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Introduction

Periodontal disease is one of the most common diseases and a major problem among the oral diseases. It is also the main cause of tooth loss in adults which accounts for about 40% of the total extraction in the United States.[1] Loss of the teeth, however, is apparently to be the terminal state of the process that begun in adolescence.[2] In general, we divide periodontal disease into three major states; dental plaque formation, gingival inflammation, and collagen degradation and alveolar bone loss.[3] An alveolar bone loss is a crucial state of the disease with regard to tooth loss.

It is almost certain that bacteria and its product are a potential etiologic agents and are essentially the major virulence factors in the initiation of periodontal disease.[4] However, host responses to bacterial infection, such as inflammation, immune response, also play a critical role in regulated the course of periodontal infection.[5] Genco[5] proposed that the pathogenesis of periodontal disease involved four states; colonization, invasion, destruction, and healing. The pathogenic bacteria, however, should have almost all of this distinctive which may enable them to colonize the host, to remain alive in the periodontal pocket and to penetrate the gingival tissues despite potentially effective defense mechanisms of the host.[4]

The role of bacteria or its product in periodontal disease is apparently complex and not yet fully understood. It is evidently that *Bacteroides gingivalis* and *Actinobacillus actinomy cetemcomitans* appear to be more potent than other periodontal organism.[4] They have been implied in many cases of severe adult periodontitis and juvenile periodontitis, respectively.[4,6]

Collagen, the most abundant protein in the body, is the major organic structural component of the extracellular matrix. Collagen is subject to a normal turnover. Inborn collagen is resist to digest by most proteolytic enzymes but is promptly degraded by specific tissue collagenase including bone collagenase[7,8] and bacterial collagenase.[9,10] The normal degradation of collagen at any one site may control locally that collagenase

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is present at concentration that are of physiological significant in collagen remodeling.[11] Tamayo et al.[12] stated that the local control of collagen degradation in mammals *in vivo* is depends on three factors; the rate of active collagenase synthesis and/or of activation of inactive enzyme precursors, the action of serum and/or tissues inhibitors, and the different combination of the both factors. It has already known that serum component such as α_2 macroglobulin, β_1 anticollagenase[9] and α_1 antitrypsin[11] are collagen inhibitors.

Because of its widely distribution in tissues, collagenase is thought to be an important factor in the massive degradation of gingival collagen in periodontal disease. However, the question about the source of collagenase in the tissues during pathological condition remains unsettle. Collagenase may originate from bacteria[4] epithelial cells or connective tissue cells of gingivae. Periodontal disease is also consider as an inflammatory disease which are characterised by infiltration of numerous cells such as leukocytes and macrophage. These cells have been demonstrated capable of producing collagenase.[13,14]

This paper will try to focus on some reports of collagenolytic activity in periodontal tissues particularly in gingival tissues. The possible relationship between collagenase activity and inflammatory cells

Mammalian and Bacterial Collagenase

Collagenolytic activity was first reported in the tissue culture of bullfrog tadpole tissues, skin, gut, and gills.[15] It has been detected later in many mammalian cells and tissues[9] as well as oral tissues.[16,17] Generally, collagenase are all extracellular enzyme, molecular weight 30,000-80,000, with optimal pH at neutral and require both Ca^{2+} [9,17] and $Zn.^{2+}$ [9] Human gingival collagenase, from culture fluid, has a molecular weight of about 40,000.[17] It is increasingly evident that collagenase exist in both latent or inactive form and active form.[18]

Mammalian collagenase attack collagen molecule at specific site, producing the N-terminal three-quarter fragment and C-terminal one-quarter fragment.[9,16] Once initial cleavage has been made on collagen molecule. It is likely that proteolytic enzyme, such as elastase or neutral protease, will further hydrolyzed collagen molecule completely.

Bacterial collagenase, however, cleave collagen at multiple sites and almost totally degraded collagen molecule into a small product that can not be detected on the gel.[9,19] *Bacteroides melaninogenicus* and *Clostridium histolyticum* which are abundant in the gingival crevice can produce collagenase.[10] Collagenase originated from bacteria, however, is not much enough to account for the observed destruction of gingival collagen. So, the endogenous collagenase may play major role in tissue destruction.

Collagenase in Gingival Tissue.

Collagenolytic activity in gingival tissue had been found firstly by Gibson and Fullmer.[20] They characterized it as a heatlabile factor produce from viable cells which can reduce the viscosity of collagen solution. Pure gingival epithelial cells and pure inflamed gingival connective tissue have been found to produce collagenase on culture.[16] Collagenase was demonstrated subsequently in both viable and frozen and thawed gingival tissue from patients with acute and

chronic inflamed gingiva[21-23] but could not detected in healthy gingiva.[22]

Collagenase activity can be found in gingival crevice and periodontal pocket. It was shown that collagenolytic activity in crevicular fluid was positively correlated with the severity of periodontal disease.[24,26] Collagenase in crevicular fluid was found to originated from adjacent gingival tissue.[16,24,26] Enzymatic activity of collagenase as well as neutral protease were increase in gingival crevice during an experimental gingivitis in man.[10] However, collagenase activity in this experiment was considered to be both tissues and bacteria origin. Recently, collagenase can also detect in human whole saliva, particularly in saliva from patients with periodontal disease.[26]

Gingival Tissue Culture Study

Geiger and Harper[19] performed a tissue culture experiment to analyze and to correlate the collagenase activity in gingival tissue with the severity of the periodontal disease. Healthy and inflamed, mildly or severely, gingival tissue were obtained from patients undergoing surgical extraction of impacted teeth and from patients undergoing periodontal treatment. Gingival tissues were explants in the culture medium. The uncontaminated supernatants culture fluid were collected every 24 hours for 6 days. The activity of collagenase was assayed by the release of ^{14}C-glycine peptides obtained from radioactively-labeled guinea pig skin collagen. It was found that explants of gingivae showing clinically severe inflammation exhibited a considerably highest collagenase activity. These activity was about 3-5 times and two times higher than that activity from non-inflamed gingivae and mildly inflamed gingivae, respectively.

Polyacrylamide gel electrophoresis was used to detect the product of collagen breakdown by these collagenase. The gel electrophoretic exhibited three-fourths and one-fourth fragment which indicated the characteristic of mammalian collagenase. They also found that healthy gingivae secreted relatively low, but constant amounts of collagenase.

This experiment is one of many tissue culture studies that showed the positive correlation between collagenase activity in gingival tissue and the severity of the periodontal disease.

Immunolocalization of Collagenase in Gingival Tissue.

An attempt to localize the distribution of collagenase in inflamed gingival tissues has been done by immunolocalization techniques.[27] The gingival tissues from two groups of patients, treated and untreated periodontitis, were fixed and washed in buffer containing a sheep monospecific antibody to human collagenase. The antigen-antibody reaction was examined by the indirect method of immunofluorescence using FITC-labelled rabbit (anti-sheep IgG) immunoglobulins as a marker.

It was found that gingival tissues taken from patents, who had recieved thoroughly treatment, Gingival Index score of zero and pocketing of 4-6 mm., exhibited insignificant amounts of immunoreactive collagenase. However, a scatter number of inflammatory cells were observed within the tissues. On the contrary, immunoreactive enzyme was detected in four of eight samples taken from patients that received no treatment prior to biopsy, Gingival Index of two and pocket of 4-6 mm.. The clinical signs of chronic inflammation was observed and the speci-

mens showed a lot of inflammatory cells. The distribution of immunoreactive collagenase in tissues were variable. However, it was found to concentrate at the interface between epithelium and connective tissue, particularly at the site of lesion. Moreover, an unidentify single cell was also shown to contain immunoreactive enzyme. No immunoreactive collagenase was detected in intact epithelium even in the prolong culture.

The author suggested that collagenase production by unknown cell in connective tissue may occur briefly at specific site and may regurate by cell-cell interaction.[9] This transient production probably account for an undetectable of immunoreactive enzyme in some specimens.

What is(are) the source(s) of collagenase in gingival tissues?

Exogenous collagenase, obviously, originated from some bacteria species in subgingival plaque,[4,10] but it could consider to be a minor one. Endogenous collagenase, as mention above, is released in connective tissues by an unidentify cells. There are a lot of cell types in matrix of gingival tissue, especially fibroblast and inflammatory cells with regard to periodontal disease.

Polymorphonuclear leukocyte (PMN) is a predominant inflammatory cell in acute inflammatory reaction and formation of abscess. PMN is the only cell in the body that has been found to contain collagenase in the granular fraction.[13] Oronsky et al.[28] reported that collagenase precursor is released in large quantity during phagocytosis of aggregated human γ globulin. When experimental gingivitis was performed in man by refrained from tooth brushing for 21 days. It was found that the number of PMNs in the gingival washings fluid increased markedly.[10] Collagenase, as well as neutral protease, also increased significantly. Positive correlation was found between these two parameters. Moreover, the collagenolytic activity, assay by gel electrophoresis, of gingival washing fluid and the granular fraction of PMN are almost identical. The author suggested that collagenase in gingival washing fluid probably originated from PMNs.

Macrophages, which predominate in chronic inflammatory lesions, also produce collagenase,[14] such as alveolar macrophages.[29] It was demonstrated that macrophages obtained from peritonial exudate of guinea pig produce collagenase when exposed to bacterial lipopolysaccharide in culture.[14] It was known that *Bacteroides* species which are abundant in subgingival plaque has lipopolysaccharide on its surface. And it has been found that endotoxin can penetrate gingival sulcular epithelium.[30] However, it is unlikely to infer this results to that of gingival macrophage.

Fibroblast, which widely distributed normally in extracellular matrix, synthesise and secrete macromolecules, especially collagens.[31] It has also been reported that fibroblasts taken from bovine gingiva and human skin can produce collagenase in cultures.[32-34] More interestingly, Simpson and Mailman[35] have found that human gingival collagenase activity was inhibited by human gingival fibroblast culture medium. Both fibroblast and collagenase were obtained from gingival tissues of patients receiving periodontal therapy. Fibroblast-culture medium also inhibit collagenase derived from macrophage, monocyte, and lymphocyte. They suggested that gingival fibroblasts during culture produce a coll-

agenase inhibitor. This inhibitor might have an important regulatory function in normal turnover of collagen. Therefore, any alteration of the ratio of inhibitor to collagenase by any stimuli during pathological state might account for an increased in collagenolytic activity follow by the degradation of collagen.

Conclusion

It is almost clear that collagenolytic activity increases in gingival tissues in gingivitis and periodontitis. Enzyme probably concentrated in connective tissues just at the site of active collagen degradation. Positive correlation between collagenase activity and inflammatory cells were reported but the source of collagenase still uncertained. Fibroblast produce either collagenase or collagenase inhibitor in such a way that it can regulate the normal turnover of collagen. On the other hand, polymorphonuclear leukocytes and macrophages which predominant in inflammatory reaction can produce collagenase and probably be the most likely source of collagenase that responsible for massive destruction of collagen in periodontal disease.

References

1. Robertson, P.B. and Fullmer, H.M. Collagenolytic activity in periodontal disease. J. of Med. Sci. 10:115-118, 1973.
2. Glickman, I. Clinical periodontology. 4th ed. Philadelphia, Saunders, 1972.
3. McGhee, J.R., Michalek, S.M. and Cassell, G.H. Dental microbiology. Philadelphia, Harper & Row, 1982.
4. Slots, J. and Genco, R.J. Black-pigmented *Bacteorides* species, *Capnocytophaga* species and *Actinobacillus actinomycetemcomitans* in human periodontal disease: Virulence facters in colonization, survival, and tissue destruction. J.Dent.Res. 63:412-421, 1984.
5. Genco, R.J. Pathogenesis of periodontal disease. New concepts. Can. Dent. Assoc.J. 50:391-395, 1984.
6. Genco, R.J. and Slots, J. Host responses in periodontal disease. J.Dent. Res. 63:441-451, 1984.
7. Sakamoto, S., Sakamoto, M., Goldhaber, P. and Glimcher, M. Collagenase and bone resorption. Biochem. Biophys. Res. Comm. 63:172-178, 1975.
8. Woods,J.F. and Nichols,G. Collagenolytic activity in mammalian bone. Sciences 142:386-387, 1963.
9. Woodly, D.E. Mammalian collagenase. In extracellular matrix biochemistry ed. Pier, K.A. and Reddi, A.H., New York, Elsevier, 1984, pp. 119-150.
10. Kowashi, Y., Jaccard, F. and Cimasoni, G. Increase of free collagenase and neutral protease activity in the gingival crevice during experimental gingivitis in man. Archs. oral Biol. 24:645-650, 1979.
11. Eisen, A.Z., Bauer, E.A. and Jeffrey, J.J. Human skin collagenase. The role of serum alpha-globins in the control of activity *in vivo* and *in vitro* Proc. Nat.Acad.Sci. 68:248-251, 1971.
12. Tamayo, R.P., Montfort, I. and Pardo, A. What controls collagen *in vivo?* Med. Hypothesis 6:711-726, 1980.
13. Lazarus, G.S., Brown, R.S., Daniels, J.R. and Fullmer, H.M. Human granulocyte collagenase. Science 159:1483-1485, 1968.
14. Wahl, L.M., Wahl, S.M., Mergenhagen, S.E. and Martin, G.R. Collagenase production by endotoxin-activated macrophages. Proc. Nat. Acad. Sci. USA. 71:3598-3601, 1974.
15. Gross, J. and Lapiere, C.M. Collagenolytic activity in amphibian tissues : A tissue culture assay. Proc. Nat. Acad. Sci. USA. 48:1014-1022, 1962.

16. Fullmer, H.M., Gibson, W.A., Lazarus, G.S., Bladen, H.A. and Whedon, K.A. The origin of collagenase in periodontal tissues of man. J.Dent.Res. 48:646-651, 1969.
17. Fullmer, H.M., Taylor, R.E. and Guthrie, R.W. Human gingival collagenase, purification, molecular weight, and inhitor studies. J.Dent.Res. 51: 349-355, 1972.
18. Reynolds, J.J., Sellers, A., Murphy, G. and Cartwright, E. A new factor that may control collagen resorption. Lancet. 11:333-335, 1977.
19. Geiger, S. and Harper, E. Human gingival collagenase in periodontal disease: The release of collagenase and breakdown endogenous collagen in gingival explants. J.Dent.Res. 59:11-16, 1980.
20. Gibson, W.A., and Fullmer, H.M. Collagenolytic activity of gingival tissue *in vitro*. J.Dent.Res. 45:1225, 1966.
21. Bentner, E.H., Triftshauser, C. and Hazen, S.D. Collagenase activity of gingival tissue from patients with periodontal disease. Proc.Soc.Exp.Biol. Med. 121:1082-1085, 1966.
22. Bennick, A. and Hunt, A.M. Collagenolytic activity in oral tissues Archs. oral Biol. 12:1-9, 1967.
23. Uitto, V.J., Turto, H. and Saxen,L. Extraction of collagenase from human gingiva. J.Perio.Res. 13:207-214, 1978.
24. Golub, L.M., Kaplan, R., Mulvihill, J.E. and Ramamurthy, N.S. Collagenolytic activity of crevicular fluid and of adjacent gingival tissue. J.Dent.Res. 58:2132-2136, 1979.
25. Golub, L.M., Siegel, K., Ramamurthy, N.S. and Mandel, I.D. Some characteristics of collagenase activity in gingival crevicular fluid and its relationships to gingival disease in humans. J.Dent. Res. 55:1049-1057, 1976.
26. Iijima,K.,Ando.K.,Kishi,M., Nakashizuka,T. and Hayakawa, T. Collagenase activity in human saliva. J.Dent.Res. 62:709-712, 1983.
27. Woolley, D.E. and Davies, R.M. Immunolocalization of collagenase in periodontal disease J.Perio. Res. 16: 292-297, 1981.
28. Oronsky, A.L., Perper,R.J. and Schroder, H.C. Phagocytic release and activation of human leukocyte procollagenase. Nature 246:417-419, 1973.
29. Senior, R.M., Bielefeld, D.R. and Jeffrey, J.J. Collagenolytic activity in alveolar macrophages. Clin.Res. 20:88, 1972.
30. McGhee, J.R., Michalek, S.M. and Cassell, G.H. Dental microbiology. Philadelphia, Harper & Row, 1982, P. 754.
31. Alberts, B., Bray, D., Lewis, J., Raff, M., Robert, K. and Watson, J.D. Molecular biology of the cell. New York, Garland, 1983, P.692.
32. Birkedel-Hausen, H., Cobb, C.M., Taylor, R.E. and Fullmer, H.M. Synthesis and release of procollagenase by cultured fibroblast. J.Biol.Chem. 251:3162-3168, 1976.
33. Bauer, E.A. Cell culture density as a modulator of collagenase expression in normal human fibroblast cultures. Exp.Cell Res. 107:269-276, 1977.
34. Busiek, D.F. and Bauer, E.A. Environment pH modulation of collagenase in normal human fibroblast cultures. Bichem. Biophys. Acta. 585:389-397, 1979.
35. Simpson, J.W. and Mailman, M.L. Synthesis of collagenase inhibitor by gingival fibroblasts in culture. Biochem. Biophys. Acta. 673:279-285, 1981.

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