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Somporn Swasdison

Kittipong Dhanuthai

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Giant cell fibroma of the oral cavity. II. An immunohistochemical study

Somporn Swasdison D.D.S., Ph.D.
Kittipong Dhanuthai D.D.S., M.Sc.

Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330.

Abstract

Objective The purpose of this study was to investigate the phenotype of the mononuclear and the multinucleated cells in the oral giant cell fibroma.

Materials and methods The study was performed in thirty-one cases of the oral giant cell fibroma. The surgical specimens of the lesions were stained with a panel of antibodies using indirect immunoperoxidase technique.

Results The mononuclear and multinucleated giant cells of the giant cell fibroma showed positive staining by antibodies against vimentin and proliferating cell nuclear antigen (PCNA) while demonstrating negative staining by antibodies against S-100 antigen, leukocyte common antigen (LCA), macrophage marker (LN5), smooth muscle actin (SMA) and cell cycle proliferation marker (Ki67).

Conclusion The results suggested that the mononuclear and multinucleated giant cells of the giant cell fibroma were mesenchymal in origin and the multinucleated giant cells were probably derived from the fusion of differentiated mononuclear cells.

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Key words: dendritic mononuclear cells; giant cell fibroma; immunoperoxidase staining; multinucleated cells

Introduction

Giant cell fibroma (GCF) is a rather uncommon, benign, non-neoplastic lesion, first described in 1974 by Weathers and Callihan.[1] The GCF represented from 0.01 -2.7% of the total biopsies surveyed.[2-6] Clinically, they are asymptomatic pedunculated mass with a nodular papillary surface and usually under 1 cm. diameter.[1,2,5-7] The most common location is the gingiva, especially on the mandibular gingiva.[1-6] This lesion has distinctive histological appearance, consisting of highly vascularized loosely arranged fibrous connective tissue usually without inflammation. The consistent diagnostic feature is the presence of large dendritic mononuclear and multinucleated cells. Dendritic processes from these cells may be seen. The giant cell usually possesses a single nucleus, but sometimes contain several nuclei and they occasionally resemble Langhans giant cells.[1-7]

Even though there have been several studies about GCF, little is known about the cell type of these dendritic mononuclear and multinucleated giant cells in the GCF. In this study, we investigated the phenotype of these cells by using immunoperoxidase technique with a panel of antibodies as different markers.

Materials and Methods

Thirty-one cases of GCF were retrieved from the files of the Department of Oral Biology & Oral Pathology, Faculty of Dentistry, University of Otago, New Zealand, and the Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Thailand. Hematoxylin and eosin histopathological section of each case was reviewed to confirm the diagnosis of GCF using the criteria of Weather and Callihan.[1] For immunohistochemical analysis, 6 μ thick sections were obtained from the formalin-fixed paraffin-embedded surgical specimens of the lesions. The staining was performed on deparaffinized sections using the indirect immunoperoxidase method. Endogenous peroxidase was blocked by incubation the sections in 2% hydrogen peroxide/methanol for 10 min. Heating the tissue sections in citrate buffer (0.01 M, pH 6.0) up to 90°C by microwave oven was used as the method for antigen retrieval. Each section was incubated in humidified chamber with primary antibody and then with appropriate secondary antibody conjugated with peroxidase. 0.005 M Tris buffered saline pH 7.6 was used for washing steps between incubations. 3',3-Diaminobenzidine tetrahydrochloride was used as chromogen. Appropriate counter stain for each immunohistochemical reaction was performed. All sections were examined microscopically. The tissue sections containing appropriate antigens for the antibodies were processed in the same manner and in parallel to the GCF tissue sections serving as positive controls. For negative controls, the primary antibody was substituted with Tris buffered saline supplemented with 1% bovine serum albumin. Under this condition, no peroxidase staining was observed in the negative control sections.

The panel of antibodies with their working dilutions, incubation times used in this study were reported in Table 1

Table 1 Antibodies used in the study

Antibody	Antigen	Type of Antibody	Working dilution	Incubation time	Source
S100	S-100 protein	Polyclonal	1:400	30 min	DAKO, Denmark
Vimentin	Vimentin	Monoclonal	1:100	30 min	DAKO, Denmark
LCA	Leukocyte common antigen	Monoclonal	1:100	60 min	DAKO, Denmark
LN5	Macrophage marker	Monoclonal	1:50	60 min	Novocastra, United Kingdom
SMA	Smooth muscle actin	Monoclonal	1:50	30 min	Novocastra, United Kingdom
PCNA	Proliferating cell nuclear antigen	Monoclonal	1:200	30 min	Novocastra, United Kingdom
Ki67	Cell cycle proliferation marker	Monoclonal	1:100	30 min	Novocastra, United Kingdom

Table 2 Immunoreactivity of cell populations in the GCF

Antibody	Multinucleated giant cells	Dendritic mononuclear cells	Mononuclear stromal cells	Other components of the lesions stained positively
S100	negative	negative	negative	Positive stain at the Langerhans cells at the basal layer of epithelium
Vimentin	positive	positive	positive	Partial stain at the endothelial cells
LCA	negative	negative	negative	Negative stain for all components
LN5	negative	negative	negative	Partially positive at the endothelial cells of blood vessels
SMA	negative	negative	negative	Positive stain at the smooth muscle cells around blood vessels
PCNA	negative	positive	positive	Positive stain in some proliferating cells at the basal layer of epithelium
Ki67	negative	negative	partially positive*	Positive stain in dividing cells at the basal layer of epithelium

* The Ki67 antibody showed positive stain at the nuclei of mononuclear cells in the mitotic stage but not the cells in resting stage.

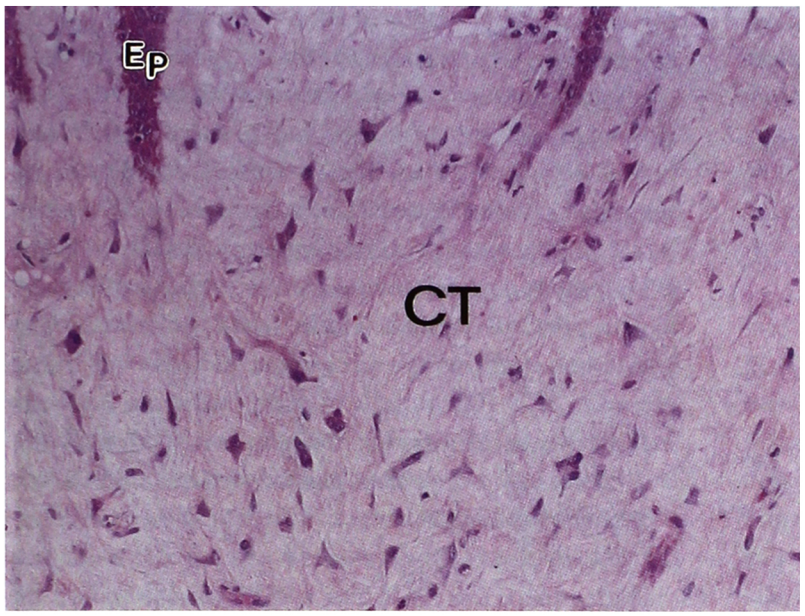

Fig. 1 Light micrograph of GCF showed the highly vascularized loose fibrous connective tissue containing large stellate mononuclear cells and dendritic multinucleated giant cells. Ep = epithelium; CT = connective tissue. (Hematoxylin and eosin stain, 180x).

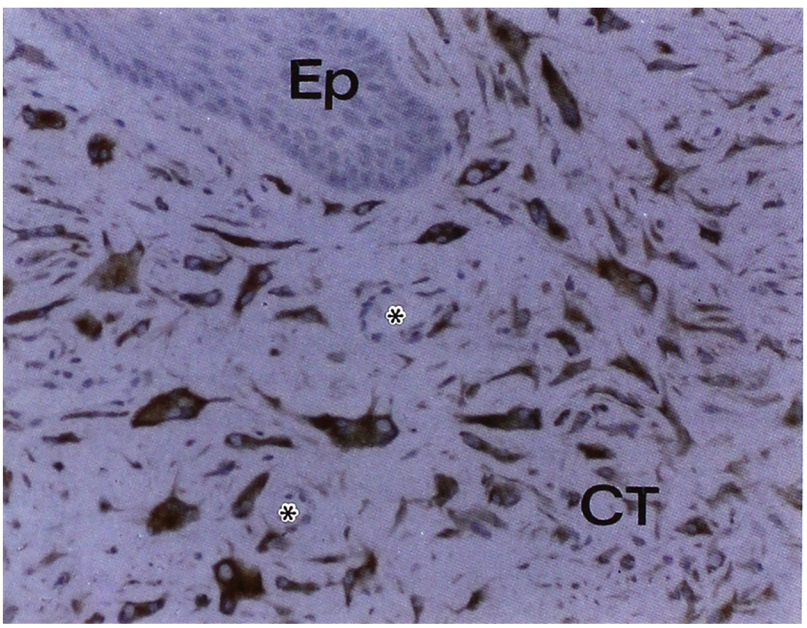

Fig. 2 Light micrograph of the positive immunohistochemical staining for vimentin showing dark brown granules in the cytoplasm of the stellate cells and the multinucleated giant cells. Ep = epithelium; CT = connective tissue. * = blood vessels. (Hematoxylin counter stain, 180x).

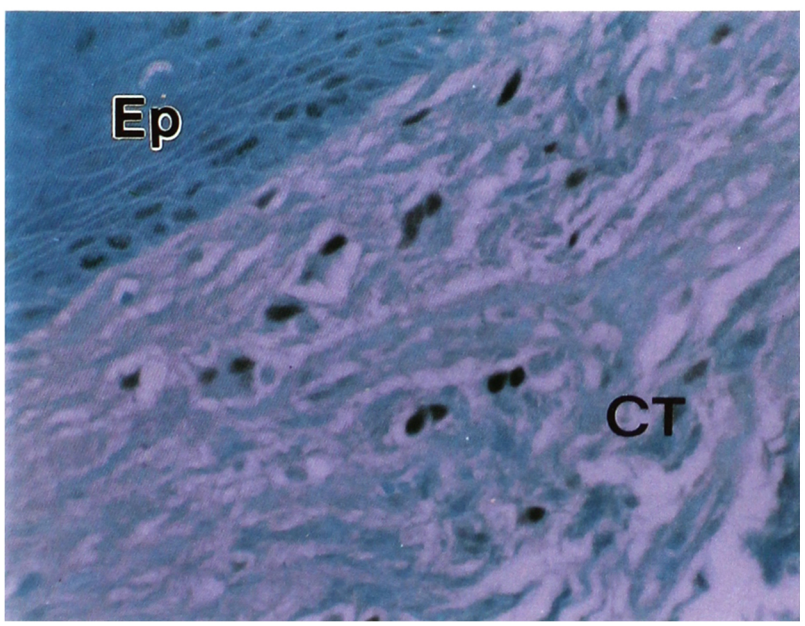

Fig. 3 Light micrograph of immunohistochemical staining for PCNA showed the positive staining reaction at the nuclei of the active cells of the basal cell layer of the epithelium and nuclei of the giant cells of the lesion. Ep = epithelium; CT = connective tissue. (Light green counter stain, 180x)

Results

Microscopic examination of the hematoxylin and eosin sections showed the typical histologic structure of the GCF which was composed of a number of large dendritic mononuclear and multinucleated giant cells scattering in the vascularized loose connective tissue. These giant cells contained either mononucleus or multinuclei (Fig. 1).

For immunohistochemical study, the immunoreactivity of cell populations in GCF was summarized in Table 2. The antibody to vimentin clearly stained both the dendritic mononuclear and multinucleated giant cells as well as the stromal fibroblasts of the lesion and the granular reaction product was strong and confined to the cytoplasm of these cells (Fig. 2). The dendritic mononuclear and multinucleated giant cells and other non-vessel associated mononuclear cells were totally negative for LCA, LN5, Ki67 and SMA. Anti -Ki67 antibody seemed to stain only the mononuclear cells in mitotic phase. Positive immunostaining for the monoclonal antibody LN5 was sometimes found at the endothelial cells of the blood vessels in the loose connective tissue of the lesion. SMA positive-stained cells appeared to be the smooth muscle cells supporting the lining of capillary spaces (the data is not shown in this report). S-100 antigen was expressed in the mononuclear cells with dendritic morphology at the basal layer of the epithelium, suggestive of the Langerhans cells. The immunoreactivity of monoclonal antibody to PCNA was found in the nuclei of the dendritic giant cells as well as in some proliferating cells at the basal layer of the epithelium (Fig. 3).

Discussion

The results of previous studies by hematoxylin and eosin stain showed that GCF seemed to contain 2 types of giant cells, the mononuclear and the multinucleated cells. These two types of cells may represent two different types or the same cell type with different functions. The cells in this lesions had also been extensively studied by other histochemical techniques, and suggested to be unusual giant fibroblasts.[1,8] But the possibility of being macrophage,[3,4,9] melanocytes[1] and myofibroblasts[9] cannot be excluded.

Weather & Callihan[1] considered the possibility that the characteristic cells in the giant cell fibroma represented dermal melanocytes because of the dendritic cytoplasmic extensions, the presence of melanin pigments and their increase in number near the epithelium, but this concept was excluded ultrastructurally[9,10] and by the negative staining for S-100 antigen.[4,7] The latter was in accordance with the negative staining for S-100 antigen in the mononuclear and multinucleated giant cells in this study. A neural or neuroectodermal origin was also excluded by negative staining for S-100 antigen in this study and for neuron specific enolase (NSE),[3] glial fibrillary acidic protein (GFAP),[7] and neurofilament[4] in the previous studies. These results thus excluding Langerhans cells as the progenitor of these cells. The presence of S-100 protein in some mononuclear cells with dendritic morphology at the basal layer of the epithelium and not at the connective tissue underneath suggested that Langerhans cells and/or their precursors did not involve in this lesion.

Cells of macrophage-monocyte lineage have previously been identified using the nonspecific markers; acid phosphatase, non-specific esterase, and lysozyme.[3,4,9] The characteristic cells in the giant cell fibroma showed negative staining for not only the above mentioned markers, but the more specific marker for macrophage-monocyte (CD31) as well.[3,7] In addition, our study revealed that the monoclonal antibody to LCA and LN5, which recognized the surface antigen of lymphoid cells and macrophage/histiocyte,[11] did show negative staining on mononuclear as well as on multinucleated giant cells. These results provided solid ground for excluding macrophage as possible progenitor of the mononuclear and multinucleated giant cells. They also clearly separated the multinucleated giant cells in GCF from those present in the peripheral giant cell granuloma, central giant cell granuloma and in the giant cell tumor of bone since the multinucleated giant cells in those lesions were believed to be of either macrophage or osteoclast in origin based upon the immunohistochemical studies.[12-15]

The possibility that these cells were derived from myofibroblasts has been proposed on the basis of their ultrastructural appearance,[9,16] but Odell et al could not demonstrate α-smooth muscle actin and desmin immunocytochemically.[7] We stained the lesion with monoclonal antibody specific for a component of myofilament of the muscle cells, SMA. We found that most of the giant cells of the lesion were negative to the monoclonal antibody to SMA. The positive immunoreactivity was observed only at the spindle-shaped smooth muscle cells around the blood vessels. Hence, the possibility that the giant cells of the lesion were

derived from myofibroblast was also excluded.

The positive immunostaining of vimentin, a mesenchymal-associated antigen, was found in the cytoplasm of the giant cells, both mononuclear and multinucleated cells, as well as the spindle-shaped stromal cells of the lesion. Therefore, we concluded from the presence of vimentin in these cells that both mononuclear cells and multinucleated giant cells were mesenchymal in origin. The mononuclear cells encompassed the spindle-shaped stromal fibroblastic cells and the dendritic cells.

Immunohistochemical investigation of PCNA and Ki67, the two nuclear proteins essential for cell proliferation in the cell cycle, indicated that Ki67 was absent from the multinucleated giant cells. The antibodies against PCNA and Ki67 differently stained some cells, but they were limited only in the mononuclear population. These results suggested that the proliferative compartment was within mononuclear cells. Anti-Ki67 antibody seemed to stain only the cells in mitotic phase whereas anti-PCNA antibody generally stained nuclei of some mononuclear cells. The differences in immunostaining pattern of these two antibodies may relate to cells in different phases of the cell cycle since these two antigens express at different locations in the nucleus and at different phases of the cell cycle.[17,18] From the immunoreactivity of Ki67 and PCNA, we concluded that the multinucleated giant cells were probably derived from the fusion of differentiated mononuclear cells and the proliferative compartment which control the behavior of the lesion might reside within the mononuclear population.

Conclusion

The results suggested that the mononuclear and multinucleated giant cells of the giant cell fibroma were mesenchymal in origin and the multinucleated giant cells were probably derived from the fusion of differentiated mononuclear cells.

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ไจแอนท์เซลล์ไฟโบรมาของช่องปาก . II. การศึกษาทางอิมมูโนฮิสโตเคมี

สมพร สวัสดิสรรพ์ ท.บ., Ph.D.
กิตติพงษ์ ดนุไทย ท.บ., วท.ม.

ภาควิชาทันตพยาธิวิทยา คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330

บทคัดย่อ

วัตถุประสงค์ เพื่อศึกษาลักษณะฟีโนไทป์ของเซลล์ชนิดที่มีนิวเคลียสเดี่ยวและชนิดที่มีหลายนิวเคลียสในเนื้องอกชนิดไจแอนท์เซลล์ไฟโบรมาของช่องปาก

วัสดุและวิธีการ ทำการศึกษาในเนื้องอกไจแอนท์เซลล์ไฟโบรมาของช่องปากจากผู้ป่วย 31 ราย โดยใช้การย้อมตัวอย่างเนื้อเยื่อจากรอยโรคของผู้ป่วยด้วยแอนติบอดีชนิดต่างๆ ตามเทคนิคการย้อมแบบอิมมูโนเปอร์ออกซิเดส

ผลการศึกษา พบว่าเซลล์ชนิดที่มีนิวเคลียสเดี่ยวและชนิดที่มีหลายนิวเคลียสในเนื้องอกไจแอนท์เซลล์ไฟโบรมามีการติดสีเมื่อย้อมด้วยแอนติบอดีต่อไวเมนทินและต่อแอนติเจนในนิวเคลียสของเซลล์ที่กำลังเพิ่มจำนวน แต่ไม่พบการติดสีเมื่อย้อมด้วยแอนติบอดีต่อ เอส-100 แอนติเจน, ลิวโคซัยต์คอมมอนแอนติเจน, แมคโครฟาจมาร์คเกอร์, แอคตินของเซลล์กล้ามเนื้อเรียบ และมาร์คเกอร์ของวงจรเซลล์ในการเพิ่มจำนวน

สรุป ผลการศึกษาแสดงให้เห็นว่าเซลล์ชนิดที่มีนิวเคลียสเดี่ยวและชนิดที่มีหลายนิวเคลียสในเนื้องอกไจแอนท์เซลล์ไฟโบรมาของช่องปากมีจุดกำเนิดมาจากพวกมีเซ็นไคมัลเซลล์และเซลล์ชนิดที่มีหลายนิวเคลียส อาจเป็นผลมาจากการรวมตัวของเซลล์ชนิดที่มีนิวเคลียสเดี่ยว

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