An immunohistochemical study of types I, III and IV collagen in fibrosis of diseased gingiva during chronic periodontitis: A light and electron microscopic study


The distribution of type I, III and IV collagen and their ultrastructural organization have been studied in fibrotic gingival connective tissue of patients with longstanding cases of chronic periodontitis. The use of an immunofluorescent procedure has shown that the diseased connective tissue was made up of both type I and type III collagen. Type I collagen was strongly fluorescent and appeared to be the main gingival collagenous component, equally distributed in all layers of the tissue, whereas type III collagen was weakly fluorescent and mostly found in gingival papillae, but not around blood vessels. Standard electron microscopy and immunolabelling using the peroxidase procedure have shown that the large and dense bundles of type I collagen of P1, which is the main pattern of organization of the gingival connective tissue, were infiltrated by small bundles of microfilaments (10-15 nm) identified as the fibrillar form of type III collagen. However, a P2, the second pattern of organization of the gingival connective tissue, type collagen fibers were predominant and gave a fibrous feature to this area. Type V collagen was exclusively located in the thickened, degraded Lamina densa of basement membranes.

Introduction

Gingival connective tissue is one of the main tissue components of the healthy human periodontium. The highly collagenous nature of this connective tissue has been demonstrated (Page 1972, Schlager et al. 1977), and we know that the two main interstitial collagen types present in the human gingival connective matrix are types I and II (Ballard & Butler 1974) with a ratio of 7:1 (Nathan et al. 1979). These two interstitial collagen types account for 99% of the total extractable gingival collagen, whereas type IV collagen, the main component of basement membranes (Kefalides 1977) accounts for less than 1% (Narayanan & Page 1983).

Previous immunolabelling studies demonstrated the distribution, ultrastructure and organization in 2 patterns of these different types of collagen in healthy human gingiva (Chavrier et al. 1981, 1984, 1985) (Fig. 1), but a description in gingival connective tissue during chronic periodontitis is lacking. In this disease, gingival fibrosis is the predominant feature of slowly progressive, long-standing cases in humans (Page & Schroeder 1976, Page et al. 1978, Narayanan & Page 1983). This study was undertaken in order to observe possible modifications in distribution, ultrastructure and organization of these collagen types in fibrotic gingiva of patients with chronic periodontitis, clinically characterized by thickening of gingiva.

Material and Methods

Gingival biopsies

Tissue samples were obtained from 5 patients, ranging in age from 45 to 52 years, with slowly progressive, longstanding chronic periodontitis at the time of periodontal surgery. The affected teeth had probing depths of 4-6 mm and an alveolar bone loss, visually assessed, of approximately 30% on radiographs. Gingival samples from each donor were clinically classified as deriving from fibrotic gingiva and removed from the vestibular and interproximal areas of the maxillary posterior teeth. Immediately after surgery each sample was cut into 4 blocks. Three of the 4 were prepared as described below for standard electron microscopy and immunohistochemical studies. At the same time, the last block was fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. In all cases the gingival connective tissue could be divided into 2 zones. The 1st, located under the pocket epithelium,
from normal and fibrotic human livers, according to Grimaud et al. (1980), washed again, and then reacted with 1% dilution of fluorescein-isothiocyanate conjugated goat anti-rabbit gamma globulins (ref: 75451 Institut Pasteur, Paris, France). Briefly, type-specific antibodies were determined by affinity chromatography on the specific collagen type and/or absorption of the anti-serum on the other type of collagen and controlled by ELISA technique.

Finally, after thorough washing, the sections were mounted and observed with an appropriately equipped (FITC filter combination; PB 450 – 490, LP 520, FT 510) Zeiss Universal microscope. Control sections were incubated with non-immune serum or immune serum previously saturated with its specific antigen.

Standard electron microscopy

The blocks of fibrotic gingiva were placed in 2% glutaraldehyde, 0.1M sodium cacodylate buffer (pH 7.4) for 2 h at 4°C. After washing, they were postfixed in 1% osmium tetroxide, 0.1M sodium cacodylate buffer (pH 7.4) for 2 h at 4°C. Dehydration was performed with ethanol and samples were embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate, then observed with a Philips 300 electron microscope.

Immunolabelling for electron microscopy

For indirect immunolabelling using peroxidase procedure, the blocks of fibrotic gingiva were immediately fixed with 4% paraformaldehyde, 0.1M phosphate buffer (pH 7.4) for 8 h at 4°C, washed and frozen. Cryostat sections (10 µm) were cut and treated with 0.3% hydrogen peroxide (bovin testis type I Sigma) for 30 min at room temperature and with 0.1M sodium azide in the same condition. Sections were washed and placed overnight in 0.07% bovine serum albumin at 4°C, then incubated in specific antisera to humans IgG during 5 h at 4°C. After washing, the sections were incubated as for immunofluorescence, washed and then reacted with peroxidase-conjugated antisera. The bound peroxidase complexes were visualized by treatment with DAB according to Graham & Karnovsky (1966). Sections were then fixed with 1% osmium tetroxide, dehydrated and flat embedded in Epon. Ultrathin sections were prepared and observed, with no further staining in a Philips 300 electron microscope. Control sections were incubated with 0.1M phosphate buffer without immunoserum and in peroxidase-conjugated antisera alone.

Results

Immunofluorescence staining procedure

The connective matrix of fibrotic gingiva appeared to be made up of type I and III collagen. Type I collagen is strongly fluorescent and equally distributed in all layers of the gingival connective tissue (Fig. 2). On the other hand, type III collagen was weakly fluorescent and mainly found in the upper part of the gingival connective tissue and seemed to disappear around the blood vessels set walls (Fig. 3). Type IV collagen was exclusively located in basement membranes and was increased because of vascular neofomation characteristic of the inflammatory procedure (Fig. 3).

Standard electron microscopy

The great density of the collagenous material observed in the two patterns organization, I and III, constituted the typical ultrastructural feature of fibroconnective tissue during chronic periodontitis (Figs. 6 and 7).

Figs. 8 and 9) In P, the dense bundles of long, thick striated collagen fibers (60 – 70 µm) were intermingled with an unstained, thick, and collagenous type III collagen fibers were predominantly and little fibrillary network was observed. Such a feature gives a fibrous aspect to this pattern organization. In addition, gingiva basement membrane were basememembres of blood vessels were thinned and quite often broken down (Fig. 7).

Immunoperoxidase electron labelling

P, of fibroconnective tissue was a mixed pattern of type I and III collagen where type I collagen was predominant. This collagen type was distributed in both transverse and longitudinal sections, of thick collagen fibers (60 – 70 µm) arranged in large dense bundles (Figs. 10 – 12). Type III collagen was associated with the fibrillar and fibrous forms. The fibrillar forms were predominant in the diseased tissue and consisted of thin microfibrils (10 – 15 nm) revealed in small isolated bundles, scattered
Collagen in fibrosis

Discussion
Gingival fibrosis during chronic periodontitis is characterized, like most fibrotic disorders, by an increase in collagenous material composed of types I and III collagens (Ballard & Butler 1974). Previous biochemical studies have shown that type III collagen predominates in granulation tissue in the

type I collagen was predominant (Figs. 13, 18). The type III collagen was rare and mainly represented by its fibrillar form (Figs. 16, 17). In addition, the peroxidase deposits resulting from antitype IV collagen labelling were exclusively limited to the thickened, degraded lamina densa of basement membranes (Figs. 19, 20).

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early stage of wound healing, while type I predominates when healing is complete (Bailey et al. 1973, Gay et al. 1978).

In advanced lesions, such as cirrhosis of the liver, pulmonary fibrosis or systemic progressive sclerosis of the skin, the proportion of type III collagen is abnormally low and the type I/III ratio increases (Seyers et al. 1976, 1977, Gay & Miller 1978, Gay et al. 1978).

This ratio has never been studied in fibrosis of gingiva during chronic periodontitis, but it seems likely that advanced gingival fibrosis, the scarring procedure of gingival connective tissue during periodontal disease, works in the same manner. According to the present data, the absence of type III collagen around blood vessel walls and the strong fluorescent contrast between types I and III collagens are consistent with the common finding that the necrotic states are characterized by an accumulation of type I collagen molecules. Furthermore, although types I and III collagens always seem to be interwoven at the ultrastructural level, thick type III collagen fibers become predominant in P1, so that this pattern of organization associated with thickened basement membranes, loses a great part of its classical remodelling ability. On the o
10. Ultrastructural immunolocalization of type I collagen in a cross section of P. Observe the perifibrous granular deposit around thick collagen fibers (60-70 nm) arranged in large and very dense bundles.

11. Ultrastructural immunolocalization of type III collagen in a cross section of P. Only the bundles of microfilaments (10-15 nm) are labeled, no positive reaction is observed on the coarse fibers.

12. Ultrastructural immunolocalization of type I collagen in a longitudinal section of P. Note the densely disposed peroxidase labeled deposit along type I collagen fibers (thin arrows). x 20 000.

13. Ultrastructural immunolocalization of type III collagen in a longitudinal section of P. Observe fibrous form of type III collagen made up of isolated 40 nm fibers (thick arrows). In both cases, peroxidase deposits are periodically arranged. x 20 000.

14. Ultrastructural immunolocalization of isolated type III microfibrillar material in P. Observe the effective staining of isolated type III collagen microfibrils (thin arrows) with periodically arranged peroxidase deposits. x 20 000.

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