Phagocytosis of Collagen by Fibroblasts Incident to Experimental Tooth Movement

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Received October 6, 1984

Summary. The upper molars of adult Wistar rats were moved lingually by a wire spring for 5 and 9 days. Collagen-containing fibroblasts in the pressure zone caused by the tooth movement were investigated morphologically (forms characterized by type A and type B compartments) and cytochemically (location of acid and alkaline phosphatase activity). The following results were obtained: 1) The distribution of collagen-containing fibroblasts with type A or type B compartments could not be distinguished clearly in either 5-day or 9-day specimens; 2) Acid phosphatase activity was recognized in the Golgi apparatus, lysosomes, and elliptical bodies associated with type B compartments; 3) Alkaline phosphatase activity was positive in the plasma membrane of collagen-containing profiles and in both intracellular and extracellular collagen fibrils.

These results suggest that fibroblasts are capable of phagocytosing collagen fibrils in all areas of the cytoplasmic membrane and that digestion of collagen fibrils in fibroblasts may be associated with acid and alkaline phosphatase.

Since Ten Cate (1972) detailed the ultrastructure of phagocytosed collagen fibroblasts in oral connective tissues, a number of investigators have reported the presence of intracellular collagen within fibroblasts in vivo (Ten Cate and Deporter, 1974, 1975; Garant, 1976a; Beertsen and Everts, 1977; Melcher and Chan, 1981) or in vitro (Rose et al., 1978, 1980; Svoboda et al., 1979a, b). In particular, Garant (1976a) demonstrated fine features of fibroblasts containing intracellular collagen fibrils associated with two types of membrane-limited compartments. These features were characterized by type A and type B compartments. The former was frequently located in the periphery of the cell and especially within pseudopodal extensions of the fibroblast cytoplasm, and one or more cross-striated collagen fibrils were contained within smooth-walled and elongated profiles. The latter was usually located in the cell body proper with elliptical and/or spherical bodies containing a dense granular material. Yajima and Rose (1977) clarified these two types in vitro and demonstrated the location of acid phosphatase in various forms of intracellular collagen. The reaction products of acid phosphatase activity were located in Golgi cisternae, dense lysosomal granules, and elliptical bodies.
These findings suggest that intracellular collagen fibrils of fibroblasts represent phagocytosed collagen and digestion by lysosomal enzyme.

The purpose of this study was to elucidate the distribution of phagocytosed collagen fibroblasts characterized by type A and type B compartments in the pressure zone incident to experimental tooth movement, and to demonstrate the location of acid and alkaline phosphatase activity in these fibroblasts.

MATERIALS AND METHODS

The upper right first molars of adult male Wistar rats (30 rats, each weighing 220-240 g) were moved lingually by a wire spring (a force of about 10 g) (NAKAMURA, 1981; OKUMURA, 1982) for 5 and 9 days. This period of force loading was chosen to check the distribution of phagocytosed collagen fibroblasts characterized by type A and type B compartments, because during this time period the remodelling of the periodontium by various cells has been known to be most active (REITAN, 1962; OKUMURA, 1982).

Anesthetized rats (6 rats each in the 5 and 9 day groups) were perfused with 1.0% paraform-glutaraldehyde mixture in 0.1 M cacodylate buffer (pH 7.2) through the ascending aortae for 30 min. The upper jaws were subsequently excised and were cut

Fig. 1.  a and b. Fibroblasts with type A profiles have numerous vacuoles containing collagen fibers at one pole of the cell (5-day experiment). a: ×19,000, b: ×12,000
into a few small blocks, as described in previous reports (HIRASHITA, 1976; HIRASHITA et al., 1980). After decalcification in EDTA-Na₂ solution containing 7.0% sucrose (0°–4°C) for 2 weeks, these blocks were sectioned at 50–100 μm with a Vibratome. The specimens were incubated in Gomori’s incubating medium (GOMORI, 1950) for the demonstration of acid phosphatase (ACPase) activity, and the method described by YOSHIKI and KURAHASHI (1971, 1972) was used for the demonstration of alkaline phosphatase (ALPase) activity. Control sections for ACPase activity were incubated in the same medium containing 10 mM sodium fluoride, and control sections for ALPase activity were incubated in the absence of substrate from the medium. After incubation, all sections were rinsed briefly with 0.1 M cacodylate buffer containing 7.0% sucrose, followed by post-fixation with 1.0% OsO₄ for 60 min. They were dehydrated in a graded ethanol series and embedded in Epon 812. Specimens for morphological examination (9 rats in each experimental group) were prepared by conventional procedures; perfusion with 2.0% glutaraldehyde, decalcification with EDTA-Na₂, post-fixation with 1.0% OsO₄, dehydration, and embedding. Ultrathin specimens cut with glass knives were stained with uranyl acetate and lead citrate. Observations focused on the fibroblasts in the pressure zone incident to the experimental tooth movement.

RESULTS

A force of 10 g is heavy enough for an adult rat molar (NAKAMURA, 1981, OKUMURA, 1982) to give rise to hyalinized tissues containing necrosed cells, broken capillaries and necrosed collagen fibers (fibrils) locally in the maximal pressure zone of the periodontium, as described in previous reports (REITAN, 1962; RYGH, 1974; OKUMURA, 1982; NODA et al., 1983). In such hyalinized areas, phagocytosed collagen fibroblasts could only rarely be observed.

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Fig. 2. Location of ACPase activity in the 5-day experiment. The reaction products are located in the Golgi apparatus (Go), and lysosomes (ly). x3,820
They were recognized, however, in the areas surrounding the hyalinized tissues. Figure 1 (a, b) shows fibroblasts with type A profiles containing banded collagen filaments which are cut in cross-section or in longitudinal section in the 5-day experiment. These fibroblasts have numerous vacuoles containing banded collagen in the periphery or in the center areas of the cytoplasm, especially at one pole of the cell. Type B compartments were not observed in these fibroblasts. The reaction of ACPase activity in fibroblasts with the type A profiles can be observed in Figures 2 and 3. The enzyme activity was demonstrated markedly in the Golgi apparatus, lysosomes, and the membrane of vacuoles. Reaction products were also located in the lysosomes which fused with vacuoles containing banded collagen. Figure 4 (a, b) shows fibroblasts with the type B profile from the 5- and 9-day experiment. They have elliptical or spherical bodies containing a dense material in the cell body proper. However, the type A compartments were rarely observed in the cytoplasm of fibroblasts with type B profiles. A weak enzyme activity was observed in elliptical or spherical bodies in fibroblasts with the type B profile (Fig. 5), and the reaction was stronger in the Golgi apparatus and lysosomes. In sum, the distribution of fibroblasts with type A or type B compartments could not be distinguished clearly in the specimens from either the 5-day or the

Fig. 3. ACPase activity in type A profiles seen in the lysosomes (ly), and the membrane of vacuoles (v). Co collagen. ×20,000
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9-day experiment, and type B compartments could not be found in fibroblasts containing numerous type A compartments (Fig. 1-3). Figures 6 and 7 show the enzyme reaction of ALPase activity in fibroblasts with type A and type B profiles.

The reaction products were located on the surface of plasma membranes contacting the collagen fibrils and also in both intracellular and extracellular collagen fibrils. However, there were hardly any reaction products of ALPase activity in the elliptical or spherical bodies or in the lysosomes. Control sections for both ACPase and ALPase activity were negative.

DISCUSSION

1. Distribution of phagocytosed collagen fibroblasts with type A and type B profiles in experimental tooth movement

SVOBODA et al. (1981) described the importance of the phagocytosis of collagen fibrils by fibroblasts in turnover or remodelling of normal soft tissues.
In particular, they noted that phagocytic fibroblasts increased markedly in inflamed parts of such tissues (Ten Cate and Freeman, 1974). In this context, no difference was noted between the ultrastructure of phagocytic fibroblasts in normal tissue turnover and that in inflamed tissues. On the other hand, the features of type A and type B compartments demonstrated in vivo by Garant (1976a) were confirmed in vitro by Yajima and Rose (1977). Therefore, we attempted to check in this study whether fibroblasts with these two types increase or decrease in the periodontium during experimental tooth movement.

The duration of experimental tooth movement (5 and 9 days) was thought to be favorable for the observation of tissue remodelling, as indicated in previous reports (Reitan, 1962; Hirashita, 1976; Nakamura, 1981; Okumura, 1982).

Generally, hyalinized tissues including necrosed cells or fibers occur in the maximal pressure zone of the periodontium incident to experimental tooth movement. Therefore, collagen-containing fibroblasts can not be observed in such areas. Fibroblasts with type A and type B profiles were observed in the areas surrounding the hyalinized tissues, and fibroblasts located on the bone side of the periodontal ligament.
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1. Location of ACPase and ALPase activity in phagocytosed collagen fibroblasts

DEPORTER and TEN CATE (1973), GARANT (1976a, b), BEERTSEN and EVERTS (1977), YEE (1979) and MELCHER and CHAN (1981) demonstrated in vivo that phagocytosed collagen fibrils of fibroblasts could be digested by lysosomal enzymes. The findings of this study agree with those results. In type A profiles, ACPase activity was located in many lysosomes, in the membranes of vacuoles, and in the spherical bodies. In type B profiles, ACPase activity was weak in the spherical bodies, but strong in the Golgi apparatus and lysosomes. From these findings, it seems that lysosomes in fibroblasts play an important role in the digestion of collagen fibrils. Fibroblasts may phagocytose the surrounding useless collagen fibrils through their membranes or by extending their processes due to sudden environmental changes (i.e., the pressure on the cementum). These fibroblasts were fewer than those observed by SVOBODA et al. (1981) and OIKAWA (1982).

Type A and type B profiles could be clearly distinguished in fibroblasts containing intracellular collagen. However, there was no difference in number between the fibroblasts with type A and those with type B profiles in the two experimental periods. Additionally, changes from type A to type B profiles or from type B to type A profiles were obscure.

2. Location of ACPase and ALPase activity in phagocytosed collagen fibroblasts

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**Fig. 6.** ALPase activity in type A profiles in the 5-day experiment. The reaction is located on the surface of plasma membranes (arrows) and in intracellular and extracellular collagen fibrils (Co). Ly Lysosome. ×21,400
periodontium caused by experimental tooth movement). In such fibroblasts, many lysosomes are produced which immediately migrate to the phagocytosed collagen fibrils and digest them (Garant, 1976a, b; Melcher and Chan, 1981; Piao et al., 1983).

Collagen-containing fibroblasts are observed both in normal and abnormal tissues. The fibroblasts in the pressure zone of the tooth movement did not always phagocytose all the extracellular collagen fibrils. This seems to imply that it may be not always necessary for fibroblasts to phagocytose these useless collagen fibrils, for collagen fibrils are not only phagocytosed by fibroblasts, but also destroyed extracellularly by collagenase activity (Beertsen and Everts, 1977; Deporter and Ten, Cate, 1973; Ten Cate and Sybru, 1974).

On the other hand, there are few reports about ALPase activity in collagen-containing fibroblasts. Deporter and Ten Cate (1973) used the demonstration of ALPase activity as circumstantial evidence of collagenase activity on the basis of Woessner's (1968), Fullmer and Lazalus's (1969) and Perez-Tamayo's (1970) reports. They suggested that collagenase hydrolyses a peptide bond whereas ALPase hydrolyses a phosphate ester, and that since collagenase activity disrupts a peptide bond, and as the collagen molecule after such rupture contains no phosphate groups, it is most likely that ALPase exerts its effect before collagenase. In this study, we did not check the collagenase activity, but ALPase activity seemed to break down collagen as Deporter and Ten Cate (1973) described (Fig. 6, 7).

Certainly, fibroblasts are capable of both synthesizing and degrading collagen as Lapierre (1967) described. When fibroblasts in the periodontium are stimulated by

Fig. 7. ALPase activity in type B profiles (arrow) in the 5-day experiment. The reaction was rarely detected in the elliptical or spherical bodies or lysosomes. Co collagen. x15,000
experimental tooth movement, they would then be engaged in the rapid synthesis of collagen or in collagen degradation as the cells increase. However, it is not known what mediators may regulate the reverse activities of fibroblasts. A more accurate study of the fibroblast ultrastructure might identify these mediators.

In summary, the results of this study suggest that fibroblasts are capable of phagocytosing collagen fibrils in all areas of the cytoplasmic membranes and that digestion of collagen fibrils in fibroblasts is associated with the enzymes ACPase and ALPase.

REFERENCES

Beertsen, W. and V. Everts: The site of remodelling of collagen in the periodontal ligament of the mouse incisor. Anat. Rec. 189: 479–498 (1977).
Deporter, D. A. and A. R. Ten Cate: Fine structural localization of acid and alkaline phosphatase in collagen-containing vesicles of fibroblasts. J. Anat. 114: 457–461 (1973).
Fullmer, H. M. and G. S. Lazarus: Collagenase in bone of man. J. Histochem. Cytochem. 17: 793–798 (1969).
Garant, P. R.: Collagen resorption by fibroblasts: A theory of fibroblastic maintenance of the periodontal ligament. J. Periodontol. 47: 380–390 (1976a).
Garant, P. R.: An electron microscopic study of the periodontal tissues of germfree rats and rats mono-infected with Actinomyces naeslundii. J. periodont. Res., Suppl. 15 (1976b).
Gomori, G.: An improved histochemical technique for acid phosphatase. Stain Technol. 24: 81–85 (1950).
Hirashita, A.: The aspect of ultrastructural changes of the osteoblasts and surface areas of alveolar bone appearing in experimental tooth movement. Bull. Tokyo Med. Dent. Univ. 23: 245–260 (1976).
Hirashita, A., Y. Nakamura, E. Okumura and Y. Kuwabara: Microanalysis of mitochondrial granules in bone cells incident to experimental tooth movement. Acta histochem. cytochem. 13: 343–358 (1980).
Lapiere, Ch. M.: Mechanism of collagen fibre remodelling. In: (ed. by) D. J. Anderson, J. E. Eastoe, A. H. Melcher and D. C. A. Picton: The mechanism of tooth support. Wright and Sons, Bristol, 1967 (p. 20–24).
Melcher, A. H. and J. Chan: Phagocytosis and digestion of collagen by gingival fibroblasts in vivo: A study of serial sections. J. Ultrastr. Res. 77: 1–36 (1981).
Nakamura, Y.: Electron microscopy of bone cells incident to experimental tooth movement. (In Japanese). Tsurumi Univ. Dent. J. 8: 95–123 (1981).
Noda, K., A. Hirashita and Y. Kuwabara: Three dimensional structure of multinucleated giant cell "Osteoclast." Jap. J. oral Biol. 25: 814–818 (1983).
Okawa, H.: Experimental study on ultrastructural changes in the rat molar periodontal membrane after discontinuation of occlusal function (In Japanese). Jap. J. oral Biol. 24: 993–1018 (1982).
Okumura, E.: Light and electron microscopic study of multinucleated giant cells related with the resorption of hyalinized tissues. (In Japanese). J. Jap. Orthodont. Soc. 41: 531–555 (1982).
Perez-Tamayo, R.: Collagen resorption in carrageenin granulomas. II. Ultrastructure of collagen resorption. Lab. Invest. 22: 142–159 (1970).
Piao, Y. J., K. Ogawa, K. Ono and M. Abe: The relationship between heterophagy and autophagy in the splenic macrophage of rats after γ-ray irradiation. Acta histochem. cytochem. 16: 353–367 (1983).
Reitan, K.: Bone formation and resorption during reversed tooth movement. In: Vistas in orthodontics. Lea and Febiger, Philadelphia, 1962 (p. 69–84).
Rose, G. G., T. Yajima and C. J. Mahan: Microscopic assay for the phagocytotic-collagenolytic...
performance (PCP index) of human gingival fibroblasts in vitro. J. dent. Res. 57: 1003–1015 (1978).

Rose, G. G., T. Yajima and C. J. Mahan: Human gingival fibroblast cell lines in vitro. I. Electron microscopic studies of collagenysis. J. periodont. Res. 15: 15–70 (1980).

Rygh, P.: Elimination of hyalinized periodontal tissues associated with orthodontic tooth movement. Scand. J. dent. Res. 82: 57–73 (1974).

Svoboda, E. L. A., D. M. Brunette and A. H. Melcher: In vitro phagocytosis of exogenous collagen by fibroblasts from periodontal ligament: An electron microscopic study. J. Anat. 128: 301–314 (1979a).

Svoboda, E. L. A., A. H. Melcher and D. M. Brunette: Stereological study of collagen phagocytosis by cultured periodontal ligament fibroblasts time course and effect of deficient culture. J. Ultrastr. Res. 38: 195–208 (1979b).

Svoboda, E. L. A., A. Shiga and D. A. Deporter: A stereologic analysis of collagen phagocytosis by fibroblasts in three soft connective tissues with differing rates of collagen turnover. Anat. Rec. 199: 473–480 (1981).

Ten Cate, A. R.: Morphological studies of fibrocytes in connective tissues undergoing rapid remodelling. J. Anat. 112: 401–414 (1972).

Ten Cate, A. R. and D. A. Deporter: The role of the fibroblast in collagen turnover in the functioning periodontal ligament of the mouse. Arch. oral Biol. 19: 339–340 (1974).

Ten Cate, A. R. and D. A. Deporter: The degradative role of the fibroblast in the remodelling and turnover of collagen by the fibroblast. Anat. Rec. 182: 1–14 (1975).

Ten Cate, A. R. and E. Freeman: Collagen remodelling by fibroblasts in wound repair. Preliminary observations. Anat. Rec. 179: 543–546 (1974).

Ten Cate, A. R. and S. Sybru: A relationship between alkaline phosphatase activity and the phagocytosis and degradation of collagen by the fibroblasts. J. Anat. 117: 351–359 (1974).

Woessner, J. F.: Biological mechanisms of collagen resorption. In: (ed. by) B. S. Gould: Treatise on collagen. Part B. Biology of collagen. Academic Press, New York, 1968 (p. 253–330).

Yajima, T. and G. G. Rose: Phagocytosis of collagen by human gingival fibroblasts in vitro. J. dent. Res. 56: 1271–1277 (1977).

Yee, J. A.: Response of periodontal ligament cells to orthodontic force: ultrastructural identification of proliferating fibroblasts. Anat. Rec. 194: 603–614 (1979).

Yoshiki, S. and Y. Kurahashi: A light and electron microscopic study of alkaline phosphatase activity in the early stages of dentinogenesis in the young rat. Arch. oral Biol. 16: 1143–1154 (1971).

Yoshiki, S., T. Umeda and Y. Kurahashi: An effective reactivation of alkaline phosphatase in hard tissues completely decalcified for light and electron microscopy. Histochemie 29: 296–304 (1972).