms of RNA was separated on an agarose gel, transferred to nitrocellulose, and hybridized with probes. Lane 1, RNA from case 1. Lane 2, RNA from case 2. Various levels of mRNA for TGF-β1 and TGF-β type II receptor were observed.*

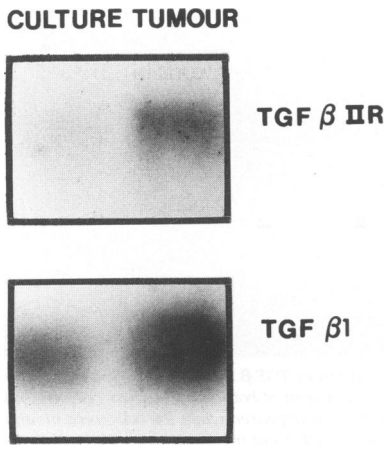

Figure 6. *Northern blot analyses of TGF-β1 and TGF-β type II receptor gene transcripts in both cultured and solid tumor from case 2. Twenty micrograms of RNA were separated on an agarose gel, transferred to nitrocellulose, and hybridized with the probes. Lane 1, RNA from spindle-shape stromal cells at ninth passage. Lane 2, RNA from the solid tumor. Spindle-shaped stromal cells do not express TGF-β type II receptor mRNA.*

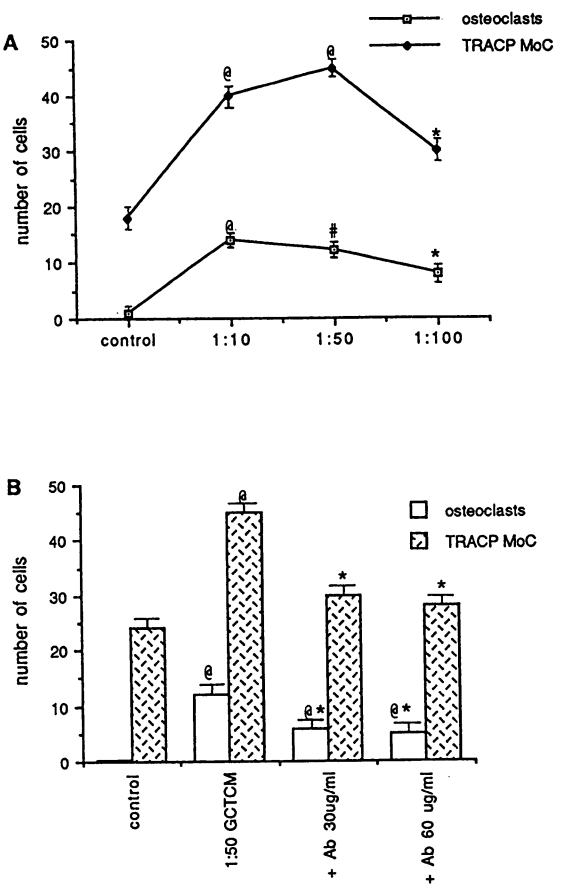

Figure 7. *Chemotactic action of GCTCM on osteoclasts and TRACP-positive mononuclear cells (TRACP MoC). Rat osteoclasts and TRACP MoC were assayed for their ability to respond to GCTCM, as described in Materials and Methods. For negative controls, medium (1:50 of 199 medium to DMEM) in the presence of 5% FCS was used.* A: *Dose response of GCTCM on chemotaxis of osteoclasts and TRACP MoC.* B: *Effect of monoclonal antibody against TGF-β on GCTCM-induced chemotaxis. The antibody was used at a concentration of 30 and 60 μg/ml, respectively, to block GCTCM (1:50)-induced chemotaxis of osteoclasts and TRACP MoC. Data shown are from a representative experiment. For osteoclast chemotaxis, data represent the mean ± SEM per coverslip. For TRACP MoC chemotaxis, data represent the mean ± SEM per 25 × objective.* @ $P < 0.005$ (versus *control*), # $P < 0.01$ (versus *control*), * $P < 0.05$ (versus *control in* A *or* versus *control and 1:50GCTCM in* B)

Evidence of TGF-β Type II Receptor on Rat Osteoclasts

To determine whether rat osteoclasts possess TGF-β type II receptors that may be involved in chemotaxis, *in situ* hybridization was used to detect mRNA transcripts for TGF-β type II receptor. The results indicate that rat osteoclasts obtained from preparations similar to those used in the experiments for osteoclast chemotaxis express TGF-β type II receptor mRNA transcript in their cytoplasm. However, fibroblasts in the same culture showed no evidence for the presence of TGF-β type II receptor mRNA (Figure 8a). Treatment with RNAse showed a significant loss of signal in the osteoclasts (Figure 8b).

Discussion

By using *in situ* hybridization and Northern blot analysis, the results of this study have clearly demonstrated that TGF-β1 gene transcript was consistently detected in tumor cells and reactive components, such as osteoclast-like cells and macrophage-like cells. By contrast, TGF-β type II receptor mRNA transcript was only present in the reactive components (osteoclast-like cells and macrophage-like cells). These findings show that distinct molecular differences exist between the various cell types in GCT. Moreover, the chemotactic activity of GCTCM for rat osteoclasts and their putative mononuclear osteoclast precursor cells (TRACP-positive mononuclear cells) indicates that tumor cells of GCT are capable of recruiting the reactive components (osteoclasts, osteoclast precursors) by chemotactic mechanisms.

TGF-β1 is an extremely potent chemotactic agent for monocytes,[30] macrophages,[30] fibroblasts,[31,32] and osteoblasts.[33] It is believed that the chemotactic action of GCTCM on osteoclasts and TRACP-positive mononuclear cells is at least in part due to the presence of bioactive TGF-β. The TGF-β1 in GCTCM may be activated by a plasmin-mediated proteolytic system (MHZ et al, unpublished data) and by stromelysin 2 and 3 produced by the neoplastic cells of GCT.[22] It is not surprising that monoclonal antibody against TGF-β did not completely block the chemotactic action of GCTCM. Many other cytokines, including macrophage colony-stimulating factor, tumor necrosis factor-α, interferon-γ, and interleukin-1 are found in GCT[22,42] and may possibly influence osteoclast chemotaxis.[42] It has been shown that these cytokines are up-regulated by TGF-β1 in other cells such as macrophages.[30] It is therefore possible that TGF-β1 acts as a key factor in concert with one or several of these cytokines to attract osteoclasts and their precursor cells to the neoplastic lesion.

There are several TGF-β receptors mediating the biological effects of TGF-β1. Five TGF-β receptors have been identified and include type I (53 to 65 kd), type II (83 to 110 kd), type III (250 to 310 kd), type IV (60 kd), and type V (400 kd) receptors.[44–46] The type IV receptor has only been found on pituitary cells,[46] whereas the others are co-expressed on most cell types including tumor cells.[29] It is believed that the type I and type II receptors mediate the majority of the activities of TGF-β1 because loss of cellular response

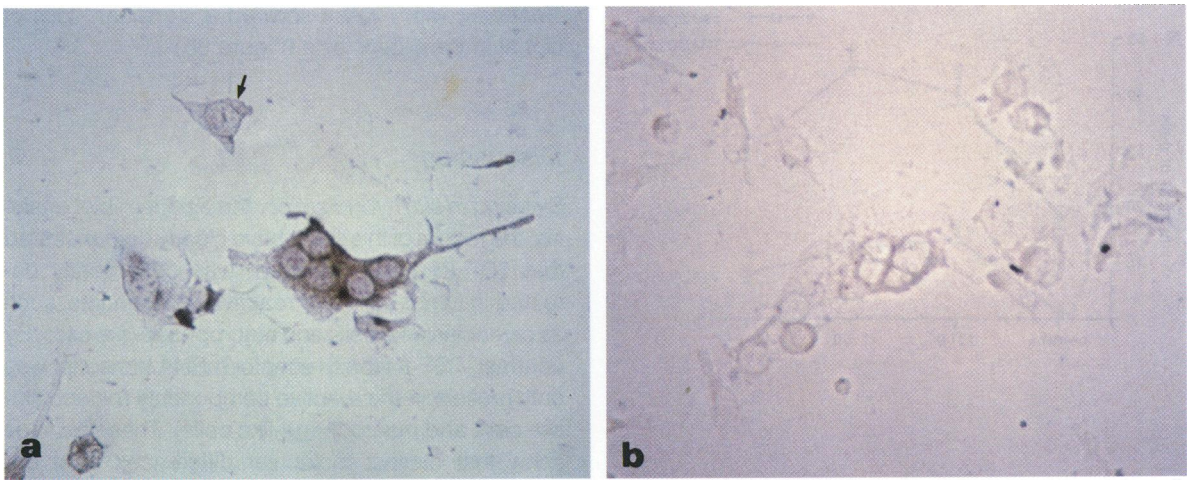

Figure 8. *Location of TGF-β type II receptor gene transcripts in cultured rat osteoclasts.* a: *TGF-β type II receptor mRNA is seen in cultured osteoclasts, whereas fibroblasts (arrow) in the cultures were negative.* b: *TGF-β type II receptor mRNA could not be demonstrated when the cells were incubated with 100 μg/ml of RNase before hybridization (×250).*

to TGF-β1 correlates with loss of type I and/or type II receptors.[47] Recent findings have demonstrated that the type II receptor functions as a transmembrane serine-threonine kinase and is required for the antiproliferative activity of TGF-β1, whereas the type I receptor mediates the induction of several genes involved in cell-matrix interactions.[49] The finding that both human osteoclast-like giant cells and rat osteoclasts express TGF-β type II receptor gene transcripts, together with the chemotactic effect of TGF-β1 on rat osteoclasts suggests that the type II receptor is involved in the chemotactic actions of TGF-β1.

Our observations showed that only osteoclasts with less than three nuclei migrate through the 12-μ filters in the osteoclast chemotactic assay. This may be due to limitations posed by the 12-μ pore size in the filters. Alternatively, osteoclasts with a small number of nuclei may have more active chemotactic responses or move more rapidly than those with a larger number of nuclei. Interestingly, small osteoclasts have a higher level RNA synthesis[36] and resorb bone more easily than large osteoclasts.[43]

The pathological significance of tumor cells having a chemotactic effect on osteoclasts and osteoclast precursors is, however, unknown. The finding that osteoclast-like cells in GCT excavate bovine bone slices *in vitro* indicates that they may be largely responsible for bone destruction. It is uncertain whether bone destruction by osteoclast-like cells is directly associated with local recurrence or metastases. Histologically, the number of osteoclast-like cells in GCT does not relate to the behavior of the tumor.[20,39] However, it appears likely that the osteoclast-like cells attracted by tumor cells promote the expansion of the tumor because they will produce osteolysis. The ability of tumor cells to attract osteoclasts suggests that GCTs evolve either from a primitive marrow stromal cell before it is committed to the osteoblast lineage or a subset of bone marrow stromal cell[40] that encourages osteoclast differentiation but is distinct from the osteoblast lineage.

Despite the fact that GCT are composed of a heterogenous population of cells including macrophage-like cells, spindle-shaped mononuclear cells, and osteoclast-like giant cells, we observed that longer-term GCT cultures only contain mononuclear cells. It seems that these growing cells were the neoplastic cells, because nonneoplastic cells cannot be maintained over an extended period in culture. Moreover, the failure to detect multinuclear osteoclast-like cells after the second passage suggests that these spindle-shaped cells are not the principal precursors of the osteoclast-like cells. These observations confirmed other investigators' finding that cells growing out from GCT were neoplastic stromal cells.[16,22] Furthermore, it was noted that multinuclear giant cells in GCT contain more nuclei than authentic osteoclasts. This may be related to the paucity of bone matrix and the sustained effects of the closely located tumor cells (A. Malcolm, personal communication).

In conclusion, we found that the TGF-β1 gene transcript was consistently detected in both mononuclear cells and multinuclear giant cells of GCT. The TGF-β type II receptor gene transcript, however, was present only in multinuclear giant cells. Conditioned medium from the cultures of GCT had potent chemotactic activity for osteoclasts. Administration of TGF-β1 monoclonal antibody significantly reduced but did not abolish the chemotactic action. These findings in the two

GCT we examined suggested that TGF-β1 acts as an autocrine/pancrine agent, which in concert with other factor(s), recruits the reactive osteoclast-like multinuclear giant cells (or their mononuclear precursors) in GCT. However, it is possible that not all GCT are cytologically identical and syncytia in one tumor may have a different origin than those in another. Obviously, the cytokine profile in several GCT needs to be elucidated before the nature of this enigmatic neoplasm is resolved.

Acknowledgments

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