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Gene Expression of Monocyte Chemoattractant Protein-1 in Giant Cell Tumors of Bone Osteoclastoma: Possible Involvement in CD68$^+$ Macrophage-Like Cell Migration

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Abstract Giant cell tumor of bone (GCT) is one of a few neoplasms in which the macrophage/osteoclast precursor cells and osteoclast-like giant cells infiltrate the tumor mass. Monocyte chemoattractant protein 1 (MCP-1) is a potent chemotactic factor specific for monocytes. In search of relevant cytokines that may enhance the recruitment of these reactive cells, we evaluated the localization and regulation of MCP-1 mRNA and protein in GCT by using Northern blot analysis, in situ hybridization and immunohistochemistry. We also determined whether conditioned medium obtained from GCT cultures can recruit human peripheral blood monocytes (CD68$^+$) in an in vitro chemotactic assay. Using Northern blot analysis, we detected the specific gene transcript for MCP-1 in all GCT samples tested. In situ hybridization and immunohistochemistry revealed that both MCP-1 gene transcript and protein were consistently present in the cytoplasm of stromal-like tumor cells of GCT. Treatment of mononuclear cells from GCT at third passage with TGF-β1 for 24 h increased the level of MCP-1 mRNA in a dose-dependent manner, with the maximum effect at 1 ng/ml. 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 conditioned medium. Thus, the results of this study suggest that recruitment of CD68$^+$ macrophage-like cells may be due to the production MCP-1 by stromal-like tumor cells. These CD68$^+$ cells may originate from peripheral blood and could have the capability of further differentiating into osteoclasts in the tumor. J. Cell. Biochem. 70:121–129, 1998.

Key words: giant cell tumor of bone; MCP-1; TGF-β; CD68$^+$; chemotaxis

Monocyte chemoattractant protein 1 (MCP-1) is a potent chemotactic factor specific for monocytes [Valente et al., 1984; Graves et al., 1986]. The MCP family includes MCP-2, MCP-3, MCP-4, MCP-5, RANTES, macrophage inflammatory protein (MIP)-α, and MIP-β [Oppenheim et al., 1991; Schall, 1991; Uguccioni et al., 1996; Sarafi et al., 1997]. The production of MCP-1 is constitutively dependent on stimulation by inflammatory mediators, such as interleukin-1, tumor necrosis factor α, interferon γ, or platelet-derived growth factor [Satriano et al., 1993; Hoshino et al., 1995; Sica et al., 1990; Villiger et al., 1992; Zhu et al., 1994]. MCP-1 gene expression and/or protein production have been detected in a variety of human diseases, such as atherosclerosis [Yu et al., 1992; Wysocki et al., 1996], rheumatoid arthritis [Villiger et al., 1992a,b], idiopathic pulmonary fibrosis [Antoniades et al., 1992], tissue injury [Furie and Randolph, 1995], periodontal infections [Tonetti et al., 1994], glioblastoma [Desbaillets et al., 1994], cervical carcinoma [Riethdorf et al., 1996], ovarian carcinoma [Negus et al., 1995], Kaposi's sarcoma [Sciacca et al., 1994], fibrous histiocytoma [Tabeya et al., 1991], and prostate adenocarcinoma [Mazzucchelli et al., 1996b]. The current view is that MCP-1 production is associated with the degree of chronic inflammation and macrophage infiltration in neoplasms. Moreover it has been shown that a number of tumor cell lines are capable of producing MCP-1,

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which appears to stimulate monocyte-mediated inhibition of tumor growth [Bottazzi et al., 1983; Graves et al., 1989]. In support of this hypothesis, there is also a correlation between the level of MCP-1 activity and the number of macrophages within tumors [Bottazzi et al., 1983].

Giant cell tumor of bone (GCT; also known as osteoclastoma) is a benign but locally aggressive neoplasm that has a predilection for the epiphysis of long bones. GCT is one of a few neoplasms in which the macrophage/osteoclast precursor cells and osteoclast-like giant cells infiltrate the tumor mass. The majority of these infiltrating cells are $CD68^+$ and belong to the macrophage/osteoclast lineage [Burmester et al., 1983; Athanasou et al., 1985]. Our previous studies showed that the tumor cells of GCT are capable of producing transforming growth factor-β1 (TGF-β1), which stimulates recruitment of reactive osteoclasts [Zheng et al., 1994]. The osteoclast-like giant cells express TGF-β type II receptor, reflecting paracrine action. Conditioned medium from tumor cell culture has been shown to chemoattract osteoclasts and tartrate-resistant acid phosphatase-positive mononuclear cells [Zheng et al., 1994]. Interestingly, administration of monoclonal antibody against TGF-β1 only partly abolishes the chemotactic activity of this 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, using in situ hybridization, Northern blot analysis and immunohistochemistry. We also determined whether the conditioned medium obtained from cultures of GCT can recruit human peripheral blood monocytes ($CD68^+$) in an in vitro chemotactic assay and if this chemotactic activity can be abolished by the administration of MCP-1 antibody.

MATERIALS AND METHODS

Materials

Four cases of GCT were investigated as previously described [Zheng et al., 1995]. KP-1 (CD68) monoclonal antibody was obtained from DAKO (Botany, Australia). Monoclonal antibody against recombinant human and recombinant human and mouse MCP-1 proteins were obtained from R & D (Minneapolis, MN). The cDNA probe for MCP-1 was purchased from ATCC (Rockville, MD). All other chemicals were standard reagents.

Immunohistochemistry

GCT imprint slides were washed in 0.2 M Tris-buffered saline (TBS), blocked for endogenous peroxidase activity by incubation in 35% H_2O_2 for 5 min, washed in TBS, and then preincubated with 20% normal horse serum for 20 min before incubation overnight at 4°C with a 1 in 10 dilution of 5D3-F7 (monoclonal antibody to human MCP-1) or KP-1 (CD68) as previously described [Wysocki et al., 1996; Zheng et al., 1995]. Slides were then washed twice with TBS, incubated for 1 h at room temperature with a 1 in 200 dilution of biotin-conjugated sheep anti-mouse immunoglobulin (Silenus, Melbourne, Australia), followed by two washes in TBS and incubation for 1 h at room temperature with a 1 in 200 dilution of peroxidase conjugated streptavidin (Silenus). Chromagen solution containing 6 mg of 3,3′-diaminobenzidine (BDH Chemicals, Poole, UK) in 10 ml of TBS/0.03% H_2O_2 was added and color allowed to develop for 5 min before being stopped by washing in TBS. Slides were counterstained in hematoxylin, dehydrated in alcohol, and mounted in Depex. Preparations in which the primary antibody to MCP-1 was omitted served as negative controls.

Tissue Culture of Giant Cell Tumor of Bone

To examine the effect of TGF-β1 on regulation of gene expression of MCP-1 in GCT, mononuclear spindle-shaped cells at the third passage derived from case 4 of GCT were subcultured in Dulbecco's minimum essential medium (DMEM) containing 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Upon reaching confluence, the culture medium was removed and replaced for 24 h with DMEM containing 2% FCS in the presence and absence of TGF β1 (0.1 ng to 5 ng/ml).

RNA Extraction and Northern Blot Analysis

Total cellular RNA was isolated by the RNAzol method (Biotech, Houston, TX) from both solid tumors and tumor cultures prepared from the one GCT preparation. Twenty-five micrograms of total cellular RNA from each extract 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 h at 42°C in a buffer containing 50% formamide and five-fold

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. The cDNA probe for MCP-1 was a 0.7kb EcoR1 fragment of MCP-1 [Yoshimura et al., 1989] labeled with [^{32}P]dCTP (3,000 ci/mmol; Amersham) using a random primer kit (Amersham). Filters were washed sequentially in 2 × SSPE/0.1% sodium dodecyl sulfate (SDS) at room temperature and 1 × SSPE/0.1% SDS, 0.1 × SSPE/0. 1% SDS at 65°C and then exposed to X-ray films at -70°C in cassettes using intensifying screens. MCP-1 mRNA levels were normalized by using GAPDH mRNA as an internal standard.

In Situ Hybridization

Inserts of MCP-1 (0.7 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 [Zheng et al., 1994, 1995]. The hybridization solution consisted of 45% deionized formamide, 10% dextran sulfate, five-fold standard saline citrate (SSC), and 1 mg/ml of denatured and sonicated salmon sperm DNA.

Isolation of Monocytes From Human Peripheral Blood

Heparinized peripheral blood from a single normal donor was mixed with one volume of phosphate-buffered saline (PBS) and one volume of hydroxyethyl starch (Plasmasteril; Fresenius, Bad Homburg, Germany) in a cylinder and placed for 30 min at 37°C to allow sedimentation of erythrocytes. The resulting supernatant was centrifuged at 400*g* for 7.5 min and the pellet was washed and suspended in PBS. This cell suspension was loaded carefully on three volumes of Ficoll-sodium metrizoate (Lymphoprep; Nyegaerd, Oslo, Norway; density = 1.077 g/ml) and centrifuged at 400*g* for 30 min. The layer of mononuclear cells was washed and suspended in Hanks' balanced salt solution, supplemented with 0.1% human serum albumin (HBSS + 0.5% HSA) and used as a source of monocytes.

Chemotactic Assay

Conditioned medium for the human monocyte chemotaxis assay was collected from early passage GCT cells cultured at a density of 10^4 cells/ml in DMEM containing 5% FCS for 3 days before collection of GCT-conditioned medium (GCTCM), which was filtered through 2.5 µm pore size filters prior to storage at -70°C for use in the chemotactic assay. Human monocytes at a density of 10^4 cells/well in DMEM were allowed to settle for 90 min at 37°C onto a polycarbonate filter with 5 µm pores within the upper well of the microchamber [Zheng et al., 1994]. The GCTCM was diluted in medium DMEM (1:10 and 1:50) and placed in the lower well of the microchamber. For negative controls, medium DMEM in the presence of 5% FCS for osteoclasts and monocytes respectively was placed in the lower wells. For positive controls, rhMCP-1 at concentrations of 100 ng/ml and 10 ng/ml of medium were used. Monoclonal antibody against MCP-1 at 150 ng/ml was used to neutralise MCP-1 activity in GCTCM. KP-1 antibody was used for immunohistochemistry as previously described [Zheng et al., 1995] to detect the effect of MCP-1 on $CD68^+$ monocyte chemotaxis.

RESULTS

Distribution of $CD68^+$ Positive Cells in GCT

As previously described [Zheng et al., 1994, 1995], tumors from all four cases of GCT displayed typical morphological features and consisted of multinuclear osteoclast-like giant cells scattered among a mass of mononuclear cells (Fig. 1a). Immunohistochemistry of GCT imprints revealed that the osteoclast-like giant cells were consistently positive for $CD68^+$. A small portion of mononuclear cells identified as round-shaped macrophage-like cells were also positive for the same marker (Fig. 1b). To further determine the number of $CD68^+$ cells in GCT, the $CD68^+$ cells in tissue imprints were counted under the 25× objective field. Table I shows the percentage and absolute number of $CD68^+$ positive cells per field in the four cases. Interestingly, $CD68^+$ positive cells account for approximately 30–40% of the mononuclear cell population.

Detection and Localization of MCP-1 mRNA and Protein in GCT

To detect the presence of MCP-1 gene transcript in GCT, total RNA extracted from snap-frozen samples of four cases of GCT was hybridized with radiolabeled MCP-1 cDNA probe.

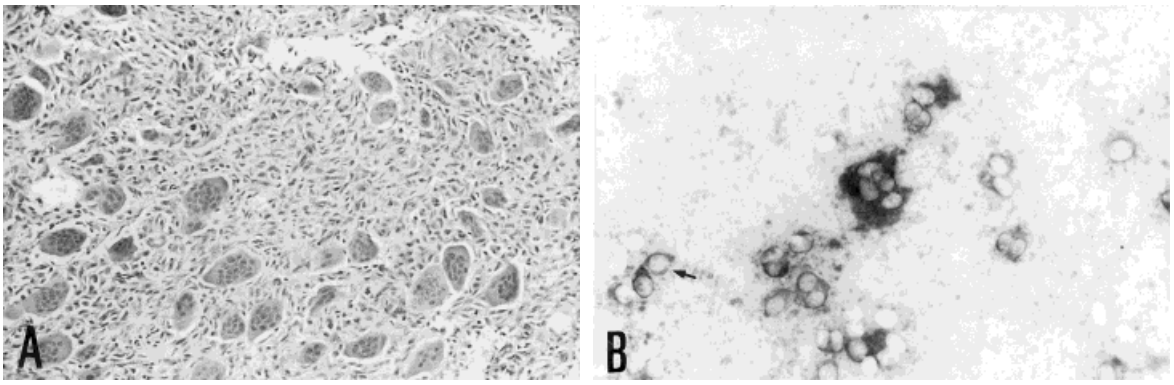

Fig. 1. Histology and CD68$^+$ immunoreactivity of giant cell tumor of bone (GCT). **A**: Histological feature of GCT. Note that many osteoclast-like giant cells are scattered among a mass of mononuclear cells. The stromal-like tumor cells are either polygonal or spindle shaped. The macrophage-like cells display a rounded appearance. **B**: Immunoreactivity of CD68$^+$ of GCT imprints. Note that both osteoclast-like giant cells and round-shaped macrophage-like cells (arrow) are positive for CD68$^+$, whereas stromal-like tumor cells are negative (×250).

TABLE I. Number and Percentage of CD68$^+$ Cells in GCT[a]

	Multinuclear giant cells		Mononuclear cells	
	CD68$^+$	CD68$^+$	CD68$^+$ macrophage-like	CD68$^-$ stromal-like
Case 1	4.1 ± 0.8 (6.8%)	0 (0%)	19.5 ± 2.6 (32.3%)	36.7 ± 4.9 (60.9%)
Case 2	2.7 ± 0.36 (5.3%)	0 (0%)	16.8 ± 2.5 (32.7%)	31.8 ± 3.1 (62%)
Case 3	9.8 ± 1.5 (5.8)	0 (0%)	64 ± 11.5 (37.9%)	94.8 ± 20 (56.2%)
Case 4	4.0 ± 0.55 (6.3%)	0 (0%)	21.4 ± 1.5 (33.5%)	38.4 ± 3.3 (60.1%)

[a]CD68$^+$ and CD68$^-$ cells in tissue imprints of GCT were counted under the 25× objective field. At least 10 fields per slide were counted. Results are expressed as mean number of cells per field + SEM. The % represents percentage of the cells in total cell population per field.

Representative Northern blot analyses of MCP-1 in GCT (cases 1 and 2) are shown in Figure 2. Autoradiograms demonstrated that all of tumors expressed MCP-1 gene transcripts at high levels. The size of the MCP-1 gene transcript in these tumors was approximately 800 base pairs, a similar size as previously described [Sica et al., 1990].

To determine the localization of MCP-1 gene transcript in GCT, we performed in situ hybridization using a digoxigenin-labeled cDNA probe [Zheng et al., 1994, 1995]. Figure 3a shows that mononuclear, mainly spindle-shaped, tumor cells express MCP-1 gene transcripts, while multinucleated osteoclast-like giant cells demonstrate minimal expression. For the tumor cells the dark brown reaction product was diffusely distributed throughout the cytoplasm. Treatment with RNase resulted in a loss of signals in all cells, indicating that the labeled probe was specifically recognizing mRNA sequences (Fig. 3b).

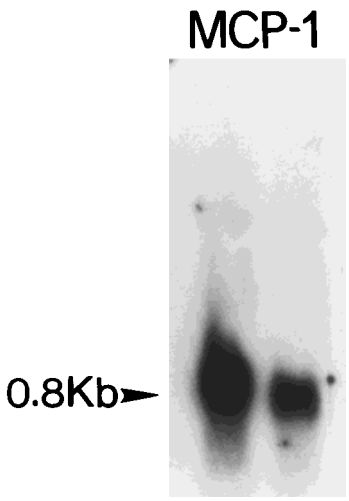

Fig. 2. Northern blot analysis of MCP-1 in GCT. Case 1 (**left**) and Case 2 (**right**) are shown. Twenty-five micrograms of total RNA were separated on an agarose gel, transferred to nitrocellulose, and hybridized with probes. Note that both cases express significant levels of MCP-1 mRNA (0.8 kb).

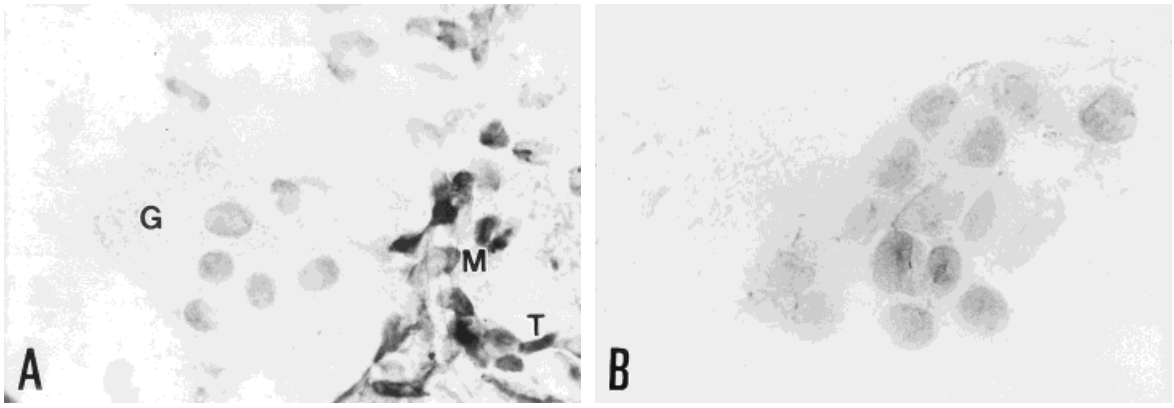

Fig. 4. Immunohistochemistry of MCP-1 in GCT imprints of Case 3. **A**: MCP-1 protein was detected in spindle-shaped stromal-like tumor cells (T) and some macrophage-like cells (M), but not in osteoclast-like giant cells (G). This result is consistent with the detection of MCP-1 mRNA by in situ hybridization. **B**: Negative control of MCP-1 immunohistochemistry cells were treated with normal preimmune goat IgG. The nuclei were stained with hematoxylin (×450).

To further determine whether MCP-1 protein was present in GCT, immunohistochemistry was performed on GCT imprints. The result showed that MCP-1 immunoreactivity closely correlated with the findings by in situ hybridization. MCP-1 protein was consistently detected in spindle-shaped mononuclear cells and a few round mononuclear cells. In contrast, multinucleated osteoclast-like giant cells were consistently negative for MCP-1 protein in all four cases (Fig. 4a). Treatment with preimmune goat IgG showed no staining on GCT imprints, indicating the specificity of immunoreaction with MCP-1 antibody (Fig. 4b).

Regulation of MCP-1 Gene Expression by TGF-β1 in GCT Cultured Cells

Previous studies have shown that tumor cells of GCT produce abundant TGF-β1 [Zheng et al., 1994]. To further examine whether TGF-β1 is involved in the regulation of MCP-1 gene expression in GCT, mixed cultures of GCT (case 4) at the third passage were used. Such cultures consisted of mononuclear cells but not osteoclast-like giant cells. The results indicated that treatment with TGF-β1 for 24 h increased the level of MCP-1 mRNA in these cultures (Fig. 5) in a dose-dependent manner, with the maximum effect was observed at a concentration of 1 ng/ml. The findings suggest that TGF-β1 upregulates MCP-1 production.

Conditioned Media From GCT Culture (GCTCM) Induced CD68$^+$ Blood Monocyte Migration

To examine the hypothesis that the accumulation of CD68$^+$ cells in GCT may be derived from peripheral blood monocytes, we tested the ability of GCTCM to stimulate normal CD68$^+$ monocyte migration. GCTCM was collected from fifth passage cultures (cases 1 and 2) and used in a Boyden Chamber as previously described

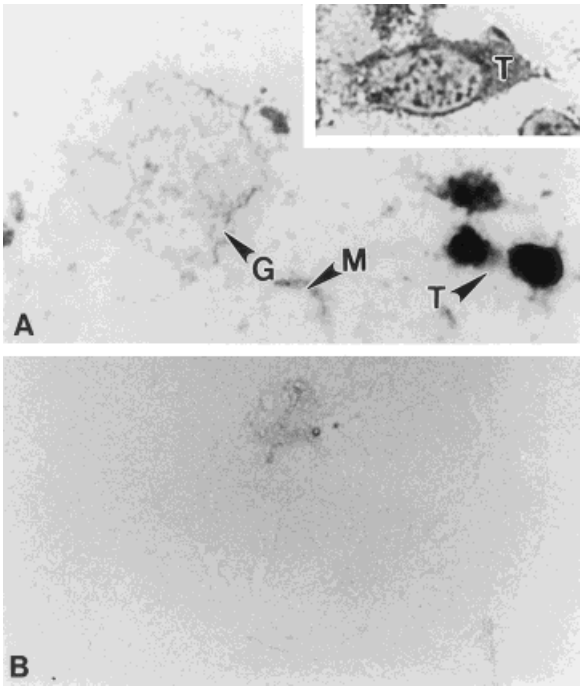

Fig. 3. In situ hybridization of MCP-1 gene transcript in GCT imprints of Case 3. **A**: The location of MCP-1 mRNA is in spindle-shaped stromal-like tumor cells (T) and some macrophage-like cells (M), but not osteoclast-like giant cells (G). Under high magnification the signal localization is observed in the cytoplasm of stromal-like tumor cells (window). **B**: The signal disappears when cells were incubated with 100/ng/ml of RNase before hybridization (×450).

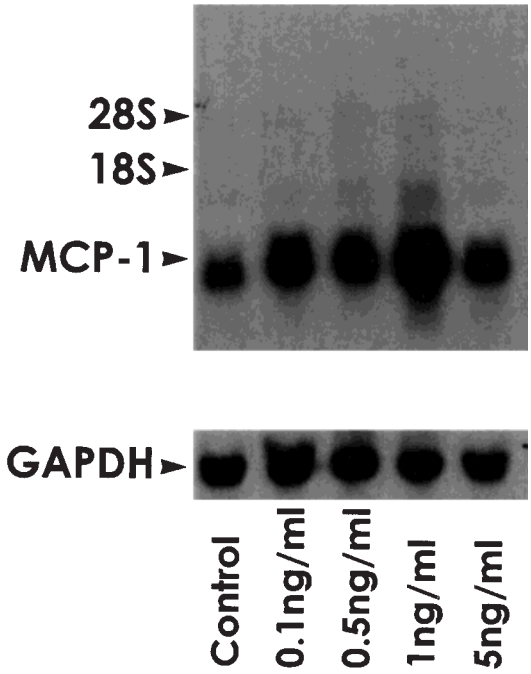

Fig. 5. Northern blot analysis for MCP-1 gene transcripts in third passage cultures from GCT (Case 4). Cells were treated with TGF-β1 at various concentrations for 24 h. Twenty micrograms of total RNA were extracted and separated on an agarose gel, transferred to nitrocellulose, and hybridized with the appropriate probes. A housekeeping gene, GAPDH, was used as an internal reference to demonstrate that the changes of MCP-1 gene expression were not due to uneven loading of RNA samples. Note that treatment with TGF-β1 increases the level of MCP-1 mRNA in a dose dependent manner, with maximum induction observed at 1 ng/ml.

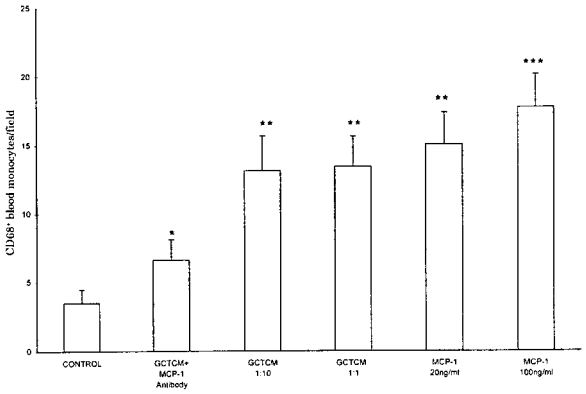

Fig. 6. Chemotactic effects of conditioned media obtained from giant cell tumor of bone (GCTCM) on $CD68^+$ positive peripheral blood monocytes. Human peripheral blood monocytes were assayed for their ability to respond to GCTCM and recombinant human MCP-1 (rhMCP-1), as described in Materials and Methods. For a negative control, DMEM containing 5% FCS was diluted with IMEM 1 to a ratio of 1:10. Cells were stained with Diff-quick stain or reacted with $CD68^+$ immunohistochemistry. Notes that both GCTCM and rhMCP-1 increase the chemotaxis of $CD68^+$ monocytes. Monoclonal antibody against MCP-1 abolished the chemotactic activity of GCTCM ($^*P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ verse control).

[Zheng et al., 1994]. The results indicated that GCTCM is able to induce cell migration of normal $CD68^+$ peripheral blood monocytes to the same extent as that produced by MCP-1 at a concentration of 10 ng/ml. Addition of monoclonal antibody against MCP-1 (125 ng/ml) to the conditioned medium abolished the chemotactic activity of GCTCM (Fig. 6).

DISCUSSION

In this report, we demonstrated for the first time that stromal-like tumor cells in GCT produce MCP-1. Using Northern blot analysis, we have detected the specific gene transcript for MCP-1 in all of GCT tested. In situ hybridization and immunohistochemistry revealed that both MCP-1 mRNA and protein were consistently present in the cytoplasm of stromal-like tumor cells of GCT. Interestingly, conditioned media from GCT cultures promoted chemotactic migration of $CD68^+$ peripheral monocytes, an activity abolished by the addition of MCP-1 antibody to the conditioned medium. MCP-1, however, had no effect on the chemotaxis of osteoclasts [Zheng et al., unpublished data]. Thus, the results of this study suggest that the recruitment into GCT of $CD68^+$ positive cells, which account for approximately 30–40% of the total cell mononuclear population, may be due to the production MCP-1 by stromal-like tumor cells.

It has been shown that many growth factors and cytokines regulate the production of MCP-1 at both transcriptional and posttranscriptional levels. For example, in mouse mesangial cells MCP-1 is increased in a dose-dependent manner after incubation with IFN-γ and TNF-α [Satriano et al., 1993]. Interestingly, changes in MCP-1 mRNA expression parallel activation of the transcriptional factor NF-kB [Satriano et al., 1994]. Furthermore, it has been shown that MCP-1 production by mouse osteoblasts was regulated by IL-1 [Rahimi et al., 1995; Zhu et al., 1994]. The production of MCP-1 by osteoblasts has been considered to be one of the steps by which osteoblasts/stromal cells regulate osteoclastic bone resorption in inflamed bone [Rahimi et al., 1995]. In this report, we find that TGF-β1 increases the level of MCP-1 gene transcripts in cultured mononuclear cells of GCT.

Since our previous studies showed that tumor cells of GCT do not possess TGF-β type II receptor, the effect of TGF-β1, on the production of MCP-1 in GCT would appear to be mediated via other types of TGF-β receptor [Chen et al., 1993]. Our finding that TGF-β1 induced MCP-1 gene expression is consistent with other reports that induction of MCP-1 mRNA in both bone marrow stromal cells and osteoblasts is modulated by TGF-β [Zhu et al 1994; Gautam et al., 1995].

MCP-1 has been detected in many solid tumors associated with macrophage infiltration. In human malignant glioma, MCP-1 mRNA and protein were found to be localized in tumor cells but not in infiltrating macrophages [Takeshima et al., 1994]. A similar pattern of MCP-1 expression has been demonstrated in Kaposi's sarcoma [Sciacca et al., 1994], fibrous histiocytoma [Takeya et al., 1991], and prostate adenocarcinoma [Mazzucchelli et al., 1996b]. A study by Negus et al. [1995], however, showed that not only do tumor cells produce large amount of MCP-1, but that other cell types present in the neoplastic mass, including macrophage and monocytes, can also secrete detectable levels of the chemokine. In our study, MCP-1 was clearly found in stromal-like tumor cells and some macrophage-like cells, but not in osteoclast-like giant cells. The lack of MCP-1 expression in multinuclear osteoclast-like giant cells is in contrast to another report that murine osteoclasts are capable of producing MCP-1 [Rahimi et al., 1995]. This inconsistency may be due to the species differences, tissue differences, or experimental conditions.

Host defense cells infiltrate many solid tumors [Bottazzi et al., 1983]. In giant cell lesions of bone and soft tissue, the infiltrating cells are predominantly macrophages and osteoclasts. Tumor-associated macrophages and osteoclastic giant cells were found to be diffusely distributed throughout lesions [Doussis et al., 1992]. Although many studies have shown that reactive macrophages may be cytotoxic to tumor cells both in vivo and in vitro [Fidler and Schoit, 1984; Brunda et al., 1991], the exact biological role of these reactive components in GCT is unknown. Previous studies have shown that macrophage-like cells in GCT may contribute to tumor spread by producing urokinase-plasmogen activators [Zheng et al., 1995]. Others have found that the macrophages produce IL-1α, IL-6, and TNF that stimulate bone resorption [Kito et al., 1993; Reddy et al., 1994; O'Keefe et al., 1997]. Thus, the biological significance of infiltration by macrophages or osteoclast-like cells in GCT is likely to be complex. It may be that the infiltration of macrophage or osteoclast-like giant cells in GCT determines tumor expansion, rather than inhibiting tumor growth.

$CD68^+$ is an intracellular glycoprotein with a molecular mass of 110 kD, that is probably associated with lysosomal granules [Pulford et al., 1989]. $CD68^+$ is expressed in tissue macrophages and in monocytes, granulocytes, and myeloid precursor cells [Pulford et al., 1989; Wagner, 1994]. Macrophages positive for $CD68^+$ constitute a major subset of the mononuclear phagocyte system. It has been shown that $CD68^+$ macrophages are rather heterogeneous [Wagner et al., 1994, 1996]. Thus, although we demonstrated that conditioned medium from cultures derived from GCT are capable of recruiting $CD68^+$ peripheral blood monocytes, the precise nature of the $CD68^+$ macrophage-like cells within GCT is unknown. Whether they are derived directly from bone marrow precursor cells or from peripheral blood needs to be further clarified. However, because GCT is a highly vascular neoplasm, it is not unreasonable to assume that peripheral $CD68^+$ monocytes emigrate from neighboring blood vessels to reach the tumor under the influence of a monocyte chemotactic activity gradient generated by tumor cells of GCT. It is noteworthy that peripheral monocytes can undergo differentiation toward osteoclasts [Fujikawa et al., 1996]. Thus, it is possible that a proportion of the $CD68^+$ macrophage-like cells in GCT, are infiltrated monocytes that originate from peripheral blood, attracted by tumor-derived MCP-1, and these circulating monocytes have the capability of further differentiating into osteoclasts in the tumor.

In conclusion, tumor cells of GCT are capable of producing MCP-1, which may induce the recruitment of $CD68^+$ macrophage-like cells into the lesion. The production of MCP-1 by tumor cells may be regulated by TGF-β1 in an autocrine fashion.

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