IMMUNOCHARACTERISATION OF GIANT CELL LESIONS OF THE JAWS

RAMESH MOHAMMAD ALIZADEH HOSHYAR, D.D.S. (IRAN)

Thesis submitted in partial fulfilment of the requirements for the degree of Master of Dental Surgery (Paediatric Dentistry)

DEPARTMENT OF DENTISTRY
FACULTY OF DENTISTRY
THE UNIVERSITY OF ADELAIDE
AUSTRALIA
OCTOBER 1997

TABLE OF CONTENTS

	PAGE
SUMMARY	ii
DECLARATION	vi
ACKNOWLEDGEMENTS	viii
LIST OF TABLES	ix
LIST OF FIGURES	x
ABBREVIATIONS	xi
CHAPTER 1 REVIEW OF THE LITERATURE	1
CHAPTER 2 MATERIALS AND METHODS	17
CHAPTER 3 RESULTS	25
CHAPTER 4 DISCUSSION	54
APPENDIX A	66
BIBLIOGRAPHY	67

ADDENDUM

The following material is to be inserted after the first paragraph in page 11.

O'Malley *et al.* (1997) compared numbers of cells in cell cycle in aggressive and non-aggressive CGCGs and concluded that it is not yet possible to predict behavior of CGCGs from known histologic, immunophenotypic, or proliferation parameters.

The following material is to be inserted at the end of the second sentence of the third paragraph in page 57.

While Mighell *et al.* (1995) reported an almost equal sex distribution with 30 male and 32 female patients.

The following material is to be inserted at the end of the first sentence of the last paragraph in page 59.

, and the work of Carvalho *et al.* (1995) who reported that CD68 was present in the majority of multinucleated giant cells of PGCG.

The following material is to be inserted before the last paragraph in page 62.

The mononuclear cells in CGCGs, PGCGs and GCTs studied were found to be unreactive with CD34 (endothelial cell marker). This confirms the work of O'Malley *et al.* (1997) and tends to exclude an endothelial origin for this cell group.

SUMMARY

Many bone and soft tissue tumors and tumor-like lesions are characterised by the presence of a variable number of multinucleated giant cells. Diagnosis of these giant cell lesions, in particular those arising in bone such as central giant cell granuloma and giant cell tumor, is based on an evaluation of the clinical history of the patient, together with the radiological and histologic findings.

The occurrence of the central giant cell granuloma is largely confined to the jaws. However, isolated cases of this lesion in the small tubular bones of the hands and feet, facial bones and the skull have been reported. Giant cell tumors occur mainly in the long bones and although infrequent, they have also been reported in the jaws and other sites in the cranial skeleton.

Although many of central giant cell granulomas may be distinguished from giant cell tumors on a histologic basis, these lesions are often difficult to differentiate using conventional histologic methods as they can exhibit a variety of morphological appearances and, with the common feature of numerous multinucleated giant cells, may closely resemble each other. Therefore, identification of histologic parameters that could distinguish central giant cell granulomas from giant cell tumors would be valuable in planning optimal therapy.

Furthermore, the origin and nature of the cellular components in giant cell lesions has not been satisfactorily established and is still debatable.

Therefore, further characterisation of these lesions is necessary before their pathogenesis can be determined.

The aims of the present study were to:

a. Perform a retrospective clinical analysis on a subset of giant cell lesions taken from a South Australian population.

b. Further evaluate the antigenic profile of the cellular components of giant cell lesions of the jaws using a panel of monoclonal and polyclonal antibodies, and enhanced antigen retrieval techniques using microwave fixation.

c. Further investigate the possible differences between the antigenic profile of the cellular components of the giant cell lesions occurring in the jaws and those of extragnathic sites.

A total of 55 cases of giant cell lesions were recovered from archives. The histories of all cases were reviewed and pertinent data regarding age, sex and site of the lesions were tabulated, analyzed and compared with international frequency studies. For the antigenic profile studies, 38 formalin-fixed and paraffin-embedded specimens (20 peripheral giant cell granulomas, 15 central giant cell granulomas, and 3 giant cell tumors) were sectioned at 5 μm intervals and immunoincubated using microwave antigen retrieval methods. Sections were immunostained using avidin-biotin-peroxidase complex immunohistochemical techniques, with a panel of markers including CD68, MAC 387, CD34, HAM 56 and Factor XIIIa. Immunoreactivity was semi-quantitatively graded on a - to + + + scale. The approximate percentage

range of cells reacting positively (in the one section) to each antibody was also evaluated in order to assess uniformity of staining.

Results revealed that the age and site distribution of South Australian cases of central giant cell granuloma, and gender distribution of South Australian cases of central and peripheral giant cell granuloma, showed some differences from those reported by other investigators. This may have reflected sampling differences.

In the immunohistochemical study, multinucleated giant cells exhibited a strong positive reaction for CD68, thus supporting the view of their origin from macrophages. However, the giant cells did not stain for MAC 387 and HAM 56, supporting the concept that these cells may be a subset of specialized giant cells derived from the mononuclear phagocyte system, such as osteoclasts.

The positive reaction of mononuclear stromal cells for CD68, MAC 387 and HAM 56 suggests that these cells originate from the macrophage (or the mononuclear phagocyte system). This relationship was reinforced by the strong positive reaction of mononuclear stromal cells to the dermal dendrocytic marker, Factor XIIIa.

Endothelial cells reacted positively for CD34 and HAM 56. The blood vessels located deeper within the giant cell lesions stained as intensely for these antibodies as those at the periphery of the lesions.

There were no obvious differences between the immunostaining patterns of the cellular components of central and peripheral giant cell granuloma.

Except for the increased vascularity of giant cell tumor, no major differences were found in the immunostaining patterns between the central giant cell granuloma and the giant cell tumor. Whereas further work needs to be done comparing the vascularity of these lesions, the results of this study support the view that central giant cell granuloma and giant cell tumor represent part of a spectrum of a single disease process and that differentiating central giant cell granulomas from giant cell tumors remains a difficult diagnostic exercise.

DECLARATION

This thesis is submitted in partial fulfilment of the requirements for the Degree of Master of Dental Surgery (Paediatric Dentistry) in The University of Adelaide, Adelaide, South Australia.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

RAMESH M. A. HOSHYAR

1997

To my husband, Mansour

for his unfailing encouragement and caring support

and my son, Yashar

for his understanding and patience

ACKNOWLEDGEMENTS

My sincere gratitude to The Ministry of Health and Medical Education, Iran, for granting me a scholarship to further my studies. I deeply appreciate this vital contribution from them.

I wish to express my most sincere appreciation to Dr. Angela M. Pierce, Senior Lecturer in Oral Pathology, and Associate Professor David F. Wilson, Reader in Oral Pathology, at The University of Adelaide, for their valuable supervision and guidance. My special thanks to Dr. Pierce who has devoted considerable time and effort during the study, and for her editorial assistance in the preparation of this thesis.

I am also grateful to Dr. Margaret Evans and Dr. Kevin R. Allen, my clinical supervisors for their consistent encouragement and support.

The study would not have been possible without the laboratory instruction and help of Mr. James Milios, Immunohistochemistry Laboratory, Institute of Medical and Veterinary Science, Adelaide. With gratitude I acknowledge his helpful advice and practical assistance.

I also wish to thank Mrs. Margaret Leppard and Ms. Sandie Powell for their willing technical guidance and assistance in the preparation of histologic and photographic material.

LIST OF TABLES

	PAGE
Table 1. Antibodies used: sources, dilutions and types	20
Table 2. Primary antibodies, related secondary antibodies and dilutions	23
Table 3. Summary of data from patients with CGCG	27
Table 4. Summary of data from patients with PGCG	28
Table 5. Summary of data from patients with GCT	29
Table 6. Immunoreactivity of cellular components in 20 cases of PGCG	33
Table 7. Immunoreactivity of cellular components in 15 cases of CGCG	34
Table 8. Immunoreactivity of cellular components in 3 cases of GCT	35

LIST OF FIGURES

		PAGE
Figure 1.	Age and sex distribution of 16 cases of CGCG	30
Figure 2.	Age and sex distribution of 35 cases of PGCG	30
Figure 3.	Photomicrograph showing a representative field of a PGCG	36
Figure 4.	Photomicrograph showing a representative field of a CGCG	36
Figure 5.	Photomicrograph showing a representative field of a GCT	38
Figure 6.	Photomicrograph of PGCG incubated for CD68	39
Figure 7.	Photomicrograph of CGCG incubated for CD68	39
Figure 8.	Photomicrograph of GCT incubated for CD68	41
Figure 9.	Photomicrograph of PGCG incubated for MAC 387	41
Figure 10.	Photomicrograph of CGCG incubated for MAC 387	43
Figure 11.	Photomicrograph of GCT incubated for MAC 387	43
Figure 12.	Photomicrograph of PGCG incubated for CD34	45
Figure 13.	Photomicrograph of CCG incubated for CD34	45
Figure 14.	Photomicrograph of GCT incubated for CD34	47
Figure 15.	Photomicrograph of PGCG incubated for HAM 56	47
Figure 16.	Photomicrograph of CGCG incubated for HAM 56	49
Figure 17.	Photomicrograph of GCT incubated for HAM 56	49
Figure 18.	Photomicrograph of PGCG incubated for Factor XIIIa	51
Figure 19.	Photomicrograph of CGCG incubated for Factor XIIIa	51
Figure 20.	Photomicrograph of GCT incubated for Factor XIIIa	53

ABBREVIATIONS

CGCG	central giant cell granuloma
PGCG	peripheral giant cell granuloma
GCT	giant cell tumor
AnBC	Aneurysmal bone cyst
ABC	avidin-biotin-peroxidase complex
μm	micrometre
IMVS	Institute of Medical and Veterinary Science
LCA	leukocyte common antigen
NHS	normal horse serum
PBS	phosphate-buffered saline solution

CHAPTER 1

REVIEW OF THE LITERATURE

CHAPTER 1 REVIEW OF THE LITERATURE

1.1 GIANT CELL GRANULOMAS

1.1.1 Introduction

1.1.2 Central giant cell granuloma

1.1.3 Peripheral giant cell granuloma

1.2 RELATED LESIONS

1.2.1 Aneurysmal bone cyst

1.2.2 Giant cell lesion (brown tumor) of hyperparathyroidism

1.2.3 Giant cell tumor of long bones

1.2.4 Relationship between CGCG and GCT

1.3 ORIGIN OF CELLULAR COMPONENTS

1.4 IMMUNOHISTOLOGICAL STUDIES

1.5 RATIONALE AND AIMS OF THE PRESENT STUDY

This review will examine the central and peripheral giant cell granulomas of the jawbones, as well as related lesions and differential diagnoses.

1.1 GIANT CELL GRANULOMAS

1.1.1 Introduction

Giant cell lesions of the jaws were mainly diagnosed as giant cell tumors before the early 1950s (Whitaker & Waldron 1993). In 1953 the term "giant cell reparative granuloma" was proposed by Jaffe to differentiate these lesions from the giant cell tumors which mostly occur at the ends of long bones. The word "reparative", however, is not accepted any longer due to the actual destructive nature of the giant cell granuloma (Auclair *et al.* 1988). The classic giant cell lesions of the jaws are the central giant cell granuloma (CGCG) and the peripheral giant cell (PGCG) granuloma.

1.1.2 Central giant cell granuloma

The central giant cell granuloma is a relatively common lesion (McDonald 1994) that is considered a benign process and appears almost totally within the jaws (Stewart 1993). However, isolated cases of this lesion in the small tubular bones of the hands and feet (Panico *et al.* 1994), facial bones (Stewart 1993) and the skull (Ratner & Dorfman 1990) have been reported.

Clinical features. The CGCG appears most commonly in children and young adults (Stewart 1993, Hall 1994) with about 75% of cases occurring prior to the age of 30 (Stewart 1993), and approximately 50% in patients under 16 years of age (McDonald 1994). The lesion appears more often in the mandible than maxilla, with the majority of cases arising anterior to the molar

teeth. The CGCG affects females more frequently than males in a ratio of approximately 2:1 (Stewart 1993, Hall 1994).

Radiographic features. The lesion may appear as a multilocular (51%) or unilocular radiolucency of bone. There is a correlation between size and locularity, with larger lesions assuming a multilocular appearance (Kaffe *et al.* 1996). Displacement and non-eruption of teeth and, less frequently, root resorption may be observed (Stewart 1993, McDonald 1994).

Histologic features. The CGCG is composed of a delicate to dense fibrillar connective-tissue stroma containing a proliferation of spindled mononuclear cells. Multinucleated giant cells are prominent throughout the connective tissue and may be focally aggregated or diffusely dispersed. Hence, the cellular compartment of these lesions consists of mononuclear stromal cells and multinucleated giant cells. The size of the giant cells can vary within a single lesion, as well as between different cases. The giant cells may contain only a few or dozens of nuclei. Numerous small, dilated vascular channels appear throughout the lesion, and extravasated erythrocytes as well as hemosiderin-laden macrophages are often prominent. Inflammatory cells are not frequently observed, but when present, they seem to be secondary in nature. Foci of new trabeculae of osteoid or bone may be present, particularly around the periphery of the lesion (Shafer *et al.* 1983, Stewart 1993, McDonald 1994).

Treatment and recurrence. Complete curettage and enucleation is usually considered as the preferred treatment (Stewart 1993). However, *en bloc* resection including the surrounding bone has been suggested for treatment of large and more aggressive lesions (Kermer *et al.* 1994). In recent years, two

alternative non-surgical approaches have also been described: local subcutaneous injection of corticosteroids (Jacoway *et al.* 1988, Kermer *et al.* 1994), and calcitonin therapy (Harris 1993).

Radiation therapy is not indicated due to sarcomatous transformation that may occur (Quick *et al.* 1980). However, on some occasions when complete surgical removal is impossible due to the location of the lesion, radiation therapy may be used in combination with the surgical procedure. Nevertheless, the potential for sarcomatous changes must be considered (Potter & Tiner 1993). Auclair *et al.* (1988) pointed out that the recurrence rate following curettage may be higher than that previously documented. In their study, 20% of the patients had developed recurrence. A higher rate of recurrence has been noted in children and young teens (Auclair *et al.* 1988, Stewart 1993).

1.1.3 Peripheral giant cell granuloma

The peripheral giant cell granuloma is thought to represent an abnormal hyperplastic connective tissue response to injury of gingival tissues (Regezi & Sciubba 1993a).

Clinical features. The PGCG, 34% of which occur between 5 and 15 years of age (Andersen *et al.* 1973), is found exclusively in the oral cavity, presenting only on the gingiva or the alveolar mucosa of edentulous areas. PGCG involves the mandible more frequently than the maxilla (McDonald 1994). The typical appearance of the lesion is a bluish-red, broad-based

mass which is usually about 1 cm in diameter. It may occur at any age and is more common in females (Regezi & Sciubba 1993a).

Histologic feature. Histologically, the PGCG is identical to its intraosseous counterpart, the CGCG. However, these two microscopically identical lesions can be separated on the basis of their clinical features (Regezi & Sciubba 1993a, McDonald 1994).

Treatment and recurrence. Complete surgical excision is the treatment of choice and recurrence is not common (Regezi & Sciubba 1993a). Harris (1993) reported that aggressive recurrent peripheral lesions can be treated by calcitonin therapy followed by excision.

1.2 RELATED LESIONS

1.2.1 Aneurysmal bone cyst

Clinical features. The aneurysmal bone cyst (AnBC) represents a non-neoplastic expansile bone lesion (Vergal De Dios *et al.* 1992) that may appear in the jaws or other bones. When the AnBC occurs in the mandible or maxilla, it tends to be found predominantly in the molar region. It is typically seen in patients under 30 years of age with the peak incidence in the second decade of life. A slight female predilection has been noted (Struthers & Shear 1984, Regezi & Sciubba 1993b).

Radiographic features. The aneurysmal bone cyst usually presents as a unilocular radiolucency. However, a multilocular pattern has also been observed in some cases (Regezi & Sciubba 1993b).

Histologic features. AnBC is composed of a fibrous connective tissue stroma containing frequent cavernous or sinusoidal spaces filled with blood.

The connective tissue also contains numerous young fibroblasts as well as multinucleated giant cells with similar distributions to that seen in CGCG. However, in the CGCG the cavernous spaces are not present. Varying amounts of hemosiderin as well as new osteoid and bone formation are found in AnBC (Struthers & Shear 1984, Regezi & Sciubba 1993b).

Relationship between AnBC and CGCG. When AnBC occurs in the posterior region of the mandible, especially in cases presenting a multilocular radiographic appearance, it may be impossible to distinguish from the CGCG radiographically. In these cases, CGCG must be differentiated on the basis of its histologic appearance (Regezi & Sciubba 1993b).

1.2.2 Giant cell lesion (brown tumor) of hyperparathyroidism

The brown tumor is a focal bony lesion which appears as the result of the direct effect of parathyroid hormone on bone (Parrish & O'Day 1986). This lesion appears microscopically identical to the CGCG. However, the giant cell lesion of hyperparathyroidism can be differentiated from CGCG on the basis of serum biochemical tests. In the case of hyperparathyroidism, serum calcium and alkaline phosphatase are increased, while serum phosphorus is decreased. With CGCG, however, normal serum chemistry values are expected (Stewart 1993, Hall 1994).

1.2.3 Giant cell tumor of long bones

Giant cell tumors (GCT) are considered to be true neoplasms and may demonstrate a broad range of biologic behavior from benign to malignant (Stewart 1993).

Clinical features. These tumors occur mainly in the long bones, especially in the area of the knee joint (Stewart 1993). Although infrequent, GCTs have also been reported in the jaws and other sites in the head and neck including the sphenoid, ethmoid and temporal bones (Quick *et al.* 1980, Stewart 1993). The most common age for the occurrence of these tumors is during the third and fourth decades of life (Stewart 1993, Schajowicz 1994). The sex ratio is 1.5:1, with females predominating over males (Schajowicz 1994).

Radiographic features. Radiographically, the GCT appears as a radiolucent lesion similar in appearance to the CGCG (Stewart 1993).

Treatment and recurrence. Surgical excision is usually the preferred treatment for giant cell tumor of long bones (Stewart 1993). However, surgical ablation (as complete as possible) with postoperative radiation therapy has been reported to be the treatment of choice for the giant cell tumor of the skull bones (Bertoni *et al.* 1992). Giant cell tumors of long bones exhibit a high rate of recurrence following treatment (30% following curettage) (Shafer *et al.* 1983, Stewart 1993). Schajowicz believes that the GCTs represent a progressive, potentially malignant process that can recur in about 20%-50% of cases (after curettage), exhibit sarcomatous transformations in about 5%-10% of patients, and even produce metastases without apparent previous malignant changes (Schajowicz 1981, 1993).

Histologic features. Like the CGCG, the GCT is composed of numerous multinucleated giant cells that are evenly dispersed among mononuclear stromal cells (Stewart 1993). Occasionally, numerous vacuoles of different sizes are found in the cytoplasm of a giant cell. The size and number of nuclei are variable, but usually there are more than 15-20, and there may be

hundreds. The tumor is rich in newly formed blood vessels, with walls composed only of endothelial cells, and in vascular channels that are lined by tumor cells themselves or by an endothelial layer (Schajowicz 1994). The multinucleated giant cells are irregularly distributed with focal aggregation around areas of haemorrhage. Mitosis is common in the spindle-shaped stromal cells, and areas of calcification can be found (Hall 1994).

1.2.4 Relationship between CGCG and GCT

CGCG and GCT of long bones

The giant cell tumor of bone has similar microscopical features to the CGCG (Whitaker & Waldron 1993). Nevertheless, some investigators believe careful examination and correct integration of clinical, radiographical, histopathological and cytometric data will allow differentiation (Ficarra *et al.* 1987, Kaw 1994, Sidoni *et al.* 1994).

Some clinical and histologic differences between the CGCG and GCT have been frequently reported such as: (1) True giant cell tumors of long bones do not occur before 20 years of age, (2) Giant cell tumors of long bones are locally more aggressive than those of the jaw, (3) Giant cell tumors have a higher recurrence rate than CGCG and have a tendency for malignant transformation, (4) The GCT contains larger giant cells (mean profile area of 1184-1230 μm^2) with greater number of nuclei (7.72 per cell profile for CGCG and 9.82 per profile for long bone GCT), (5) Giant cells in the GCT are more uniformly dispersed, (6) Foci of necrosis appear in GCT and not in CGCG, (7) The giant cells of the GCT exhibit a higher tendency for nuclei to be

aggregated centrally, (8) The giant cell tumors exhibit more prominent stromal cellularity with little inter-cellular matrix, (9) Fresh hemorrhage and hemosiderin are more commonly present in the CGCG, (10) The production of osteoid or new bone is more often present in the CGCG, and (11) The CGCG contains a greater number of spindle-shaped fibroblasts (Waldron & Shafer 1966, Hamlin & Lund 1967, Friedberg *et al.* 1969, Leban *et al.* 1971, Lucas 1972, Abrams & Shear 1974, Hirschl & Katz 1974, Smith & Ward 1978, Franklin *et al.* 1979, Mirra 1980, Fechner *et al.* 1984, Auclair *et al* 1988, Hall 1994).

GCT of the jawbones

Some authors have suggested that although the majority of CGCGs may be distinguished from GCTs on a histologic basis, a smaller subset of CGCGs may fall within the histologic profile of giant cell tumors of long bones. In contrast, some GCTs of long bones also exhibit the histologic features of the CGCGs of the jaws (Abrams & Shear 1974, Franklin *et al.* 1979, Chuong *et al.* 1986, Auclair *et al.* 1988).

According to Hall (1994), a true giant cell tumor of the jaw may not be a distinct entity, but this is still under debate. The more aggressive types of CGCG, which some classify as GCT of the jawbones, are often associated with swelling, erosion of the alveolar cortex, loosening of teeth, root resorption, pain, paraesthesia, and recurrence (Hall 1994). This author has also reported an aggressive giant cell lesion of the jaw which occurs consistently in young children (mean age 6 years). The lesion apparently

shows a degree of aggressiveness in between that of GCT and CGCG (Hall 1994). Chuong *et al.* (1986) suggested that all lesions be labeled "central giant cell lesion: clinical classification as aggressive or non-aggressive necessary".

Mintz *et al.* (1981) reported a primary malignant giant cell tumor of the mandible. The neoplasm presented as a lytic lesion which recurred following surgery and metastasized to the lungs and one lymph node. The authors emphasized that the histologic features of the tumor satisfied the diagnostic criteria of Dahlin *et al.* (1970) who proposed that "to qualify as a malignant giant cell tumor, a tumor must have histologic evidence of the benign counterpart in the lesion under study or in material removed previously from the same area". These criteria serve to differentiate the primary malignant giant cell tumor from cases of osteosarcomatous or fibrosarcomatous transformation.

As previously described, although CGCG can recur, it is almost always controlled with surgery alone, and radiation therapy is contraindicated. In contrast, radiation therapy forms an integral part of the treatment in GCT of the skull, and surgery alone does not control the disease. Therefore, identification of histologic parameters that could distinguish CGCGs from GCTs would be valuable in planning optimal therapy.

1.3 ORIGIN OF CELLULAR COMPONENTS

The origin and nature of the lesional cells in PGCG, CGCG and GCT is controversial. It has been suggested that the giant cells may arise from mononuclear stromal cells (Hanaoka *et al.* 1970, Steiner *et al.* 1972), macrophages (Kashara *et al.* 1979, Regezi *et al.* 1987), or osteoclasts (Athanasou *et al.* 1985, Flanagan *et al.* 1988, Bonetti *et al.* 1990). Some authors proposed that the multinucleated giant cells form by fusion of the mononuclear stromal cells (Schajowicz 1961, Adkins *et al.* 1969), while others suggested that they develop from bone cells (Pepler 1958, Sapp 1972), fibroblasts (Bartel & Piatowska 1977) or myofibroblasts (El-Labban & Lee 1983). It has also been proposed that the giant cells may originate by fusion of endothelial cells (Drepper & Themann 1961) and some investigators have labeled them as foreign body giant cells (Thompson *et al.* 1983). In addition to the previous suggestions, it has been proposed that the giant cells may be present only as a reactionary component of the lesion in response to an as yet unknown stimulus from the stroma (El-Mofty & Osdoby 1985, Cohen *et al.* 1988).

It has also been suggested that the mononuclear cells represent transformed histiocytes and are derived from the mononuclear-macrophage system (Ping *et al.* 1984, Regezi *et al.* 1987). Lim and Gibbins (1995) proposed that the blood vessels in giant cell lesions may be responsible for some of the unique features of these lesions.

1.4 IMMUNOHISTOLOGICAL STUDIES

Various studies have attempted to define the origin of the cellular components of giant cell lesions using immunohistochemical techniques. Seven cases of giant cell granulomas (1 CGCG and 6 PGCG) were immunohistochemically analyzed by Flanagan *et al.* (1988) using two mouse monoclonal antibodies to human osteoclasts (13C2 and 23C6) developed by Horton *et al.* (1985), and a panel of mouse monoclonal antibodies to human cell surface antigens. (13C2 and 23C6 are not commercially available.) All of the lesions showed strong positive membrane staining of giant cells with both of the osteoclast-specific monoclonal antibodies. Monoclonal antibodies EBM11 (an antibody reacting with macrophages and osteoclasts) and My7 (an antibody which reacts with osteoclasts) stained the cytoplasm and cell membrane of multinucleated giant cells, respectively. HLA-DR antigens were only recognized on the mononuclear cell component, and they were absent from giant cells. Furthermore, multinucleated giant cells were found to excavate bone *in vitro* and possess receptors for calcitonin (Flanagan *et al.* 1988). Flanagan *et al.* concluded that these results provide strong evidence for the osteoclastic nature of the giant cell component of central and peripheral giant cell granulomas.

Bonetti *et al.* (1990) assessed the immunoreactivity of giant cells in nine cases of peripheral giant cell granulomas. In their study, giant cells were unreactive to antibodies recognizing myelomonocytic (lysozyme) and macrophage markers (MAC 387 and HAM 56). However, giant cells showed strong staining with MB1, an antibody reactive with osteoclasts.

The immunohistochemical staining pattern of giant cells from one case of giant cell tumor of bone has been compared with that of osteoclasts by Athanasou *et al.* (1985). Only EBM 11, an antibody reacting with a wide spectrum of macrophages, stained both osteoclasts and giant cells, but stromal cells and osteoblasts did not react.

Whitaker and Bouquot (1994a,b) evaluated ten cases of CGCG and ten cases of PGCG for the detection of estrogen and progesterone receptor proteins. Staining for progesterone receptors was completely negative in all cases for both giant cells and mononuclear cells. Staining for estrogen receptors was essentially negative in all cases of CGCGs. However, three cases of PGCG exhibited strong staining for estrogen receptors when examining the mononuclear cell population.

The antigenic phenotype of giant cells in giant cell lesions of bone and soft tissues was compared with that of osteoclasts and macrophage polykaryons by Doussis *et al.* (1992). These authors concluded that osteoclasts, and giant cells of GCT and GCG could be distinguished from macrophage polykaryons and giant cells in other lesions by their generalized absence of HLA-DR reaction. Doussis *et al.* (1992) found staining for leukocyte common antigen (LCA), CD68, and HLA-DR useful in distinguishing reactive histiocytic giant cells and osteoclasts from tumor giant cells.

Five cases of giant cell granulomas of distal skeletal bones were immunohistochemically evaluated by Panico *et al.* (1994). All cases showed

positive staining of the stromal spindle cells for vimentin and actin. The osteoclast-like giant cells were positive for CD68, vimentin and LCA.

Lim and Gibbins (1995) studied twenty giant cell lesions (eight CGCGs, nine PGCGs and three GCTs) using a panel of immunohistochemical markers. In this study, the multinucleated giant cells showed positive staining with MB1 (osteoclast marker), the mononuclear round cells were positive to LCA and the spindle cells were unreactive to all of the markers. In particular, the spindle cells were unreactive to smooth muscle specific actin. They also showed that the blood vessels on the periphery of the lesions stained strongly positive for Factor VIII related antigen. The blood vessels located deeper in the lesions, however, did not react to the antibody.

On the basis of this review of previous immunohistochemical studies of giant cell lesions, it would appear that:

- the mononuclear stromal cell component, which may be important in the understanding of the pathogenesis of these lesions, has largely been ignored.
- the microwave-mediated antigen retrieval methods recently introduced by Shi *et al.* (1991) as "an enhancement method for immunohistochemical staining in formalin-fixed paraffin-embedded tissues based on microwave oven heating of tissue sections" have not been utilized in such studies.
- the origin and nature of the cellular components of giant cell lesions is still the subject of debate.

1.5 RATIONALE AND AIMS OF THE PRESENT STUDY

The origin and nature of the cellular components in giant cell lesions has not been satisfactorily established and is still debatable. Further characterisation of giant cell lesions is necessary before their pathogenesis can be determined.

The aims of the present study were to:

a. Perform a retrospective clinical analysis on a subset of giant cell lesions taken from a South Australian population.

b. Further evaluate the antigenic profile of the cellular components of giant cell lesions of the jaws using a panel of antibodies, and enhanced antigen retrieval techniques using microwave fixation.

c. Further investigate the possible differences between the antigenic profiles of the cellular components of giant cell lesions occurring in the jaws and those of lesions in extragnathic sites.

CHAPTER 2

MATERIALS AND METHODS

CHAPTER 2 MATERIALS AND METHODS

2.1 CLINICAL DATA

2.2 IMMUNOHISTOCHEMICAL STUDY

2.2.1 Preparation of sections

2.2.2 Microwave-mediated antigen retrieval and immunostaining

2.2.3 Controls for immunohistochemical study

2.2.4 Evaluation of immunostaining

2.1 CLINICAL DATA

All cases diagnosed and coded as CGCG, PGCG and GCT for the period 1969-95 were recovered from the files of the Department of Oral Pathology, The University of Adelaide and the Institute of Medical and Veterinary Science (IMVS), Adelaide, South Australia. The diagnoses were reviewed by an oral pathologist and a total of 55 cases were accepted as examples of the lesions including 16 cases of CGCG, 35 cases of PGCG, and 4 cases of GCT.

The histories of all cases were reviewed and pertinent data regarding age, sex and site of the lesions were tabulated, analyzed and compared with international frequency studies. Data regarding the locations of the lesions was not available for all of the cases.

2.2 IMMUNOHISTOCHEMICAL STUDY

2.2.1 Preparation of sections

Thirty eight formalin fixed, paraffin-embedded specimens were available for the immunohistochemical part of the study, including 15 CGCG, 20 PGCG and 3 GCT of bone. The specimens were sectioned at 5 μm (micrometre) intervals, and sections were processed using previously coated (subbed) glass slides (Appendix A) for immunohistochemistry. One section from each specimen was routinely stained with haematoxylin and eosin for reference.

A panel of immunohistochemical markers including CD68/KP1 (human macrophage / monocyte marker), CD34 (human hematopoietic progenitor cell / endothelial cell marker), HAM 56 (human macrophage / endothelial cell

marker), MAC 387 (human myeloid / histiocyte / monocyte marker), and Factor XIIIa or fibrin stabilizing factor, which labels bone marrow derived dermal dendritic cells, were applied to dewaxed sections of the specimens in order to examine the immunocharacteristics of these lesions. CD68, MAC 387 and HAM 56 were selected to study the histogenesis of both the multinucleated and mononuclear stromal cell components. CD34, an endothelial cell marker (van de Rijn & Rouse 1994), was chosen to investigate the blood vessels of these lesions. Factor XIIIa was selected in order to establish a relationship between the cells of giant cell lesions and dermal dendrocytes.

The source, dilution and type of antibodies used are showed in Table 1.

Table 1. Antibodies used: Sources, dilutions and types.

Antibody	Type	Dilution	Source
CD68/KP1	monoclonal	1/2000	DAKO, Carpenteria, CA
CD34	monoclonal	1/100	Novacastra, Newcastle, UK
MAC 387	monoclonal	1/200	DAKO, Carpenteria, CA
HAM 56	monoclonal	1/600	DAKO, Carpenteria, CA
Factor XIIIa	polyclonal	1/25000	Calbiochem, La Jolla, CA

The sections were processed using microwave-mediated antigen retrieval methods (Shi *et al.* 1991) and subsequently stained using avidin-biotin-peroxidase complex (ABC) techniques (Hsu *et al.* 1981). All of these procedures were performed under the supervision of Mr. James Milios in the immunohistochemistry laboratory of the IMVS, Adelaide.

2.2.2 Microwave-mediated antigen retrieval and immunostaining

1. Sections were placed into a plastic staining rack and dewaxed using Xylene.

2. Endogenous peroxidase was inhibited by incubating the sections with 0.5% hydrogen peroxide in methanol for 30 minutes and followed by air-drying.

3. The sections were washed for 1 minute in running water, followed by several quick changes of deionised water.

4. The slides were placed into containers of citrate buffer solution (250 ml each) then microwaved on full power using a Toshiba 1000 Watt microwave oven until the buffer solutions began to boil.

5. The slide-bearing containers were transferred to an NEC microwave oven (Mode 1702) with the power setting on level 2 (Magnetron cycle: 6 seconds ON, 16 seconds OFF) for 10 minutes. This allowed the solution to reach almost boiling point in a cyclic manner, thus minimising damage to tissue sections during the bubbling process. At the completion of the microwaving, the containers were allowed to cool at room temperature for 25 minutes. Microwave safe plastic jars and staining racks were used for the microwave procedure.

6. Sections were encircled with "PAP" pen (Dakopatts, USA. Cat No.S2002), and placed in phosphate-buffered saline solution (PBS) pH 7.4 for 5 minutes, followed by 3% normal horse serum (NHS) for 20 minutes.

7. Excess NHS was drained (suctioned) and sections were incubated with primary antibody appropriately diluted with NHS, overnight at room temperature.

8. Sections were twice washed in PBS pH 7.4, 3 minutes each time.

9. Biotinylated secondary antibody was directed against primary species, diluted with NHS and incubated for 30 minutes. (The secondary antibodies and their dilutions are showed in Table 2.)

10. Sections were twice washed in PBS pH 7.4, 3 minutes each time.

11. Sections were incubated with peroxidase conjugated streptavidin (Pierce, USA) 1/1500 diluted with NHS for 60 minutes.

12. Sections were twice washed in PBS pH 7.4, 3 minutes each time.

13. Sections were incubated in peroxidase substrate solution: 25 mg diaminobenzidine tetrahydrochloride (Sigma, Cat No. D-5637) to 50 ml Tris HCL buffer pH 7.4, and 50 μl of 30% hydrogen peroxide. Reaction was controlled macroscopically and microscopically, 15-20 minutes.

14. Sections were rinsed in PBS pH 7.4

15. Sections were lightly counterstained with Mayer's haematoxylin.

16. Sections were dehydrated, cleared and mounted.

2.2.3 Controls for immunohistochemical study

Positive controls consisted of tissues with known antigenic positivity and included sections of spleen (for CD68 and MAC 387), sections of placenta (for HAM 56 and Factor XIIIa) and a section of lymphoid tissue (for CD34). Negative controls (2 sections per specimen) were obtained by omitting the primary antibody or substitution of primary antibody with a non-related antibody.

Table 2. Primary antibodies, related secondary antibodies and their dilutions.

Primary Antibody	Secondary Antibody	Dilution of Secondary Antibody
CD68/KP1 (monoclonal)	rabbit anti mouse serum	1/500
CD34 (monoclonal)	rabbit anti mouse serum	1/500
MAC 387 (monoclonal)	rabbit anti mouse serum	1/500
HAM 56 (monoclonal)	rabbit anti mouse serum	1/250
Factor XIIIa (polyclonal)	swine anti rabbit serum	1/500

2.2.4 Evaluation of immunostaining

Immunohistochemical reactivities were assessed by 3 reviewers. All sections were reviewed in an Olympus CH-2 microscope and photographed using Kodak Kodachrome 64 daylight film.

Immunoreactivity was semi-quantitatively graded depending on the degree of immunoprecipitate intensity. Intensity was graded on a - to + + + scale as follows: - negative, + weakly positive, + + moderately positive, and + + + strongly positive. The approximate percentage (quartile) range of cells

reacting positively (in the one section) to each antibody was also evaluated in order to assess uniformity of staining. The cells examined included multinucleated giant cells, mononuclear stromal cells and endothelial cells.

CHAPTER 3

RESULTS

CHAPTER 3 RESULTS

3.1 CLINICAL DATA

3.1.1 Tabulation of data

3.1.2 Analysis of data

3.2 IMMUNOHISTOCHEMICAL STUDY

3.1 CLINICAL DATA

3.1.1 Tabulation of data

Data (age, sex and location of the lesion) from patients with CGCG, PGCG and GCT are presented in Table 3, Table 4 and Table 5 respectively.

Table 3. Summary of data from patients with CGCG

Age (years)	Sex	Location
65	M	left mandible
7	M	mandible
60	F	maxilla
7	M	mandible
32	M	right mandible
55	F	*
45	M	mandible, molar region
73	F	left mandible
7	F	maxilla
9	M	left maxilla
22	M	*
*	M	right maxilla
36	F	left maxilla
30	F	left maxilla
*	M	right maxilla
9	M	left mandible
Average age = 32.6 Age range = 7 - 73 *Unknown = 2	M = 10 (62%) F = 6 (38%) Total = 16	Mandible = 7 (50%) Maxilla = 7 (50%) *Unknown = 2

Table 4. Summary of data from patients with PGCG

Age (years)	Sex	Location
65	F	*
47	M	*
*	F	right mandible
22	M	left mandible
76	M	*
71	M	*
47	M	mandible
23	F	mandible
67	F	*
64	M	*
45	F	*
69	M	*
71	F	mandible
43	F	mandible
36	F	mandible
21	F	mandible
47	M	mandible
*	F	maxilla
13	M	mandible
62	M	*
10	M	maxilla
18	M	*
49	F	maxilla
25	M	left maxilla
12	M	left maxilla
42	F	mandible
14	M	left maxilla
65	M	*
14	M	right maxilla
40	M	mandible
63	M	left mandible
72	F	right mandible
8	M	right maxilla
38	F	left maxilla
37	F	right maxilla
Average Age = 42.3 Age range = 8-76 *Unknown = 2	M = 20 (60%) F = 15 (40%) Total = 35	Mandible = 14 (58%) Maxilla = 10 (42%) *Unknown = 11

Table 5. Summary of data from patients with GCT

Age (years)	Sex	location
12	M	right radius
13	F	left fibula
21	F	*
16	M	*

* Data regarding location was not available for 2 cases.

3.1.2 Analysis of data

Age and sex distribution of patients with CGCG and PGCG are shown in Figure 1 and Figure 2 respectively. (The number of GCTs was insufficient for statistical analysis.)

a. CGCG: In the present study, 50% of lesions occurred before the age of 31 and 35% appeared before the age of 11.

b. PGCG: In the current series, 21% of lesions occurred before the age of 21 and 33% appeared prior to 31 years of age.

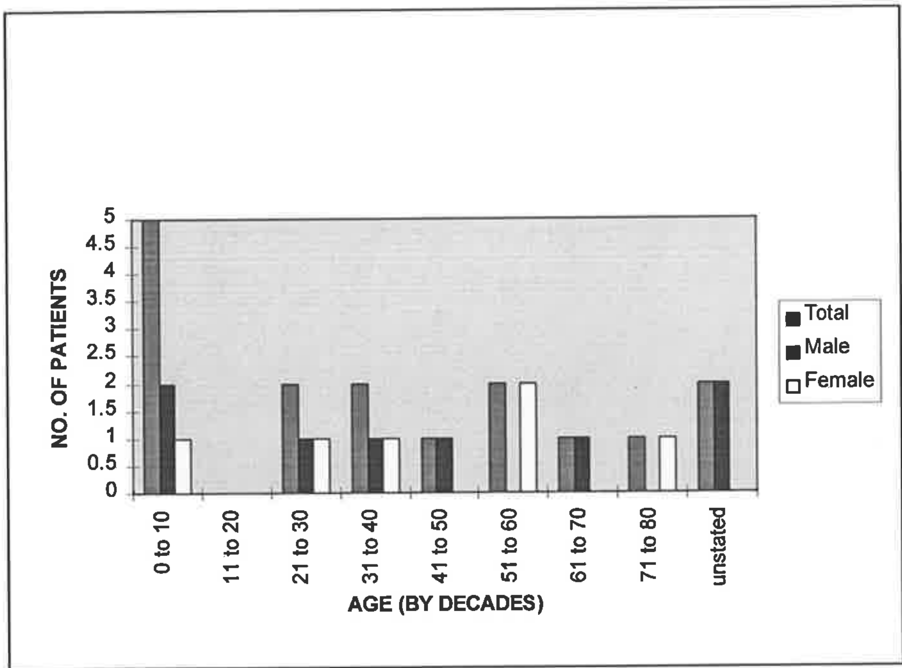


Figure 1. Age and sex distribution of 16 cases of CGCG

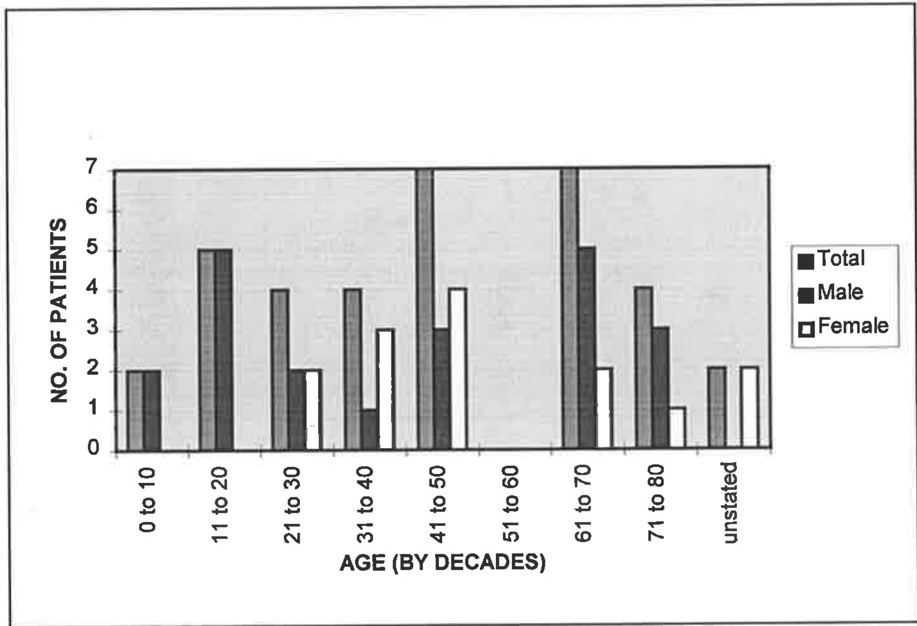


Figure 2. Age and sex distribution of 35 cases of PGCG

3.2 IMMUNOHISTOCHEMICAL STUDY

The results of immunohistochemical staining of 20 cases of PGCG, 15 cases of CGCG, and 3 cases of GCT are summarized in Table 6, Table 7 and Table 8 respectively. Immunoreactivity was semi-quantitatively graded depending on the degree of immunoprecipitate intensity. The approximate percentage range of cells reacting positively (in the one section) to each antibody was also evaluated in order to assess uniformity of staining. Representative sections of each of the three categories of lesions studied are shown in Figures 3-5. Examples of the immunohistochemical staining are illustrated in Figures 6-20.

Results of immunohistochemistry of the cellular components in CGCG and PGCG

- No difference was found in immunostaining patterns of cellular components between the CGCG and the PGCG.
- As shown in Table 6 and Table 7, 26-50% of mononuclear stromal cells exhibited strong staining with HAM 56 in 19 cases of PGCG and 13 cases of CCGC. However, mononuclear stromal cells in 1 case of PGCG and 2 cases of CGCG remained totally unreactive to HAM 56.
- The corresponding cells in all of the negative control sections remained unstained.

Comparison of results of immunohistochemistry of the cellular components in CGCG, PGCG and GCT.

- In CGCG and PGCG, 26-50% of mononuclear stromal cells exhibited strong staining with HAM 56 or Factor XIIIa. In the case of GCT, however, 51-75% of the mononuclear stromal cells of each section reacted strongly with HAM 56 or Factor XIIIa.
- Endothelial cells of 51-75% of the blood vessels showed strong immunoreactivity to HAM 56 in the cases of CGCG and PGCG. In cases of GCT, however, endothelial cells of 26-50% of blood vessels exhibited only a moderate positive reaction to HAM 56.
- More than 75% of the giant cells in CGCGs and PGCGs exhibited a weak positive reaction to Factor XIIIa. The giant cells in GCTs, however, remained unreactive to the same antibody.
- All three cases of GCT were much more vascular compared to CGCGs and PGCGs.
- The corresponding cells in all of the negative control sections remained unstained.

Table 6. Immunoreactivity of cellular components in 20 cases of PGCG. Intensity was graded on a - to + + + scale (- negative, + weakly positive, + + moderately positive, and + + + strongly positive). Percentage of cells refers to approximate proportion of cells (quartile range) reacting positively to that specific antibody within the one section (indicative of uniformity of staining).

Cellular Components	CD68		MAC 387		CD34		HAM 56		Factor XIIIa	
	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells
Giant Cells	+ + +	76-100	-		-		-		+	>75
Mononuclear Cells	+ +	51-75	+ + +	<25	-		+ + +	26-50	+ + +	26-50
Endothelial Cells	-		-		+ + +	76-100	+ + +	51-75*	-	

*Mainly endothelial cells of smaller blood vessels

Table 7. Immunoreactivity of cellular components in 15 cases of CGCG. Intensity was graded on a - to + + + scale (- negative, + weakly positive, + + moderately positive, and + + + strongly positive). Percentage of cells refers to approximate proportion of cells (quartile range) reacting positively to that specific antibody within the one section (indicative of uniformity of staining).

Cellular Components	CD68		MAC 387		CD34		HAM 56		Factor XIIIa	
	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells
Giant Cells	+ + +	76-100	-		-		-		+	>75
Mononuclear Cells	+ +	51-75	+ + +	<25	-		+ + +	26-50	+ + +	26-50
Endothelial Cells	-		-		+ + +	76-100	+ + +	51-75*	-	

*Mainly endothelial cells of smaller blood vessels

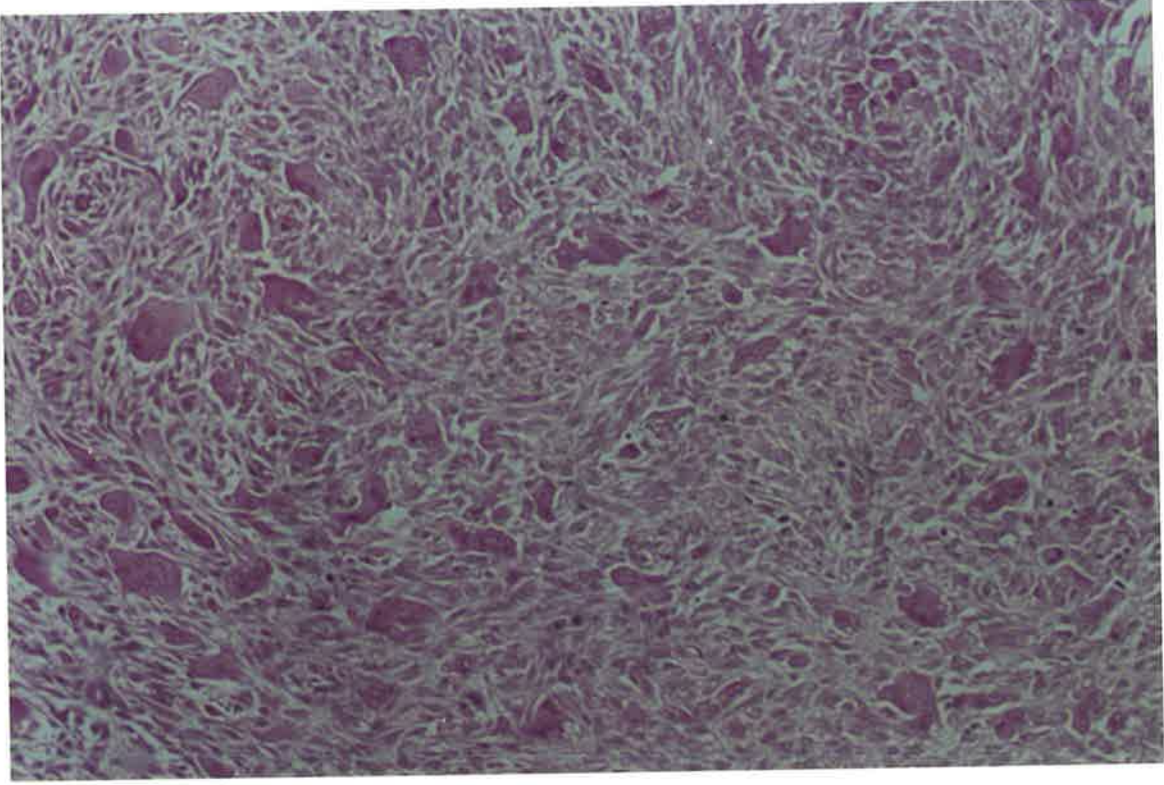
Table 8. Immumoreactivity of cellular components in 3 cases of GCT. Intensity was graded on a - to + + + scale (- negative, + weakly positive, + + moderately positive, and + + + strongly positive). Percentage of cells refers to approximate proportion of cells (quartile range) reacting positively to that specific antibody within the one section (indicative of uniformity of staining).

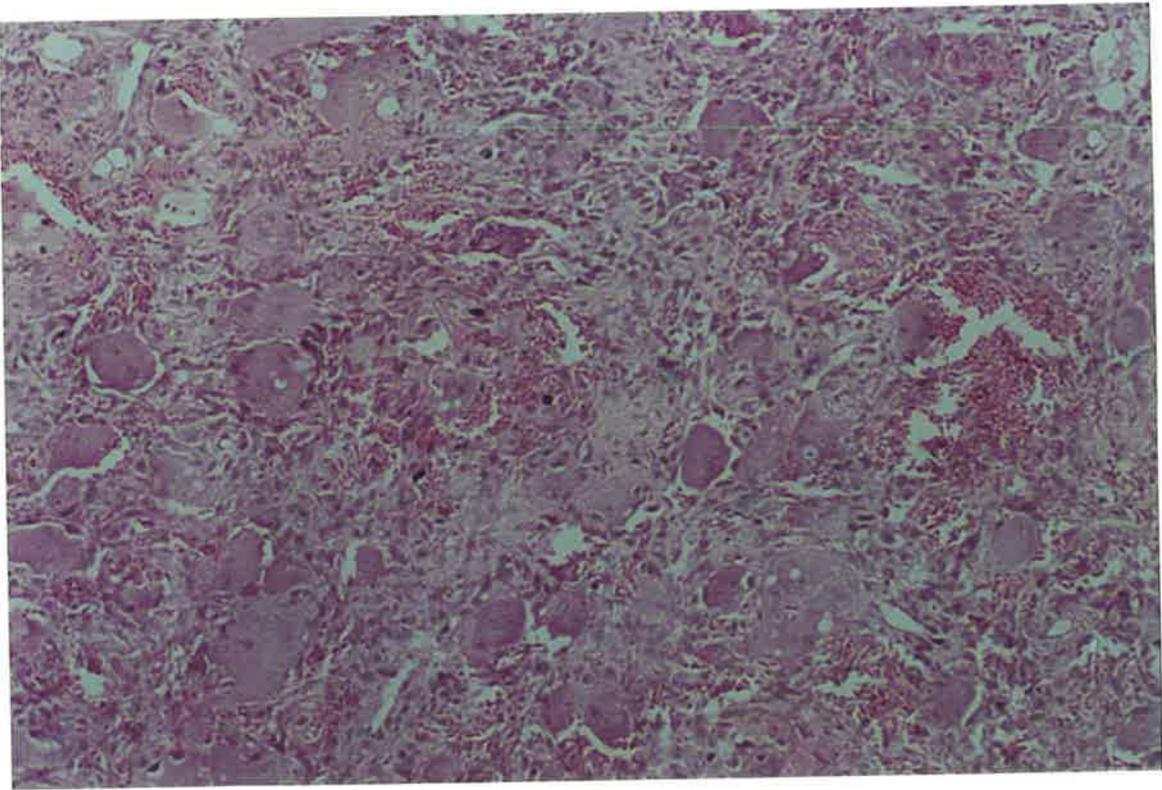
Cellular	CD68		MAC 387		CD34		HAM 56		Factor XIIIa	
Components	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells	Intensity	% Cells
Giant Cells	+ + +	76-100	-		-		-		-	
Mononuclear Cells	+ +	51-75	+ + +	<25	-		+ + +	51-75	+ + +	51-75
Endothelial Cells	-		-		+ + +	76-100	+ +	26-50*	-	

*Mainly endothelial cells of smaller blood vessels

Figure 3: Photomicrograph showing a representative field of a PGCG. Note the characteristic multinucleated giant cells and mononuclear stromal cells. (Haematoxylin & eosin, Original magnification x100)

Figure 4: Photomicrograph showing a representative field of a CGCG. Note the characteristic multinucleated giant cells and mononuclear stromal cells. (Haematoxylin & eosin, Original magnification x100)





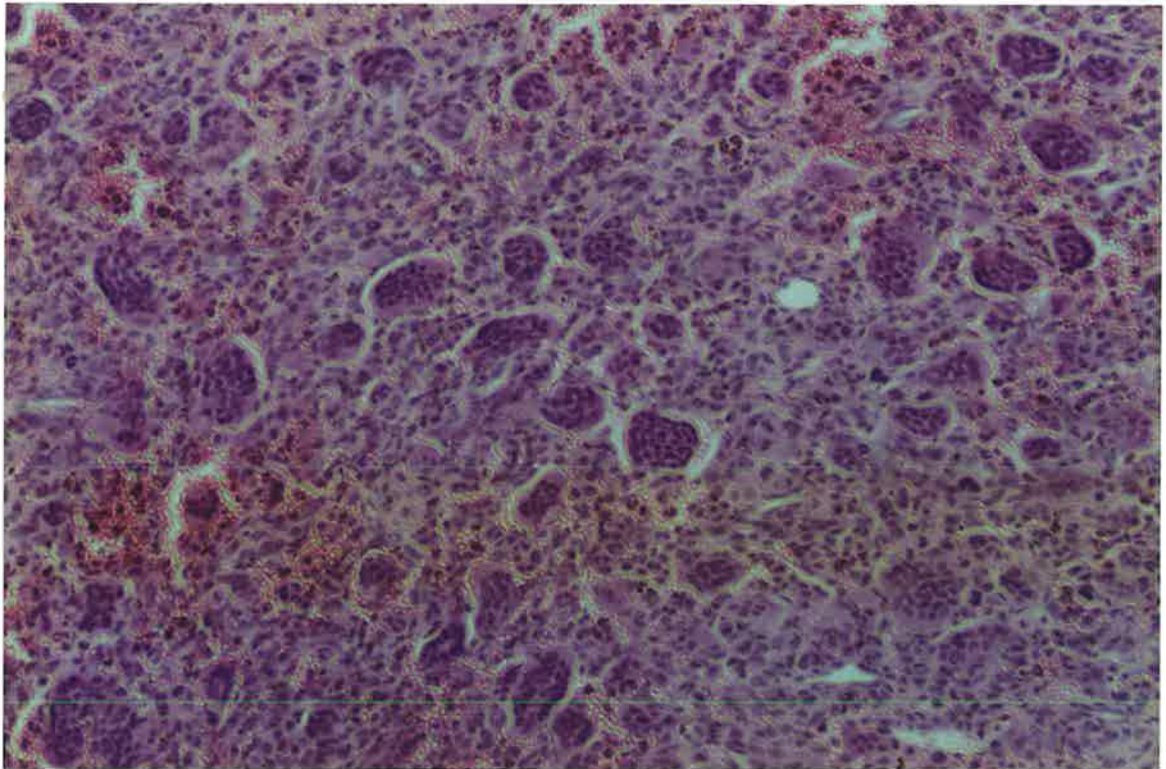
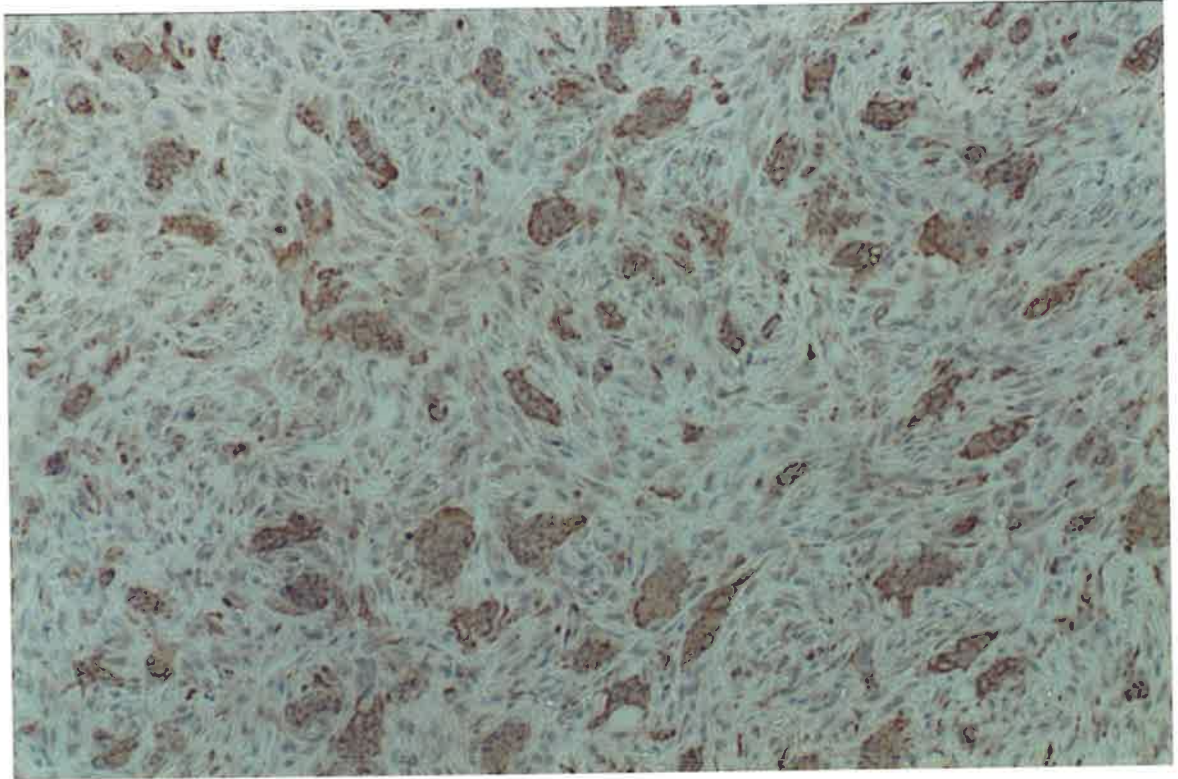


Figure 5: Photomicrograph showing a representative field of a GCT of bone. Note the characteristic multinucleated giant cells and mononuclear stromal cells. (Haematoxylin & eosin, Original magnification x100)

Figure 6: Photomicrograph of PGCG incubated for CD68 using ABC technique and counterstained with haematoxylin. Note strong positive reaction of giant cells to the antibody (Grade: + + +). Staining for giant cells was assessed as 76-100%. Note moderately positive reaction of mononuclear cells to the antibody (Grade + +). Staining for mononuclear cells was assessed as 51-75%. (Original magnification x100)

Figure 7: Photomicrograph of CGCG incubated for CD68 using ABC technique and counterstained with haematoxylin. Note strong positive reaction of giant cells to the antibody (Grade: + + +). Staining for giant cells was assessed as 76-100%. Note moderately positive reaction of mononuclear cells to the antibody (Grade + +). Staining for mononuclear cells was assessed as 51-75%. (Original magnification x100)



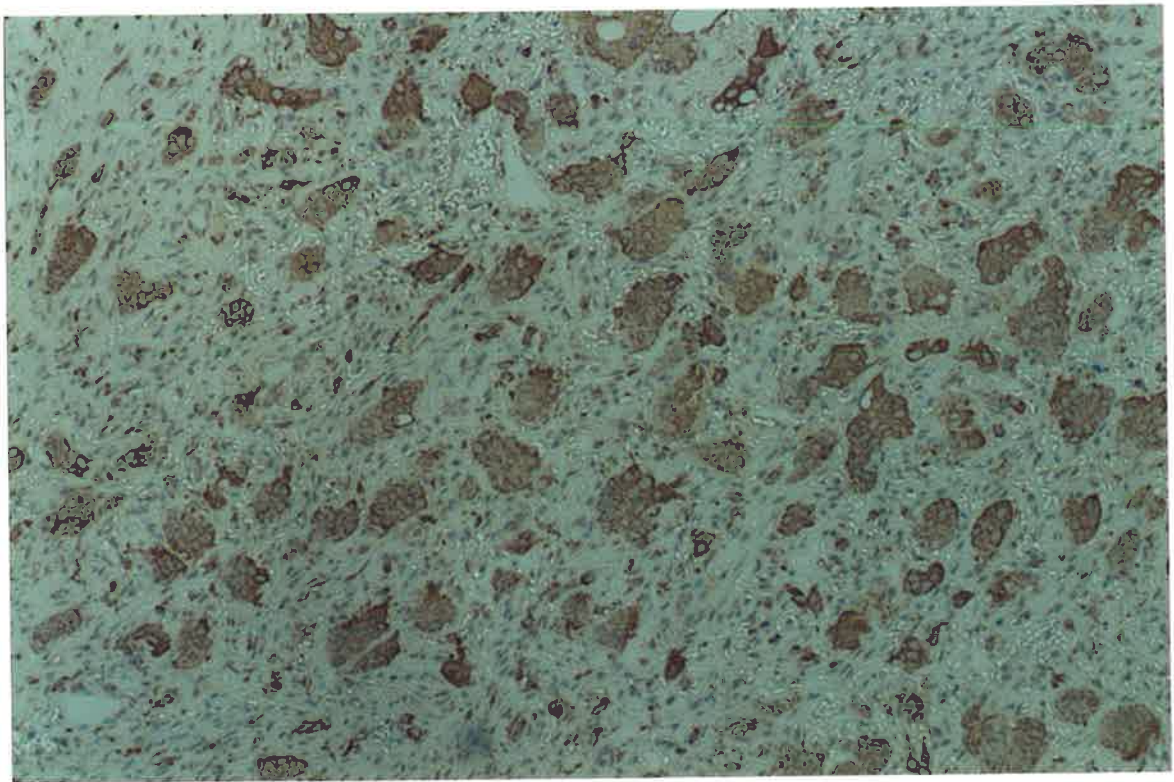
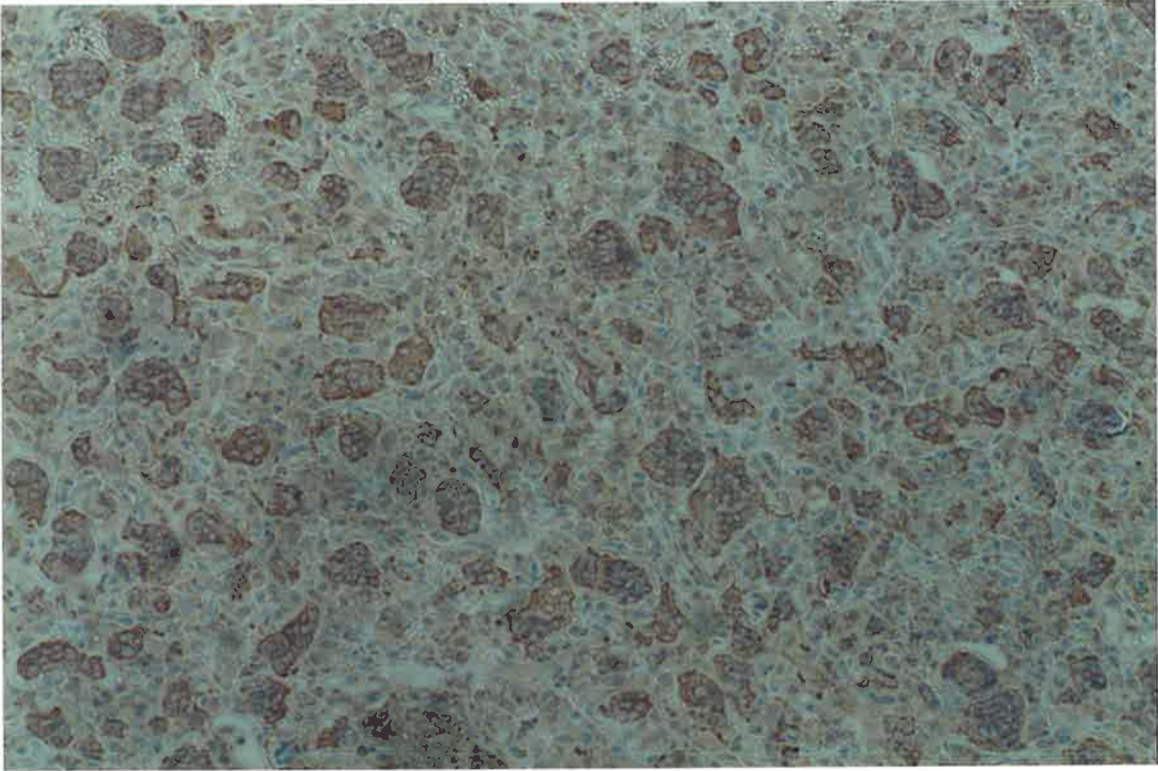


Figure 8: Photomicrograph of GCT incubated for CD68 using ABC technique and counterstained with haematoxylin. Note strong positive reaction of giant cells to the antibody (Grade: + + +). Staining for giant cells was assessed as 76-100%. Note moderately positive reaction of mononuclear cells to the antibody (Grade + +). Staining for mononuclear cells was assessed as 51-75%. (Original magnification x100)

Figure 9: Photomicrograph of PGCG incubated for MAC 387 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) to the antibody (Grade: -). Note strong positive reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as <25%. (Original magnification x100)



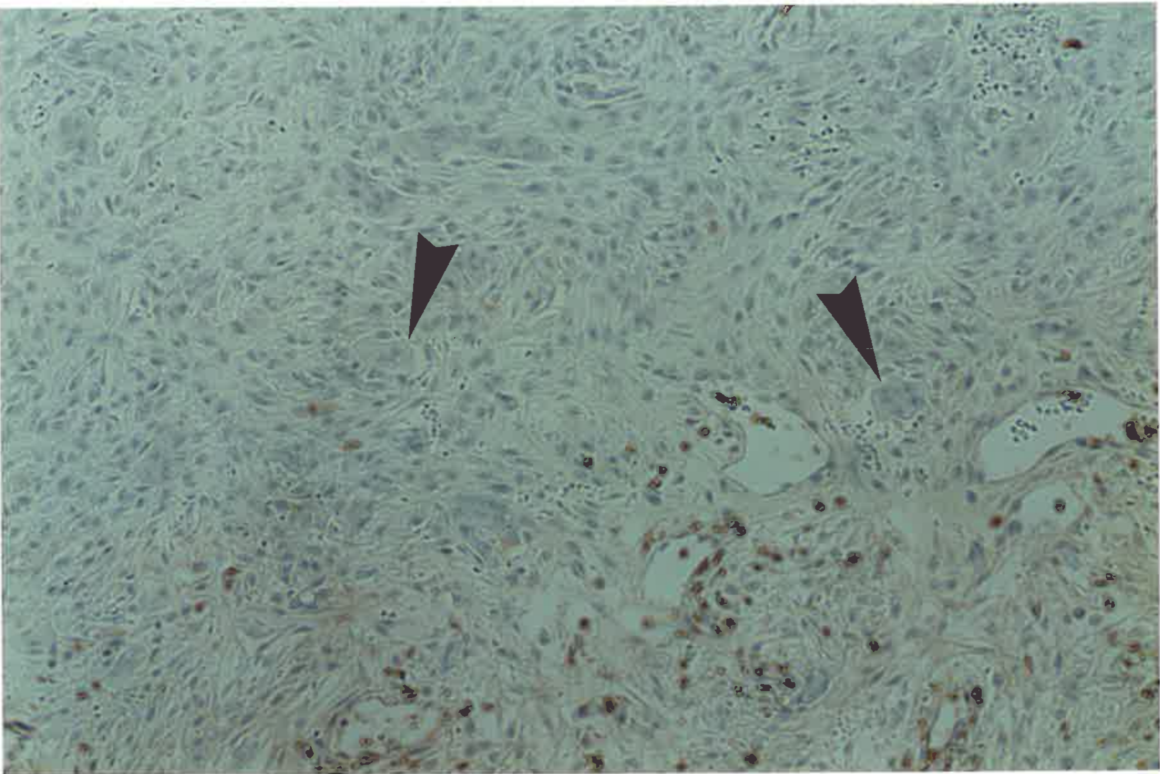
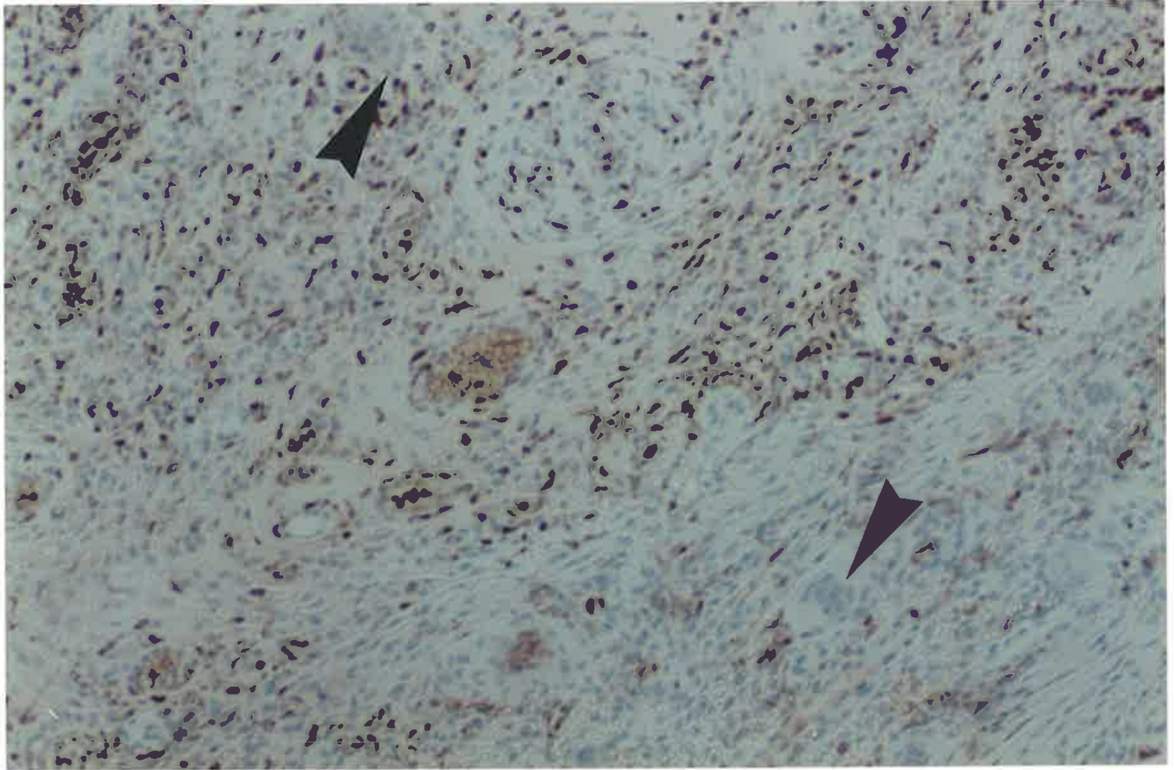


Figure 10: Photomicrograph of CGCG incubated for MAC 387 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) to the antibody (Grade: -). Note strong positive reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as <25%, as mononuclear cells did not react to the antibody in some areas of the section. (Original magnification x100)

Figure 11: Photomicrograph of GCT incubated for MAC 387 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) to the antibody (Grade: -). Note strong positive reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as <25%. (Original magnification x100)



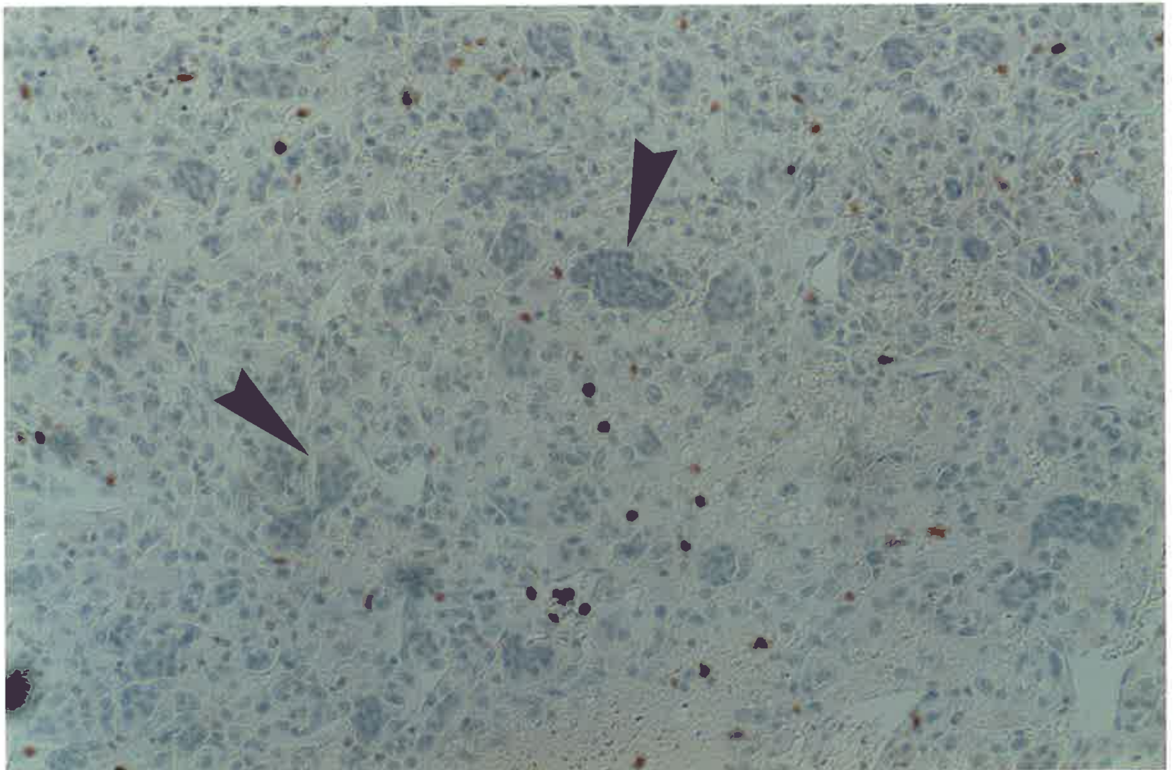
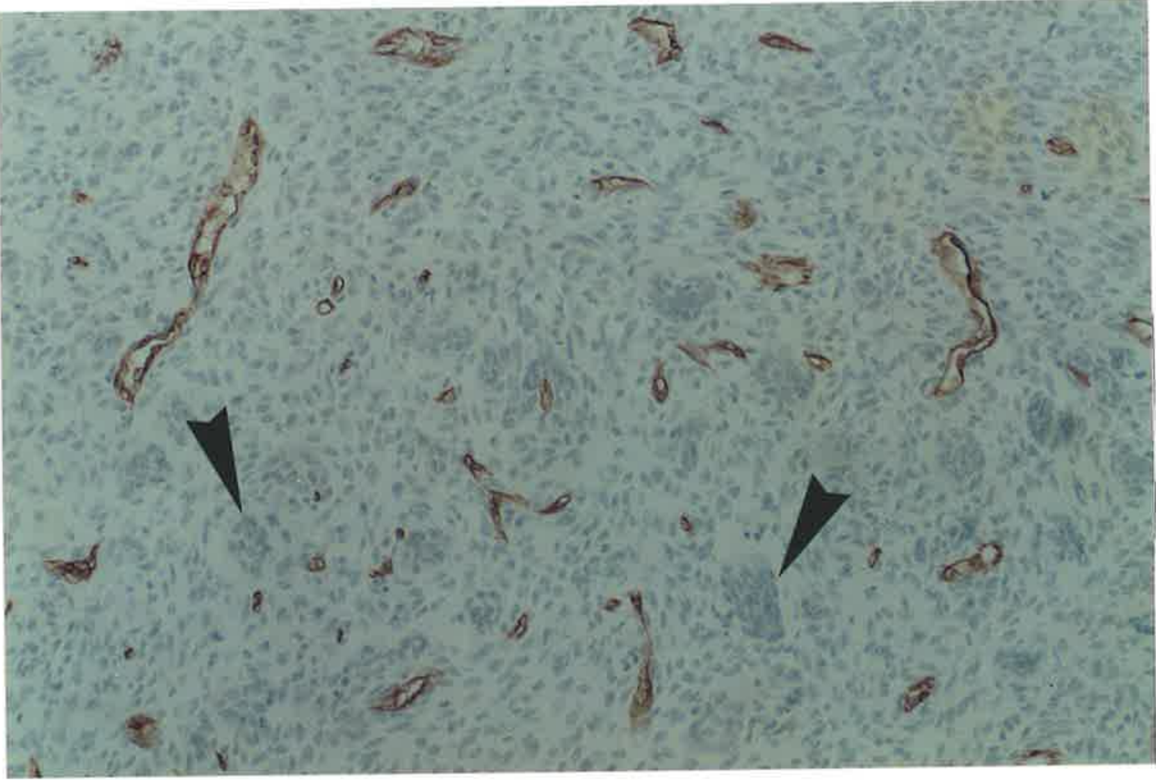


Figure 12: Photomicrograph of PGCG incubated for CD34 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) and mononuclear stromal cells to the antibody (Grade: -). Note strong positive reaction of endothelial cells to the antibody (Grade + + +). Staining for endothelial cells was assessed as 76-100%. (Original magnification x100)

Figure 13: Photomicrograph of CGCG incubated for CD34 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) and mononuclear stromal cells to the antibody (Grade: -). Note strong positive reaction of endothelial cells to the antibody (Grade + + +). Staining for endothelial cells was assessed as 76-100%. (Original magnification x100)



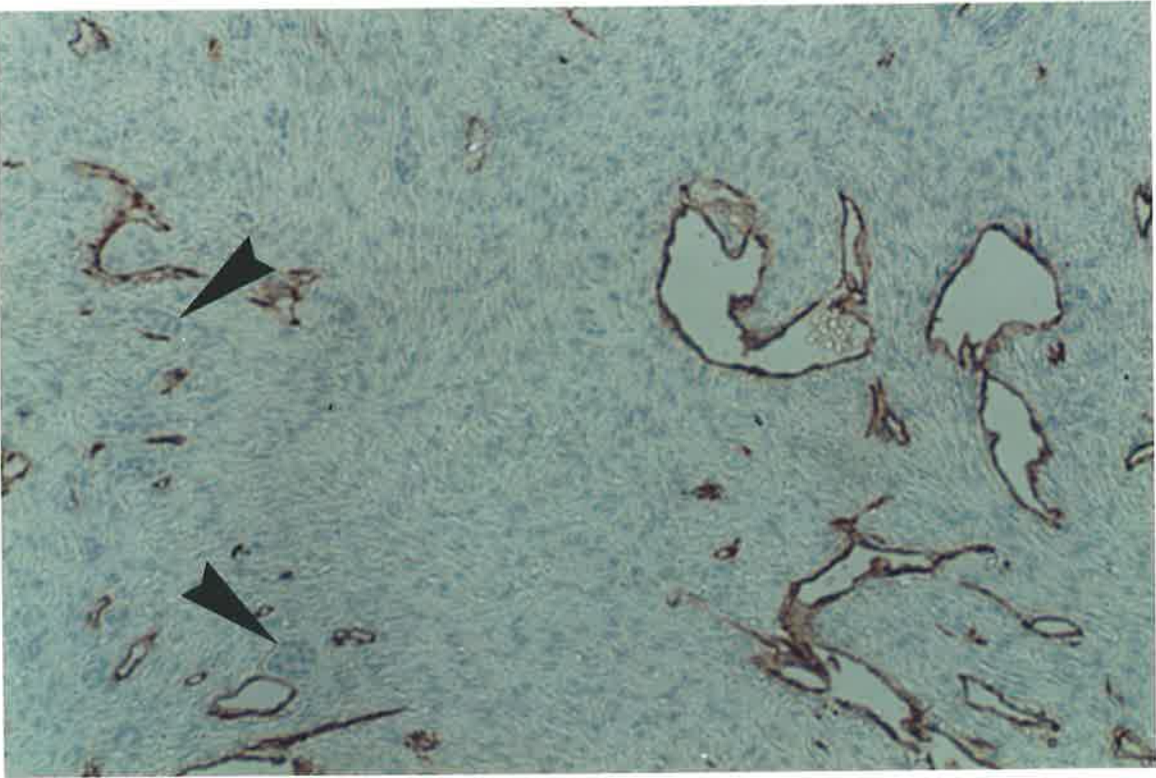
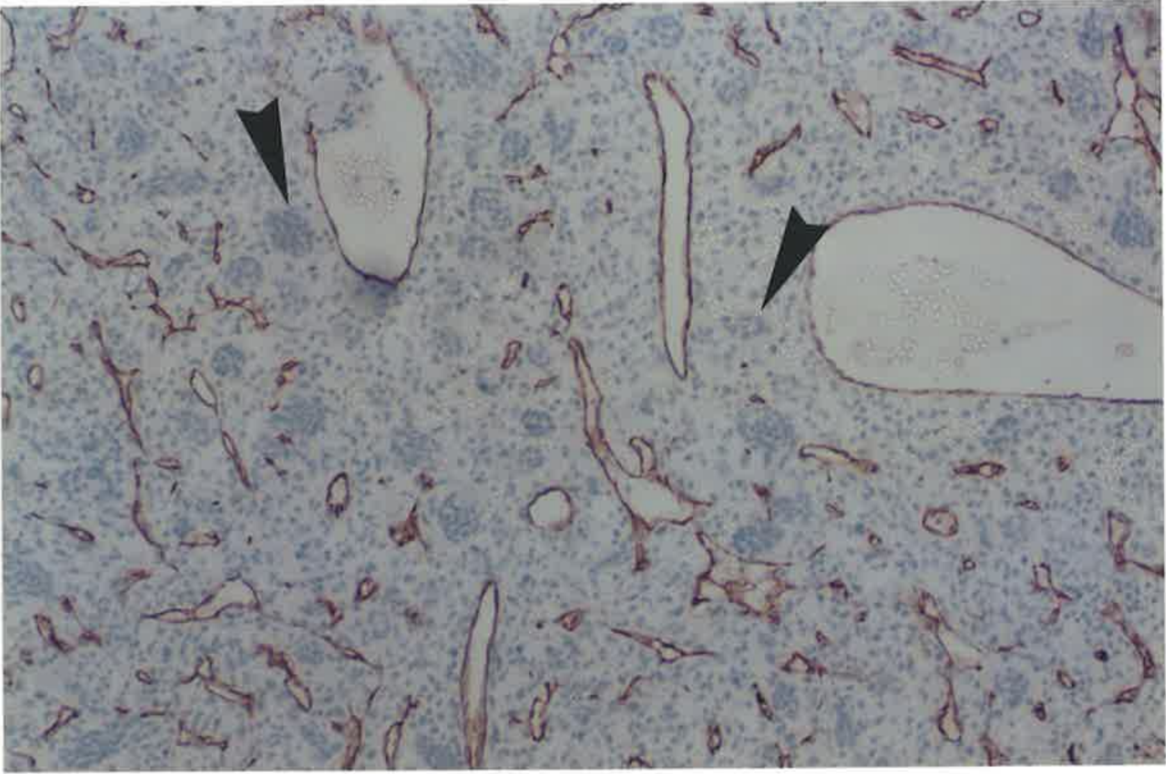


Figure 14: Photomicrograph of GCT incubated for CD34 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) and mononuclear stromal cells to the antibody (Grade: -). Note strong positive reaction of endothelial cells to the antibody (Grade + + +). Staining for endothelial cells was assessed as 76-100%. (Original magnification x100)

Figure 15: Photomicrograph of PGCG incubated for HAM 56 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (large arrows) to the antibody (Grade: -). Note strong positive reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as 26-50%. Note strong positive reaction of endothelial cells (mainly those of smaller blood vessels, small arrows) to the antibody (Grade + + +). Staining for endothelial cells was assessed as 51-75%. (Original magnification x100)



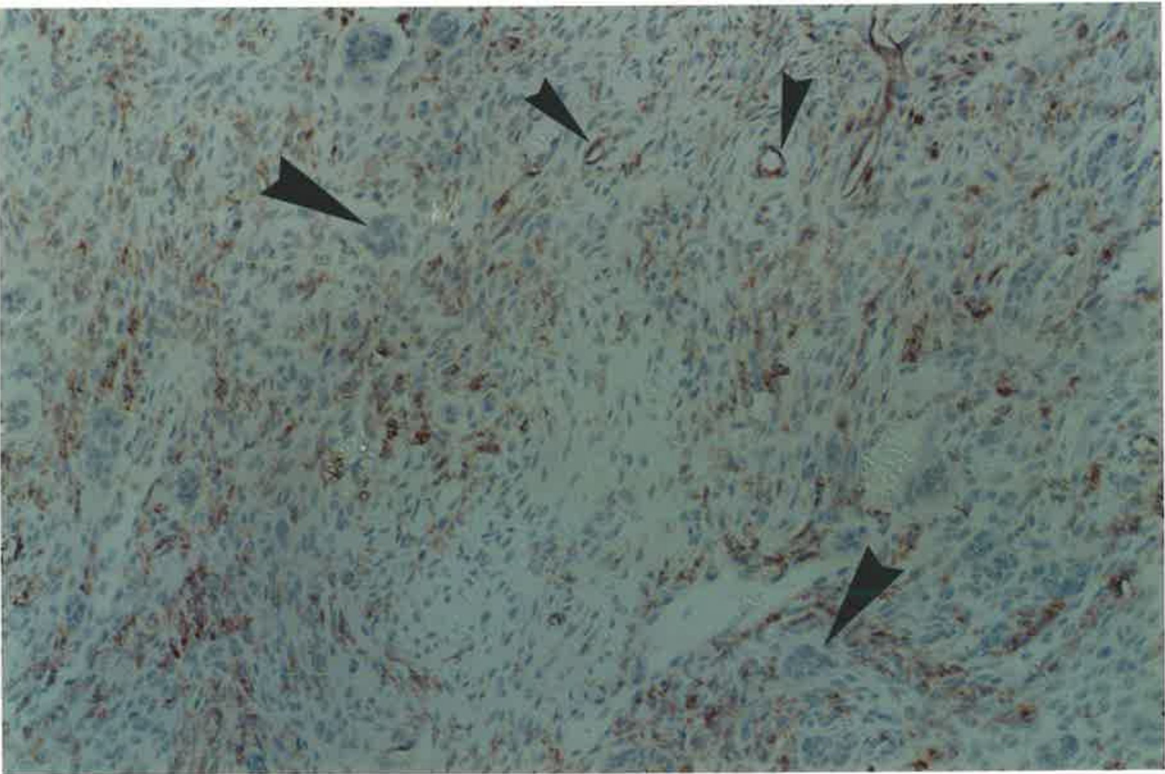
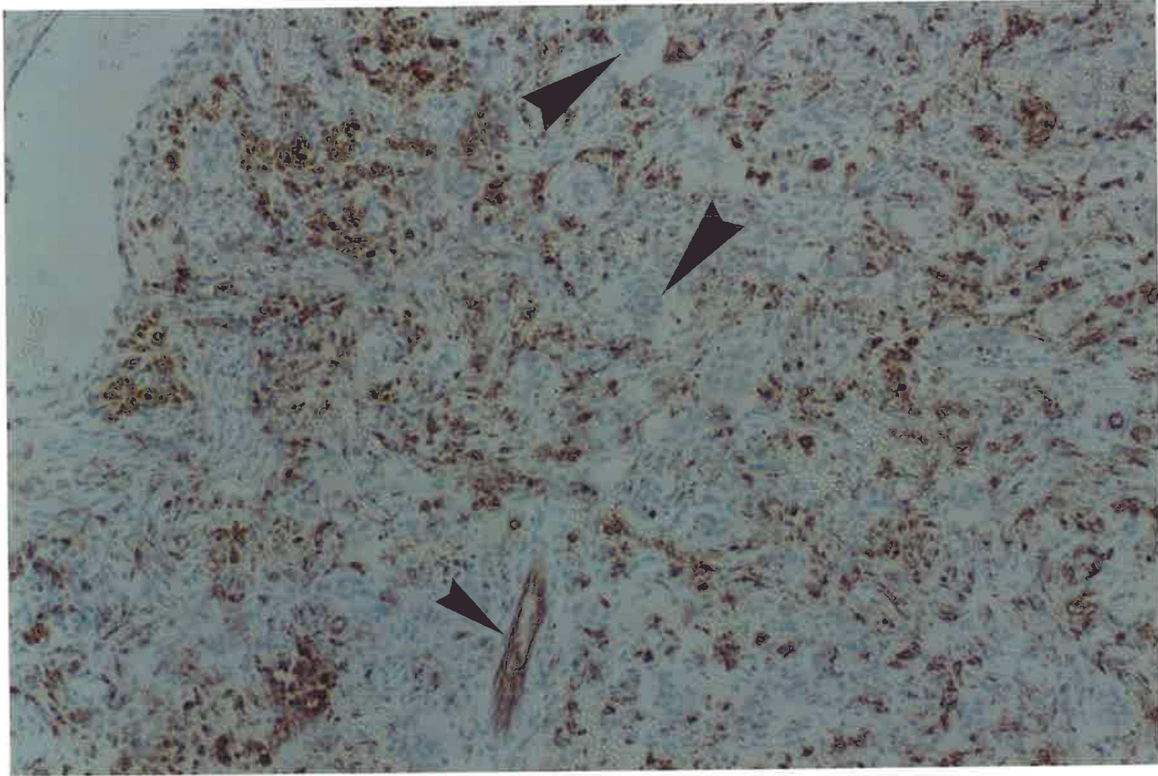


Figure 16: Photomicrograph of CGCG incubated for HAM 56 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (large arrows) to the antibody (Grade: -). Note strong positive reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as 26-50%. Note strong positive reaction of endothelial cells (mainly those of smaller blood vessels, small arrow) to the antibody (Grade + + +). Staining for endothelial cells was assessed as 51-75%. (Original magnification x100)

Figure 17: Photomicrograph of GCT incubated for HAM 56 using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) to the antibody (Grade: -). Note strong positive reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as 51-75%. Endothelial cells exhibited only moderate, focal staining (not illustrated), and was assessed as 26-50%. (Original magnification x100)



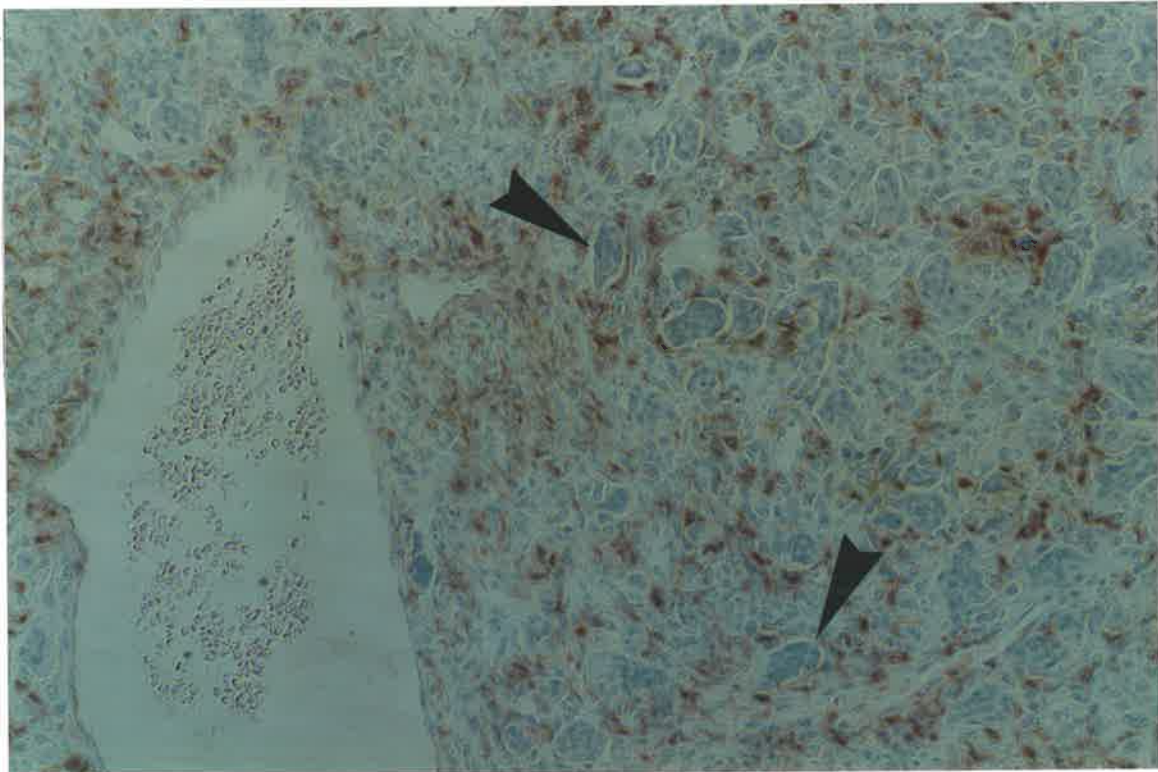
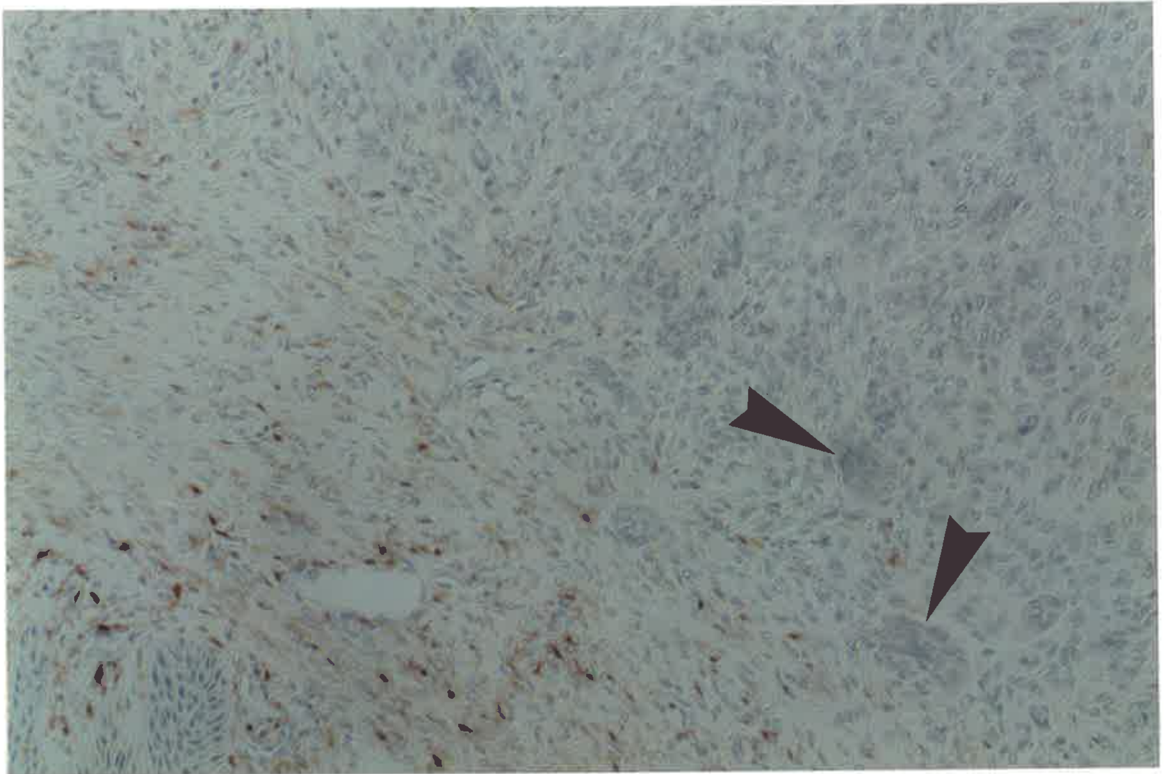
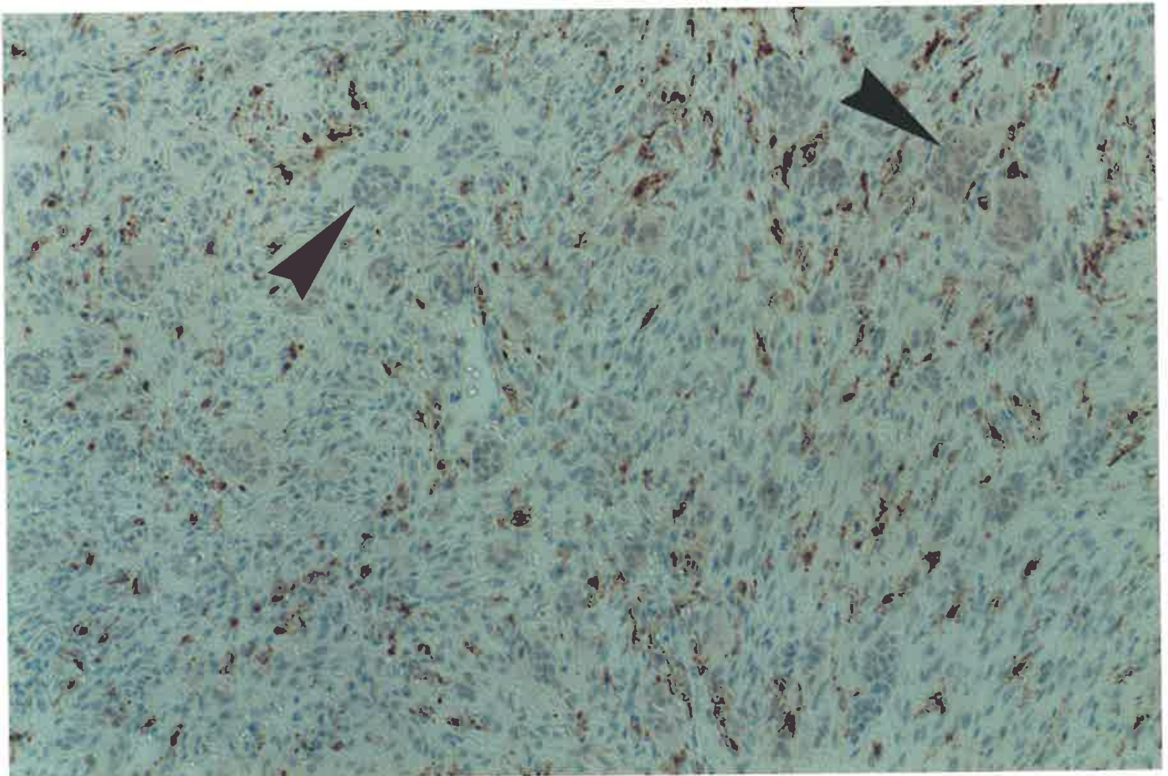


Figure 18: Photomicrograph of PGCG incubated for Factor XIIIa using ABC technique and counterstained with haematoxylin. Note weakly positive reaction of the giant cells (arrows) to the antibody (Grade +). Staining for giant cells was assessed as >75%. Note strong positive, focal reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as 26-50%. Note lack of reaction of endothelial cells to the antibody (Grade: -). (Original magnification x100)

Figure 19: Photomicrograph of CGCG incubated for Factor XIIIa using ABC technique and counterstained with haematoxylin. Note weakly positive reaction of the giant cells (arrows) to the antibody (Grade +). Staining for giant cells was assessed as >75%. Note strong positive, focal reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as 26-50%. Note lack of reaction of endothelial cells to the antibody (Grade: -). (Original magnification x100)





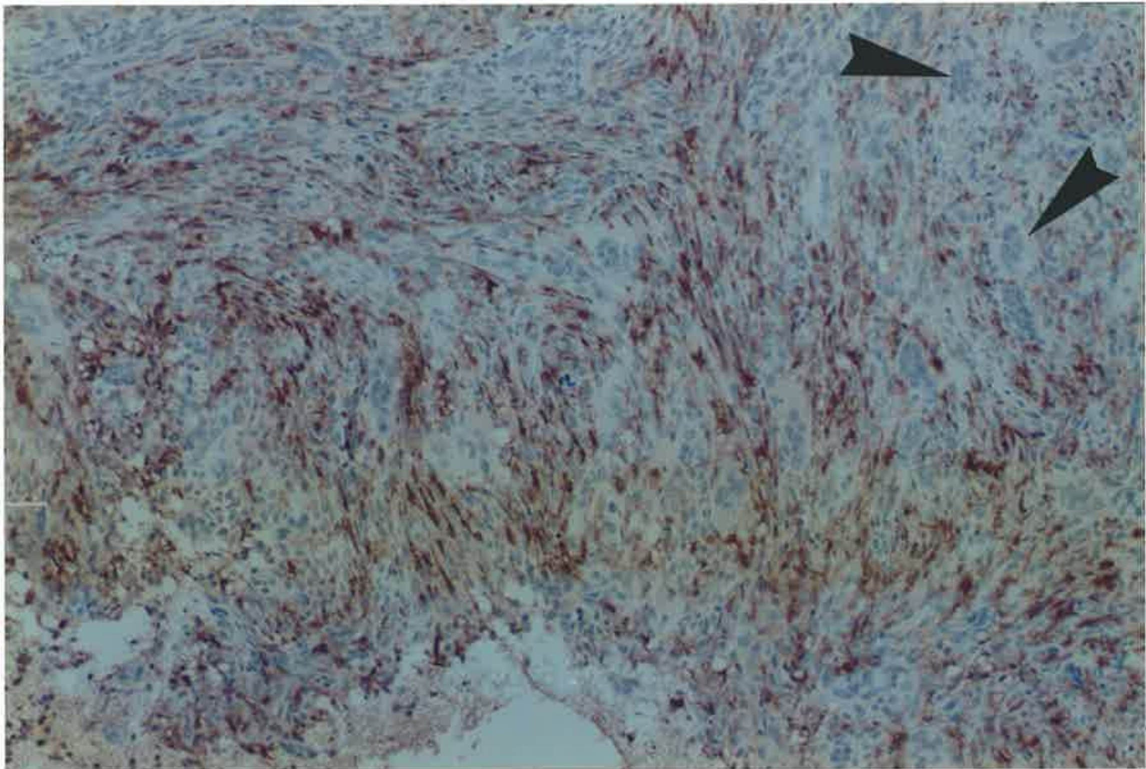


Figure 20: Photomicrograph of GCT incubated for Factor XIIIa using ABC technique and counterstained with haematoxylin. Note lack of reaction of giant cells (arrows) and endothelial cells to the antibody (Grade: -). Note strong positive reaction of mononuclear cells to the antibody (Grade + + +). Staining for mononuclear cells was assessed as 51-75%. (Original magnification x100)

CHAPTER 4

DISCUSSION

CHAPTER 4 DISCUSSION

4.1 CLINICAL ANALYSIS

4.1.1 CGCG

4.1.2 PGCG

4.1.3 Interpretation of clinical data

4.2 IMMUNOHISTOCHEMICAL STUDY

4.2.1 Discussion of methods

4.2.2 Multinucleated giant cells

4.2.3 Mononuclear stromal cells

4.2.4 Endothelial cells

4.3 CONCLUSIONS

4.1 CLINICAL ANALYSIS

The number of cases in the present study was not substantial and, accordingly, no statistical comparisons were made with other studies. Nevertheless, the following general observations are presented.

4.1.1 CGCG

Age. In the present study, the average age of patients was 32.6 years and the age range was from 7 to 73 years (age was not stated in 2 cases). Fifty percent of lesions occurred before the age of 31 and 35% appeared before the age of 11. In a previous study of 142 cases of CGCG by Whitaker and Waldron (1993), the mean age was 23 years, the range of ages was from 2 to 81 years, 64% of lesions appeared before the age of 30 and about 14% occurred before 11 years of age. Auclair *et al.* (1988) reported that in their series, 47% of 49 cases of CGCG occurred in patients under 16 years of age. Whereas the age distributions observed in the current study appear to be consistent with the ranges noted in previous reports, the disparities in sample size prevent any direct comparisons.

Sex. A sex predilection was noted in the current series, as 62% of the lesions occurred in males. A distinct female gender bias, however, was noted by Whitaker and Waldron (1993) and Waldron & Shafer (1966) as 63% (of 142 cases) and 68% (of 38 cases) respectively of their patients were female. The sex predilection in the present study, while interesting, might represent sample size differences.

Location. In the present study, 7 (50%) of lesions were mandibular in origin and 7 (50%) appeared in the maxilla. Location was not specified in 2 cases. In previous studies, however, 72% (Whitaker & Waldron 1993), 67% (Auclair

et al. 1988) and 66% (Waldron & Shafer 1966) of lesions were reported to be mandibular in origin and 25%, 31% and 34% were reported to appear in the maxilla. The site distribution in the present study might also represent sample size differences.

4.1.2 PGCG

Age. In the present study, 21% of lesions occurred before the age of 21 and 33% appeared before the age of 31. According to previous studies, 33% of 720 cases of PGCGs were noted in patients under 20 years of age (Giansanti & Waldron 1969), and 34% of 97 cases occurred between 5 and 15 years of age (Andersen *et al.* 1973). It should be noted that the limited sample size of the current study prevents direct comparison.

Sex. A sex predilection was noted in the present series, as 60% of cases were in males. Giansanti and Waldron (1969), however, reported a nearly 2:1 predilection of females to males. As with CGCG, the sex predilection in the present study, while interesting, might represent sample size differences.

Location. In the current series, 14 (58%) of lesions were mandibular in origin and 10 (42%) appeared in the maxilla. Location was not specified in 11 cases. This is in agreement with the study of Giansanti and Waldron (1969), who reported that the mandible was involved more often (55%) than the maxilla (45%).

4.1.3 Interpretation of clinical data

On the basis of analysis of the current sample of giant cell lesions of the jaws, it is concluded that:

- the age and site distribution of South Australian cases of CGCG, and gender distribution of South Australian cases of CGCG and PGCG showed some differences from those reported by other investigators.
- whether these observed differences represent a real variation in expression of these lesions, or reflect sampling differences, remains to be clarified.

4.2 IMMUNOHISTOCHEMICAL STUDY

The main purpose of this study was to further evaluate the antigenic profile of the cellular components of the giant cell lesions CGCG, PGCG and GCT. The particular components examined included the multinucleated giant cells, mononuclear stromal cells and endothelial cells.

4.2.1 Discussion of methods

ABC technique. Standard histologic processing techniques were used to obtain paraffin sections for light microscopy. The ABC technique used for immunohistochemistry is a highly sensitive method, resulting in a low background staining due to the high dilutions of antibody employed (Guesdon *et al.* 1979, Hsu *et al.* 1981). The high binding affinity of the ABC complex has enabled the detection of antigens, like immunoglobulins, in fixed material, resulting in better morphology compared with traditional preparations such as frozen sections.

Microwave-mediated antigen-retrieval method. This method of antigen retrieval, which is based on microwave heating of tissue sections attached to microscope slides to temperatures up to 100 degrees centigrade in the presence of metal solutions, was used for antigen unmasking (retrieval) prior to the immunostaining. According to Shi *et al.* (1991), the technique improves staining of antigens at increased primary antibody dilutions or reduced incubation times of primary antibodies. Adequate immunostaining has also been demonstrated by these authors in long-term formalin-fixed tissues which could fail to stain by conventional methods.

The microwave antigen-retrieval method does not appear to have been utilized in previous immunohistochemical studies of giant cell lesions. Staining patterns for CD68, HAM 56 and MAC 387 were largely consistent with those noted in previous studies. However, it was not possible to evaluate the degree of staining enhancement provided by the microwave antigen-retrieval techniques for these antigens, as comparable data for the intensity and proportion of staining were not presented in the earlier studies. Nevertheless, it is feasible that the enhanced immunostaining of blood vessels observed in the current study may have been a direct result of using microwave antigen-retrieval methods (see section 4.2.4 below).

4.2.2 Multinucleated giant cells

The multinucleated giant cells of CGCG, PGCG and GCT were found to exhibit strong positive immunostaining for CD68 (human macrophage marker), confirming the work of Doussis *et al.* (1992) who showed that CD68 was present in the giant cells of giant cell lesions of bone and soft tissue. This

finding seems to support the concept of a macrophage origin for giant cells and agrees with other studies by Kashara *et al.* (1979) and Regezi *et al.* (1987) who used different markers to identify a macrophage origin for the giant cells in these lesions.

CD68 has also been shown to be expressed by osteoclasts (Doussis *et al.* 1992, Kadoya *et al.* 1994), supporting the interpretation that the giant cells in giant cell lesions of bone and soft tissue may be osteoclasts, a concept which was previously proposed by Bonetti *et al.* (1990), Flanagan *et al.* (1988) and Athanasou *et al.* (1985). However, the giant cells in all three categories in the present study were found to remain unreactive for MAC 387 (human myeloid / histiocyte marker) and HAM 56 (human macrophage marker), which is consistent with the findings of Bonetti *et al.* (1990) who used the same antibodies to study the giant cells of PGCG. These findings may indicate that giant cells in PGCG, CGCG and GCT are a subset of specialized giant cells (such as osteoclasts) derived from the mononuclear phagocyte system.
Further study will be required to determine the biologic basis of the results obtained.

The giant cells were found to be either unreactive or stained with a weak (+) intensity for Factor XIIIa (dermal dendritic cell marker). According to Cerio *et al.* (1989), Factor XIIIa positive cells represent a specific population of bone marrow-derived dermal dendritic cells. They share a number of cytochemical and immunologic markers with cells of the mononuclear phagocyte system, as well as possessing extensive phagocytic function (Headington 1986). From the results of the current study, the possibility of giant cells being bone

marrow-derived dermal dendrocytes remains limited, unless they fail to express Factor XIIIa in their multinucleated forms.

The giant cell population in the CGCGs, PGCGs and GCTs studied was found to be unreactive with CD34 (endothelial cell marker). This finding tends to exclude an endothelial origin for this cell group, a suggestion previously put forward by Drepper and Themann (1961).

4.2.3 Mononuclear stromal cells

Whereas one of the aims of the current immunohistochemical study was to further characterise the nature of the mononuclear stromal cell component, the underlying heterogeneity of this cell population should be recognised. Within any giant cell lesion, several different mono-nucleated cell types will variably be present, including fibroblasts, myofibroblasts, endothelial cells, nerve cells, histiocytes, mast cells, etc. The mononuclear stromal cells which the current study addresses is that dense population of uniform mononuclear cells which can not readily be classified as belonging to one or more of the above cell types, and which may form the bulk of the giant cell lesion. This cell population could represent precursors of the multinucleated giant cells (Schajowicz 1961, Hanaoka *et al.* 1970, Steiner *et al.* 1972).

This study showed that the majority (51-75%) of the mononuclear stromal cells in all three categories exhibited a moderate positive reaction for CD68 in giant cell lesions. This finding supports the evidence for a macrophage origin for this group of cells. A smaller proportion of mononuclear cells, however, did not stain for CD68. Previous studies have also shown that substantial

numbers of macrophages are present in the giant cell tumor of bone (Hanaoka *et al.* 1970, Kashara *et al.* 1979). The role of these macrophages is not clear. They may, however, form part of the immunological response to the tumor or be osteoclast / giant cell precursors recruited by the tumor.

A variable proportion of mononuclear stromal cells was found to react strongly with MAC 387 and HAM 56 confirming the work of Bonetti *et al.* (1990) who showed that a population of mononuclear stromal cells was positive for MAC 387 and HAM 56 in the PGCG. This finding supports the concept that these particular mononuclear stromal cells originate from the macrophage (or mononuclear phagocyte system) as previously suggested by Ping *et al.* (1984) and Regezi *et al.* (1987). A slight difference in the percentage of cells reacting to HAM 56 in CGCGs and PGCGs versus GCTs was observed.

It was noted that mononuclear cells remained unreactive to HAM 56 in 2 cases of CGCG and 1 case of PGCG. This may have been caused by less than ideal tissue fixation or over-decalcification of tissue, both of which can negatively affect antigen expression.

In the current study, a variable proportion of mononuclear stromal cells exhibited a strong positive reaction to dermal dendritic cell marker, Factor XIIIa, with a higher proportion of GCT cells showing staining compared to CGCG and PGCG. As mentioned in the previous section, dermal dendrocytes appear to be related to cells of the mononuclear phagocyte system. Furthermore, Sueki *et al.* (1993) suggested that they contain appropriate factors to play an important role in microvascular hemostasis and in acute

and chronic phases of wound healing. Headington (1986) proposed that fibrous histiocytomas and giant cell tumors of superficial soft tissue probably take their origin from the dermal dendrocyte. It is interesting to note that in the current study, mononuclear stromal cells showed strong staining for the dermal dendrocytic marker, Factor XIIIa, in all three categories of lesions. This finding might also suggest a relationship between the stromal cells and cells of mononuclear phagocyte system.

4.2.4 Endothelial cells

Endothelial cells lining blood vessels in all studied lesions reacted positively for CD34. Endothelial cells of the capillaries and smaller blood vessels also stained (with slight differences in intensity and positive-cell proportion in CGCGs and PGCGs versus GCTs) with HAM 56. The differential reactivity of the blood vessels in the giant cell lesions which has recently been reported by Lim and Gibbins (1995) was not observed in the current series. In other words, in the current study, the blood vessels located deeper within the giant cell lesions and within the aggregations of the multinucleated giant cells stained as intensely to CD34 as the blood vessels at the periphery of the lesions. In this instance, the microwave antigen retrieval technique may have contributed to the enhanced response noted in the current study. It should also be pointed out that Lim and Gibbins (1995) used Factor VIII related antigen as a marker for blood vessels, whereas CD34 was used in the present investigation.

Interestingly, all three cases of GCT were found to be clearly more vascular than CGCGs and PGCGs, when examining for CD34. This differential was

not recorded in the study by Lim and Gibbins (1995). Further studies to quantitate and compare the vascularity of these lesions may be rewarding in order to differentiate giant cell granulomas from GCT.

4.3 CONCLUSIONS

Based on the clinical analysis of the current sample of giant cell lesions of the jaws, and the findings from the immunohistochemical study, it was concluded that:

- The age and site distribution of South Australian cases of CGCG, and gender distribution of South Australian cases of CGCG and PGCG showed some differences from those reported by other investigators. Whether these observed differences represent a real variation in expression of these lesions, or reflect sampling differences, remains to be clarified.

- The ABC technique, which was used for the immunohistochemical part of the study, produced in a low background staining due to the high dilutions of antibody used, and also provided adequate immunostaining of long-term formalin-fixed tissues.

- The microwave-mediated antigen retrieval method was a useful adjunct to the current study, being particularly suitable for examination of archival material.

- The immunohistochemical studies appeared to reinforce the concept that the giant cells of CGCG, PGCG and GCT may be a subset of specialized giant cells derived from the mononuclear phagocyte system, such as osteoclasts. Further studies on the development of specific markers for osteoclasts as well as the reevaluation of the existing markers are necessary.

- The positive immunostaining of mononuclear stromal cells to CD68, MAC 387 and HAM 56 suggests that these cells originate from the macrophage (or mononuclear phagocyte system). This relationship was reinforced by the strong positive reaction of mononuclear stromal cells for the dermal dendrocytic marker, Factor XIIIa.

- There were no obvious differences between the immunostaining patterns of the cellular components of CGCG and PGCG.

- Except for the increased vascularity of GCT demonstrated with CD34, no major differences were found in the immunostaining patterns of cellular components between the CGCG and GCT. Whereas further work needs to be done comparing the vascularity of these lesions, the results of this study support the view that CGCG and GCT represent part of a spectrum of a single pathologic process. Hence, differentiating CGCGs and GCTs remains a difficult diagnostic exercise.

APPENDIX A: GLASS SLIDE COATING (SUBBING)

Glass slide coating for adhesion of decalcified tissues and immunostaining sections using APT (3-amino propyltriethoxysaline), SIGMA CAT. NO. A3648

Requirements

100 glass slides

200 ml square container

4 clean black plastic racks

5 black plastic containers

Method

Carry out all procedures involving chemicals in a fume extraction environment

1. Pre-rinse slides placed in the racks, in 2 washes (1 minute each) in consecutive containers of xylol (xylene) or preferably an overnight soak in detergent.
2. Wash in several changes of running water. Tip the water from the container and allow to refill several times with water running to the base of the container. Drain but do not dry.
3. Rinse (30 seconds) in two consecutive containers of absolute alcohol.
4. Dip (10 seconds) in APT, 2% Solution made up with acetone. This is enough for 100 slides.
5. Rinse (30 seconds) in two consecutive containers of absolute alcohol.
6. Rinse in a large clean container of distilled water.
7. Air dry.

BIBLIOGRAPHY

Abrams B, Shear M (1974) A histologic comparison of the giant cells in central giant cell granuloma and the giant cell tumor of long bone. J Oral Pathol 3:217-23

Adkins KF, Martinez MG, Robinson LH (1969) Cellular morphology and relationships in giant cell lesions of the jaws. Oral Surg Oral Med Oral Pathol 28:216-22

Andersen L, Fejerskov O, Philipsen HP (1973) Oral giant cell granulomas: a clinical and histological study of 129 new cases. Acta Pathol Microbiol Scand 5(Sec A 81):606-16

Athanasou NA, Bliss E, Gatter KC, Heryet A (1985) An immunohistological study of giant cell tumor of bone: evidence for an osteoclast origin of the giant cells. J Pathol 147:153-8

Auclair PL, Cuenin P, Kratochvil FJ, Slater LJ, Ellis GL (1988) A clinical and histomorphologic comparison of the central giant cell granuloma and giant cell tumor. Oral Surg Oral Med Oral Pathol 66:197-208

Bartel H, Piatowska D (1977) Electron microscopic study of peripheral giant cell reparative granuloma. Oral Surg 43:82-96

Bertoni F, Unni KK, Beabout JW, Ebersold MJ (1992) Giant cell tumor of the skull. Cancer 70(5):1124-32

Bonetti F, Pelosi G, Martignoni G, Mombello A, Zamboni G, Pea M, Scarpa A, Chilosi M (1990) Peripheral giant cell granuloma: Evidence for osteoclastic differentiation. Oral Surg Oral Med Oral Pathol 70:471-5

Cerio R, Griffiths CEM, Cooper KD, Nickoloff BJ, Headington JT (1989) Characterization of Factor XIIIa positive dermal dendritic cells in normal and inflamed skin. Br J Dermatol 121:421-31

Chuong R, Kaban LB, Kozakewich H, Perez-Atayde A (1986) Central giant cell lesions of the jaws: a clinicopathologic study. J Oral Maxillofac Surg 44:708-13

Cohen MA, Grossman ES, Thompson SH (1988) Features of central giant cell granuloma of the jaws xenografted in nude mice. Oral Surg Oral Med Oral Pathol 66:209-17

Dahlin DC, Cupps RE, Johnson EW (1970) Giant cell tumor: A study of 195 cases. Cancer 25:1061-70

Doussis IA, Puddle B, Athanasou NA (1992) Immunophenotype of multinuleated and mononuclear cells in giant cell lesions of bone and soft tissue. J Clin Pathol 45:398-404

Drepper H, Themann H (1961) Electron microscopic and histochemical studies on giant cell epulis of man. Z Pathol 71:203-20

El-Labban NG, Lee KW (1983) Myofibroblasts in central giant cell granuloma of the jaws: an ultrastructural study. Histopathology 7:907-18

El-Mofty SK, Osdoby P (1985) Growth behavior and lineage of isolated and cultured cells derived from giant cell granuloma of the mandible. J Oral Pathol 14:539-52

Fechner RE, Huvos AG, Mirra JM, Spjut HJ, Unni KK (1984) A symposium on the pathology of bone tumors. In: Sommers SC, Rosen PP. Pathology Annual. Vol. : 19 Norwalk: Appleton-Century-Crofts pp. 135-40

Ficarra G, Kaban LB, Hansen LS (1987) Central giant cell lesions of the mandible and maxilla: A clinicopathologic and cytometric study. Oral Surg Oral Med Oral Pathol 64: 44-9

Flanagan AM, Tinkler SMB, Horton MA, Williams DM, Chambers TJ (1988) The multinucleate cells in giant cell granulomas of the jaw are osteoclasts. Cancer 62:1139-45

Franklin CD, Craig GT, Smith CJ (1979) Quantitative analysis of histologic parameters in giant cell lesions of the jaws and long bones. Histopathology 3:511-22

Friedberg SA, Eisenstein R, Wallner LJ (1969) Giant cell lesions involving the nasal accessory sinuses. Laryngoscope 79:763-76

Giansanti JS, Waldron CA (1969) Peripheral giant cell granuloma: review of 720 cases. J Oral Surg 27:787-91

Guesdon JL, Ternynck T, Aorameas S (1979) The use of avidin-biotin interaction in immunoenzymatic techniques. J Histochem Cytochem 27:1131-9

Hall RK (1994) Giant cell lesions of the jaw. In: Pediatric Orofacial Medicine and Pathology. London: Chapman & Hall pp. 309-14

Hamlin WB, Lund PK (1967) "Giant cell tumors" of the mandible and facial bones. Arch Otolaryngol 86:658-65

Hanaoka H, Friedman B, Mack RP (1970) Ultrastructure and histogenesis of giant cell tumor of bone. Cancer 25:1408-23

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

Headington JT (1986) The dermal dendrocyte. Adv Dermatol 1:159-71

Hirschl S, Katz A (1974) Giant cell reparative granuloma outside the jaw bone. Hum Pathol 5:171-81

Horton MA, Lewis D, McNulty K, Pringle JAS Chambers TJ (1985) Monoclonal antibodies to osteoclastomas (giant cell bone tumors): Definition of osteoclast specific cellular antigens. Cancer Res 45:5663-9

Hsu SM, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577-80

Jacoway JR, Howell FV, Terry BC (1988) Central giant cell granuloma - an alternative to surgical therapy. Oral Surg 5:572

Jaffe HL (1953) Giant cell reparative granuloma, traumatic bone cyst, and fibrous (fibro-osseous) dysplasia of the jawbones. Oral Surg Oral Med Oral Pathol 6:159-75

Kadoya Y, Al-Saffar N, Kobayashi A, Revell PA (1994) The expression of osteoclast markers on foreign body giant cells. Bone and Mineral 27:85-96

Kaffe I, Ardekian L, Taicher S, Littner MM, Buchner A (1996) Radiologic features of central giant cell granuloma of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 81(6):720-6

Kashara K, Yamamuro T, Kashara A (1979) Giant cell tumor of bone: cytological studies. Br J Cancer 40:201-9

Kaw YT (1994) Fine needle aspiration cytology of central giant cell granuloma of the jaw. A report of two cases. Acta Cytol 38(3):475-8

Kermer C, Millesi W, Watzke IM (1994) Local injection of corticosteroids for central giant cell granuloma. Int J Oral Maxillofac Surg 23:366-8

Leban SG, Lepow H, Stratigos JT (1971) The giant cell lesion of jaws: neoplastic or reparative. J Oral Maxillofac Surg 29:398-404

Lim L, Gibbins JR (1995) Immunohistochemical and ultrastructural evidence of a modified microvasculature in the giant cell granuloma of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 79(2):190-8

Lucas RB (1972) Pathology of tumors of the oral tissues. 2nd ed. Baltimore: Williams & Wilkins pp. 239-46

McDonald JS (1994) Tumors of the oral soft tissues and cysts and tumors of bone. In: McDonald RE, Avery DR, Dentistry for the child and adolescent. 6th ed. St. Louis: Mosby-Williams & Wilkins pp. 159-85

Mintz GA, Abrams AM, Carlsen GD, Melrose RJ, Fister HW (1981) Primary malignant giant cell tumor of the mandible. Report of a case and review of the literature. Oral Surg Oral Med Oral Pathol 51:164-71

Mirra JM (1980) Bone tumors. Diagnosis and treatment. Philadelphia: J.B. Lippincott pp. 344-9

Panico L, Passeretti U, De Rosa N, D'Antonio A, De Rosa G (1994) Giant cell reparative granuloma of the distal skeletal bones. A report of five cases with immunohistochemical findings. Virchows Archiv 425:315-20

Parrish CM, O'Day DM (1986) Brown tumor of the orbit. Case report and review of the literature. Arch Ophthalmol 104:1199-202

Pepler WJ (1958) The histochemistry of giant cell tumors: osteoclastoma and giant cell epulis. J Pathol Bacteriol 76:505-10

Ping L, Jia-qing Z, Chuan-han F (1984) Cytologic observation on giant cell tumor of bone. Chin Med J 97:411-8

Potter BJ, Tiner BD (1993) Central giant cell granuloma. Oral Surg Oral Med Oral Pathol 75:286-9

Quick CA, Anderson R, Stool S (1980) Giant cell tumors of the maxilla in children. Laryngoscope 90:784-91

Ratner V, Dorfman HD (1990) Giant cell reparative granuloma of the hand and foot bones. Clin Orthop 260:251-8

Regezi JA, Sciubba J (1993a) Red-blue lesions. In: Oral pathology. Clinical-pathologic correlations. 2nd ed. Philadelphia: W. B. Saunders pp. 136-60

Regezi JA, Sciubba J (1993b) Cysts of the oral region. In: Oral pathology. Clinical-pathologic correlations. 2nd ed. Philadelphia: W. B. Saunders pp. 322-61

Regezi JA, Zarbo RJ, Lloyd RC (1987) Muramidase, α-1 antitrypsin, α-1 antichymotrypsin, and S-100 protein reactivity in giant cell lesions. Cancer 59:64-8

Sapp JP (1972) Ultrastructure and histogenesis of peripheral giant cell reparative granuloma of the jaws. Cancer 30:1119-29

Schajowicz F (1961) Giant cell tumors of bone (osteoclastoma). J Bone Joint Surg Am 43:1-29

Schajowicz F (1981) Giant-cell tumor (osteoclastoma). In: Tumors and tumorlike lesions of bone and joints. New York: Springer-Verlag, pp. 205-42

Schajowicz F (1993) Giant-cell tumor (osteoclastoma). In: Histological typing of bone tumors. 2nd ed. Berlin: Springer-Verlag, pp. 20-2

Schajowicz F (1994) Giant-cell tumor (osteoclastoma). In: Tumors and tumorlike lesions of bone. Pathology, radiology, and treatment. 2nd ed. Berlin: Springer-Verlag, pp. 257-299

Shafer WG, Hine MK, Levy BM (1983) Benign and malignant tumors of the oral cavity. In: A textbook of oral pathology. 4th ed. Philadelphia: W. B. Saunders, pp. 86-229

Shi SR, Key ME, Kalra KL (1991) Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 39(6):741-8

Sidoni A, Monico S, D'Errico P, Simoncelli C (1994) Giant cell reparative granuloma of the maxillary bone: case report and review of diagnostic criteria. Pathologica 86(5):552-6

Smith GA, Ward PH (1978) Giant cell lesions of the facial skeleton. Arch Otolaryngol 104:186-90

Steiner GC, Ghosh L, Dorfman HD (1972) Ultrastructure of giant cell tumors of bone. Hum Pathol 3:569-86

Stewart JCB (1993) Benign non-odontogenic tumors. In: Regezi JA, Sciubba J, Oral pathology. Clinical-pathologic correlations. 2nd ed. Philadelphia: W. B. Saunders pp. 389-420

Struthers PJ, Shear M (1984) Aneurysmal bone cyst of the jaws. (I) Clinicopathological features. Int J Oral Surg 13:85-91

Sueki H, Whitaker D, Buchsbaum M, Murphy GF (1993) Novel interactions between dermal dendrocytes and mast cells in human skin. Lab Invest 69:160-72

Thompson SH, Bischoff P, Bender S (1983) Central giant cell granuloma of the mandible. J Oral Maxillofac Surg 41:743-6

van de Rijn M, Rouse RV (1994) CD34. A review. Applied Immunohistochem 2(2):71-80

Vergal De Dios AM, Bond JR, Shives TC, McLeod RA, Unni KK (1992) Aneurysmal bone cyst. A clinicopathologic study of 238 cases. Cancer 69(12):2921-31

Waldron CA, Shafer WG (1966) The central giant cell reparative granuloma of the jaws. Am J Clin Pathol 45(4): 437-47

Whitaker SB, Bouquot JE (1994a) Estrogen and progesterone receptor status of central giant cell lesions of the jaws. Oral Surg Oral Med Oral Pathol 77:641-4

Whitaker SB, Bouquot JE (1994b) Identification and semi-quantification of estrogen and progesterone receptors in peripheral giant cell lesions of the jaws. J Periodontol 65:280-3

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

ADDENDUM

REFERENCES

Carvalho YR, Loyola AM, Gomez RS, Araujo VC (1995) peripheral giant cell granuloma. An immunohistochemical and ultrastructural study. Oral Disease 1: 20-5

Mighell AJ, Robinson PA, Hume WJ (1995) Peripheral giant cell granuloma: a clinical study of 77 cases from 62 patients, and literature review. Oral Disease 1: 12-19

O'Malley M, Pogrel MA, Stewart JCB, Silva RG, Regezi JA (1997) Central giant cell granulomas of the jaws: phenotype and proliferation-associated markers. J Oral Pathol Med 26: 159-63