Biosynthesis of complement components in chronically inflamed gingiva

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The biosynthesis of the third and fifth components of complement in chronically inflamed gingiva is demonstrated. The technique utilized in these studies was an immunochemical detection of $^{14}$C-labeled amino acid incorporated protein from gingival explant cultures. C3 and C5 synthesis was detected in 8 of 10 individuals with periodontal disease, while no evidence of synthesis was noted in 5 control subjects. The results indicate an even greater consumption of complement occurs in periodontal disease than has been predicted previously using serum as the sole source of complement. The local production of complement could play a role in modulating the inflammatory response in the gingiva.

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Introduction

Previous studies indicate that the complement system, a principal mediator of immunologically induced inflammation, is involved in the pathogenesis of periodontal disease. The third component of complement is decreased in gingival fluid when compared with serum (Attstrom et al. 1974, Schenkein & Genco 1977a). The presence of split products of C3, C3d, and C3 pro-activator have also been identified in gingival fluid (Attstrom et al. 1974, Schenkein & Genco 1977b). The complement component profile indicates complement activation occurs by both the classical and alternate pathway in gingival fluid. Furthermore, bacteria coated with C3 and antibody have also been demonstrated in the gingival crevice. (Nisengard & Jarrett 1976).

Since it has been shown that chronically inflamed gingiva synthesizes immunoglobulins (Lally, Baehni & McArthur 1980), the present study was undertaken to investigate the biosynthesis of complement proteins in chronically inflamed human gingival tissue.

Materials and Methods

Source of tissue
Gingival tissue was obtained from patients being treated in the Periodontics Clinic of the University of Pennsylvania School of Dental Medicine. "Normal" gingival tissue was obtained from patients undergoing extractions for orthodontic purposes.

Preparation of tissue
The method utilized to demonstrate in vitro incorporation of $^{14}$C labeled amino acids is
a modification of the method of Hochwald, Thorbecke and Anofsky (1963) which has been described previously (Lally et al. 1980, Jones & Lally 1980). Briefly, gingival tissue was washed free of blood and debris. Following mincing, 100 µg (wet weight) of tissue was placed into serum free media containing the [14C]-labeled amino acids. The media used in these experiments was a modified RPMI-1640 (Selectamine, Gibco) to which 5.0 µCi/ml [14C]-lysine (specific activity 354 mCi/mmol, Amersham) and 1.0 µCi/ml [14C]-isoleucine (specific activity 354 mCi/mmol, Amersham) were the only source of these amino acids added to the media. Incubation was carried out for 48h at 37°C in 5% CO2.

Immunoelectrophoresis

After incubation the tissues were freeze-thawed three times and centrifuged; the supernatants were decanted, dialyzed, and concentrated (10 X) (Minicon, Amicon Corp., Lexington, Mass.). The supernatants were then mixed 1:1 with purified (Nilsson & Müller-Eberhard 1965) C3 (0.5 mg/ml) or purified (Nilsson, Tomar & Taylor 1972) C5 (0.5 mg/ml), and the entire mixture was subjected to electrophoresis (Williams & Chase 1971) in either agar (Difco) or agarose (Seakem, Marine Colloids, Rockland, Me.) gels. Precipitin lines were developed with rabbit anti-human C3 and rabbit anti-human C5 (kindly supplied by Dr. Ulf Nilsson). Controls consisted of tissue processed in an identical manner. However, cultures were either frozen immediately or the incubation carried out for 48 h in the presence of 2 µg/ml of cycloheximide.

Autoradiography

Slides were then washed extensively in 0.05M borate buffered saline, pH 8.4, dried, and stained with amido black. Autoradiographs were prepared on Kodak SB-5 X-ray film (Eastman Kodak, Rochester, N.Y.). The autoradiographs were exposed for 48 days and developed with Kodak D-19 developer.

Evaluation of protein-synthesis

The intensity of the precipitin arc autoradiograph, which is indicative of the amount of protein synthesized in the gingival culture, was graded on the following scale: (-) negative; (+) just visible; (+) clearly visible. All readings were double blind by two individuals.

Results

Complement biosynthesis

The analysis of autoradiographs of complement biosynthesis is normal and chronically inflamed gingiva is shown in Table 1. Synthesis of C3 and C5 was demonstrated in 8 of 10 patients with periodontal disease and none of the control patients (x2 = 8.61, p = < 0.01 > 0.001).

The amounts were small when compared

Table 1

<table>
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<tr>
<th>Patient</th>
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<td>+</td>
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</table>

The intensity of the line on the autoradiograph was graded from: (-) not visible; (+) just visible; (+) clearly visible.
Fig. 1. Biosynthesis of C3 in chronically inflamed human gingiva. (a) Immunoelectrophoresis pattern (on agar); top well, partially purified C3 (1 mg/ml) mixed 1:1 with concentrated (10X) control (cycloheximide) supernatant from patient C; bottom well, partially purified C3 (1 mg/ml) mixed 1:1 with concentrated culture fluid (10X), incubated 48 hours at 37°C. Synthesis of C3 is noted in the autoradiograph. The additional line noted in the electrophoresis is γ (C3b inactivator enhances a normal contaminant of C3 preparation and antimer). Autoradiograph exposure time: 48 days.

Control experiments. Routine controls demonstrating the specificity of the method have been described elsewhere (Lally et al., 1980, Jones & Lally 1980). Briefly, two different types of control cultures are utilized: one in which cycloheximide (2 µg/ml), a known inhibitor of protein synthesis, was used and a second in which the tissue was frozen immediately after preparation with no incubation period.

In agar gel electrophoresis both C3 and C5 exhibit β mobility (Fig. 1). The possibility that immunoprecipitation of labeled protein was non-specific due to the proximity of the precipitin arc to the well was considered. Subsequent experiments were performed using agarose gel electrophoresis. With agarose, both complement components exhibit α mobility. The results in agarose are shown in Fig. 2 for C5. Labeled protein was demonstrated in the precipitin band.
Discussion

Evidence demonstrating the biosynthesis of two complement components, C3 and C6, was obtained using the incorporation of 14C labeled amino acids into immunoprecipitable C3 and C5. Biosynthesis of these components was seen in 8 of 10 individuals with chronic periodontal disease. In none of the patients who had clinically and histologically normal gingiva.

Previous studies have demonstrated that C3 was synthesized by liver parenchymal cells (Rosen et al. 1971) and that the liver is the primary site of synthesis of C3 (Rapp & Borsos 1970). However, C3 synthesis occurs in a variety of extrhepatic sites. The cell involved in this extrhepatic synthesis is the macrophage (Stecher & Thorbecke 1967, Lui A Fat & Van Furth 1975). Synthesis of C5 has been observed in a variety of sites (Colton 1973), and recently Whaley (1980) demonstrated C5 synthesis by blood monocytes. Synthesis of both C3 and C5 occurs at mucosal sites (Colton 1976) as well.

Complement biosynthesis has also been shown to occur in lepromatous skin (Lai A Fat et al. 1979) and rheumatoid synovia (Ruddy & Colten 1974). The synthesis of complement at these inflammatory sites is probably by the mononuclear phagocytes found in the tissue. Macrophages are seen as early as 4–7 days after the accumulation of plaque (Page & Schroeder 1976), and it is possible that these cells are synthesizing the complement proteins observed in the present study.

Complement components have been found in the gingival crevicular fluid (Atstrom et al. 1974, Schenkein & Genco 1977b), coating crevicular bacteria in vivo (Nisgard & Jarrett 1976) and localized in the connective tissue of patients with periodontal disease (Genco et al. 1974). It has been assumed that the serum was the source of these proteins. However, the present studies demonstrate that there is also a local contribution to the complement in the gingival crevicular fluid. The concentration of native C3 in crevicular fluid is markedly reduced when compared with serum (Schenkein & Genco 1977a, Atstrom et al. 1974). This reduction occurs despite local synthesis of this component and indicates an even greater rate of consumption than was predicted on serum levels alone.

The role of the complement system in the pathogenesis of periodontal disease has not been defined. Complement deposits have been demonstrated in diseased gingiva (Shillitoe & Lehrer 1972, Genco et al. 1974, Pattar, Schenkein & Weinstein 1979, Tallassen & Saltved 1980), and recently the activation of complement by dental plaque has been shown (Okuda & Takazoe 1980). Several studies observed the penetration of crevicular epithelium by various componenents of dental plaque and other antigens (McDougall 1971, Kusek & Steinberg 1979) while Ranney (1970) and McDougall (1972) have demonstrated antibody responses in the gingiva to topically applied antigens. Immune complexes could result from local accumulation of antigen and antibody in these areas and as a result the complement system would be activated. Studies by Clagett & Page (1976), however, have failed to demonstrate the presence of insoluble immune complexes in chronically inflamed gingiva.

A source of the stimulation for the local production of certain complement factors could be plaque bacteria. Colten (1974) demonstrated a 10-fold increase in the synthesis of C2 and C4 by peritoneal macrophage following incubation with heat-killed pneumococci. Increases in rates of synthesis of C2 and C4 is also dependent on the mechanism of obtaining these cells (Colten & Wyatt 1972). Plaque bacteria and their products could contribute to the in-
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protein. However, the phenomenon that there is also a
mon to the complement in the fluid. The concentration
of complement is increased when compared with human
& Genco 1977a. Attstrom's reduction occurs despite the
activity and indicates the rate of consumption and depletion
on serum levels alone. The presence of complement in
peridontal disease has been complement deposits extracted in diseased gingiva
Genco et al. 1974, in & Weinstein 1979, Tol
1980, and recently by the complement by dental plaque
Okuda & Takaoze 1980, observed the penetration of
human by various components of plaque and other antigens.
Kuske & Steinberg 1979, 70) and McDougall (1972)
add these responses in serologically applied antigens.
A could result from local antigen and antibody in
in a result of the complement of activated. Studies by
(1978), however, have not rule the presence of in-
complexes in chronically stimulated for the local
complement factors activity. Collen (1974) detected an increase in the syn-
C4 by peritonal macrophage with heat-killed cells in rates of synthesis is also dependent on
obtaining these cells 1975). Bacteria and did contribute to the in-
fection of complement synthesis in chronic inflamed gingiva. Studies of such fac-
will provide interesting insights into the modulating effect of complement may exert
the host defense and plaque organisms in the gingival crevice.

Acknowledgements

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References


