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The connective tissue cells of human dental pulp: An histologic and immunohistochemical study

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SUMMARY

Twenty human healthy teeth were extracted for orthondontic purposes and processed for histological, and immunohistochemical examination. Odontoblasts were pseudostratified in depth of 1-8 cells in pulpward direction showing the zone of Weil and the cell-rich zone in coronal third pulp. In the central part of pulp tissue, fibroblasts were arranged as a network. These cells strongly immunoreacted with an antibody (monoclonal and polyclonal) directed against the intermediate filament vimentin. The product reaction was specifically located in the cytoplasm. Near vessels occasional lymphocytes and mast cells were also present. Collagen fibers formed a plexus below the cell-rich zone in middle and coronal pulp.

KEY WORDS:

Pulp - Fibroblast - Man - Histology - Immunohistochemistry.

RESUME

Vingt dents humaines saines ont été avulsées pour des raisons orthodontiques et traitees pour être examinées du point de vue histologique et par immunohistochimie. Les odontoblastes étaient pseudostratifiés (1-8 cellules) et dans le tiers coronaire de la pulpe soit la zone de Weil une zone riche en cellules est présente. Dans la partie centrale de la pulpe, les fibroblastes forment un réseau. Ces cellules sont fortement immuno-marquées par un anticorps (monoclonal ou polyclonal) dirigé contre la vimentine. Le marquage est spécifiquement localisé dans le cytoplasme. A proximité des vaisseaux, des mastocytes ainsi que des lymphocytes sont présents. Les fibres de collagène forment un plexus au-dessous de la zone cellulaire riche dans la partie coronaire et centrale de la pulpe.

MOTS CLEFS:

Pulpe - Fibroblaste - Homme - Histologie - Immunohistochimie.

INTRODUCTION

Tooth pulp occupies the tooth cavity and is the connective tissue forming dental papilla during embryonic development (Baume, 1980). Adult tooth is a unique tissue containing cells in an abundant gelatinous ground substance and many collagenous fibrils running in all directions. Adjacent to the dentin there are cells arranged in the fashion of a specialized epithelium, called odontoblasts, capable to synthetize and secrete a hard mineralized tissue. The macrophage and lymphoid cells are also present within the pulp. Many histological studies have been performed about odontoblasts (Frank 1966; Matthiessen and Von Bülow 1970; Gvozdenovic et al. 1973; Selzer and Bender 1988), and immunocompetent cells of pulp (Cahen and Frank 1970; Eifinger 1970; Zachrisson 1971; Pulver et al. 1977; Miller et al. 1978; Jontell et al. 1987). The basic cells of connective tissue of pulp are the fibroblasts. Pulpal fibroblasts are quite interesting cells. Previous studies have demonstrated that pulp possess various

fibroblast subpopulation cells (Karim 1989). However, if these cells posses different functional properties or represent different developmental stage of the same unique cell, is unknown. In view of the importance of cell heterogeneity for cell biology, the aim of this investigation was to study the connective cells of human dental pulp at histological and immunohistochemical levels, trying to better characterize these cells.

MATERIALS AND METHODS

Twenty healthy tooth (premolars and molars) were removed for orthodontic reasons.

The apex of the root was removed to facilitate fixation, with a high-speed waterspray handpiece. Ten teeth were placed immediately in 5% buffered formol saline fixative solution. Specimens were then demineralized in 10% EDTA, dehydrate and embedded in paraffin wax. Sections 5 μm thick were cut and stained with haematoxylin and eosin. For immunohistochemistry 5 teeth were fractured buccolingually and their pulps were fully extirpated and immersed in neutral buffered formol for 24 h. Pulp tissue was then dehydrated and embedded in paraffin wax. Tissue sections were cut at 6 μm and processed for immunoperoxidase.

In brief, sections were incubated for 1 h, with a primary polyclonal antibody vimentin diluted 1:100 and a monoclonal antibody (MAb) obtained from Dako Corporation, Santa Barbara, CA.

Endogenous peroxidase was inactivated by 0.03% hydrogen peroxide.

Immunoperoxidase staining was performed using the avidin-biotin complex (ABC) method. Reagents were purchased from Vector Laboratory, CA, USA and used as described in the staining procedure of Vectastain ABC kit. The sections were stained with 0.03% H_2O_2 for about 5 min. After rinsing, the sections were mounted with glycerol in PBS. For control, tissue sections were incubated replacing the first antibody with PBS or non immune serum.

The sections were examined by a Leitz microscope.

RESULTS

Odontoblasts possess a pseudostratified appearance especially in coronal part where the layer was of 5-8 cells, in the apical part was only 1-2 cells dep. Odontoblasts had a columnar morphology in coronal pulp on the contrary they were round on low cuboidal in the apical third of pulp. The cell free zone of Weil and the cell rich zone were observed in coronal part of pulps although discountinously. These zones were lacking in middle and apical regions. Fibroblasts constitute the greatest number of cells present in pulp tissue. They were embedded in a gelatinous ground substance forming a syncytium of spindle-shaped cells interconnected by intercellular bridge.

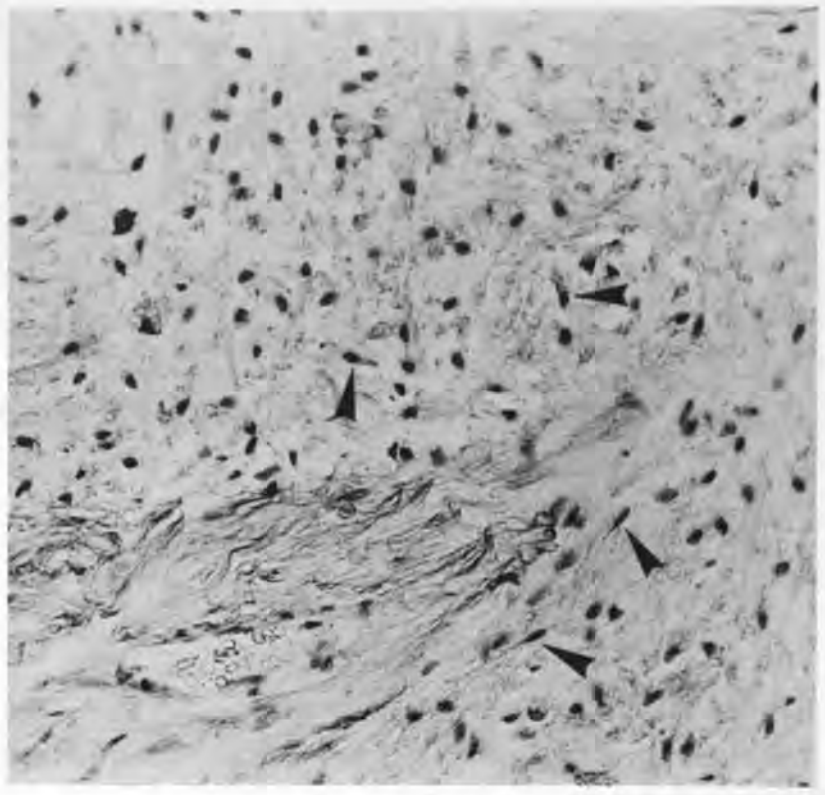

Fig. 1.: Immunoperoxidase staining for monoclonal vimentin showing fibroblasts positive (arrows) for this antigen. Immunoreactivity is present in cytoplasm. Immunoperoxidase ×200.

Fig. 1: La coloration par la technique des immunoperoxydases pour la vimentine monoclonale est positive au niveau des fibroblastes (flèches). L'immunoréactivité est présente dans le cytoplasme. Immunoperoxydase ×200.

Pulpal fibroblasts possess a well defined nucleous stained characteristically with basic stains and a light-stained cytoplasm. The immunohistochemical analysis for vimentin by the immunoperoxidase method demonstrated all cells stained positively for vimentin (Fig. 1). There was practically no difference between monoclonal and polyclonal antibody. The positive immunoreaction was localized in the cytoplasm of the cells. Also odontoblasts, when present in tissue section, showed a positive staining for vimentin (Fig. 2). The control sections exhibited no positive reaction.

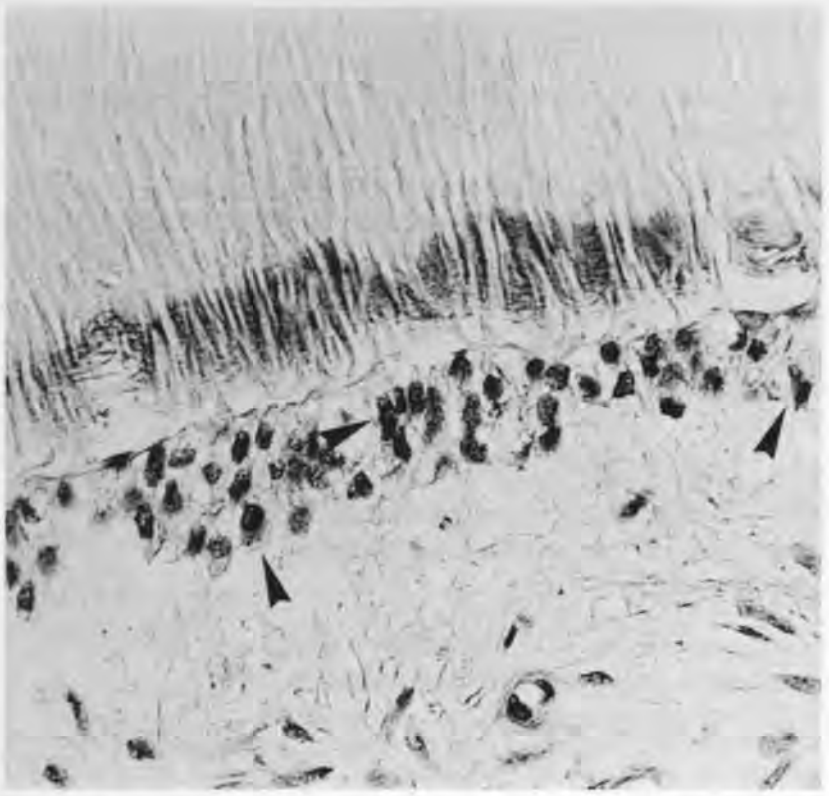

Fig. 2: Immunoperoxidase labelling for polyclonal vimentin is evident in both odontoblasts and pulp fibroblasts (arrows). Immunoperoxidase ×200.

Fig. 2: Marquage par l'immunoperoxydase pour la vimentine polyclonale est évidente à la fois dans les odontoblastes et dans les fibroblastes pulpaires (flèches). Immunoperoxydase ×200.

DISCUSSION

Generally speaking, the human dental pulp is described to possess two well defined regions: central and peripheral (Seltzer and Bender 1988). In the peripheral pulp zone different layers are apparent. Adjacent to the predentin there is a palisade of odontoblasts. Below this layer there is the subodontoblastic region called the «cell-free» zone of Weil (Avery 1971). Capillaries and small nerve fibers can be seen in this layer. Deeply to the odontoblasts, is the «cell-rich» zone. This «cell-rich» zone contains many fibroblasts and undifferentiate totipotential cells, replacing the population of odontoblasts by proliferation and differentiation (Zach et al. 1969). Nevertheless these regions differ from area to area of the same tooth being less constant and prominent near the root pulp apex (Gotjamanos 1969a; 1969b; Baume 1980).

The central pulp zone fills the area surrounded by the «cell-rich» zone. This region contains numerous large vessels and nerves supplying outer pulp layers. Principal cells within this zone are represented by fibroblasts and main extracellular component is collagen.

Pulpal fibroblasts possess a diameter of about 1-5 μm with inside the cytoplasm microfilaments of about 8 nm building the bulk of the cytoskeleton. On the contrary of odontoblasts, pulpal fibroblasts are not polarized although sometimes show a ciliary apparatus. Pulpal cells are arranged as a network and communicate by gap junctions.

Today three types of fibroblast according their morphology and their degree of differentiation are recognized (Eifinger 1970; Chaen and Frank 1970):

a) the inactive «mesenchymal cells» rounded shaped with a only short process, a large nuclear situated eccentrically in cytoplasm. These cells are usually situated near capillaries;

b) the «functional fibroblasts, with a changing pattern of cellular organites depending of the function activity. Sometimes a ciliary apparatus is present in these cells;

c) the «regressive fibrocyte» characterized by a large nucleous surrounded by a poor cytoplasm with few organelles.

Baume (1980) considering the cytostructure and the metabolic specificity as well as the embryonic origin suggested the term of «pulpoblast» to replace «functional pulpal fibroblast» and «pulpocyte» to replace «regressive pulpal fibrocyte». Pulpal fibroblast produce type I and III collagen, distributed in the subodontoblastic zone and around vessels and nerves, and also glycosoaminoglycans and proteoglycans (Han 1968; Baume 1980). Chondroitin sulfate and hyaluronic acid associated with dermatan, keratan or heparan sulfate are also present (Baume 1980). Pulpal fibroblasts not only produce the extracellular matrix, but they also participate in its turnover by the resorption mechanism of collagen fibers (Torneck 1978). By immunohistochemistry, connective tissue cell of the human pulp shows the presence of the intermediate filament protein vimentin. This protein of major intermediate filament, is considered a marker of mesenchymal cells (Osborn et al. 1977) often employed to differentiate between mesenchymal cells and those of other derivation (Leader et al. 1987). Although mesenchymal cell retains vimentin expression in all stage of differentiation, in some cases, there are cells that reduce their vementin expression in maturation process (Dellagi et al. 1983). Thus, the quantitative expression of vimentin is highly variable within different lineage

of the same cell of origin. Interestingly, pulpal fibroblasts are characterized by the presence of various subpopulation cells, probably representing different developmental stages of the same cell type (Karim 1989). This phenomenom being also found in human fibroblast population (Hassell and Stanek 1983; Komuro 1990). However, at difference of a previous report (Dellagi et al. 1983), the different subpopulation cells of human pulpal fibroblast expressed the presence of vimentin. Practically no differences concerning the labelling were observed between the polyclonal and the monoclonal antibody. Monoclonal antibodies give less background problems and eliminate undesired specificities introduced when monoclonal antibodies are produced. It is reasonably to assume that the lack of a difference in the staining obtained with polyclonal or monoclonal antibodies, is due to the widespread distribution of vimentin in pulpal cells. However, other studies need to be performed to clarify this concern. To summarize concerning the vimentin this protein is shared by the diverse cell types composing the human pulp.

Fibroblasts are traditionally considered to posses a relative uniform morphology. The identification of cytoskeletal markers has led to the recognition of a phenotypic heterogeneity between fibroblastic populations in physiologic as well as pathologic situations. For example, it has been shown that fibroblasts are also capable to express cell markers typical of muscle cells (desmin or actins) and that cytokines are probably implicated in the modulation of fibroblastic phenotypic features (Sappino et al. 1990).

In the present study carried out on healthy dental pulps we observed the presence of only intermediate filament protein vimentin; actins and desmin were specifically present in the muscle cells of vessels inside the pulp (Lombardi et al., paper submitted). However, it is interesting to speculate that pathological (caries) or physiologic (elderly) conditions might induce dental fibroblastic cells to express other cytoskeletal markers. Other investigations are necessary to establish the expression potential of these cells.

Pulp morphology reflects its function. The layering below the odontoblasts palisade must be considered as a well defined structure of human dental pulp responding to a functional need. It is clear that further studies improving our knowledge on dental pulp cells, will be useful also for clinical therapeutics.

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