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Identification of Rheumatoid Factor in Subgingival Plaque, Saliva and Peripheral Blood of Periodontitis Patients

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IDENTIFICATION OF RHEUMATOID FACTOR IN

SUBGINGIVAL PLAQUE, SALIVA AND

PERIPHERAL BLOOD OF PERIODONTITIS PATIENTS

By

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VITA

The author, Alphonse Vincent Gargiulo, Jr., D.D.S., is the son of Alphonse Vincent Gargiulo and Anna (Gizzi) Gargiulo. He was born September 24, 1953 in Chicago, Illinois.

His elementary education was obtained at William Beye Elementary School in Oak Park, Illinois, and secondary education at Oak Park and River Forest High School, Oak Park, Illinois, where he graduated in 1971.

In September, 1971, he entered College of Lake County and in May, 1973, received the degree of Associative of Science with a major in Biology. In September of 1973 he entered Loyola University of Chicago-Lake Shore Campus earning 107 hrs. toward a Bachelor of Science Degree with a major in Biology. He was accepted in September, 1974, to Northwestern University Dental School. He graduated from Northwestern University in 1979 receiving the degree Doctor of Dental Surgery.

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CHAPTER I

INTRODUCTION

The purpose of this thesis is to identify rheumatoid factor in the subgingival plaque, saliva and peripheral blood, of Type III periodontitis patients.

Also, this thesis will discuss the chronic antigenic stimulating nature of periodontal disease and its possible association with rheumatoid factor; an autoantibody present in diseases of chronic antigenic stimulation. (P.M. Johnson, W. Page Faulk, 1976, Ralph C. Williams, Jr., 1979, James Taborn, Sara E. Walker, 1979)

Furthermore, this thesis may result in the classification of periodontal disease with other chronic diseases, characterized by chronic antigenic stimulation which are associated with rheumatoid factor.

CHAPTER II

REVIEW OF LITERATURE

Accordingly, the etiologic agent sited in most types of periodontal disease seems to be microbial. Genco (1970). Two possibilities have been discussed relating to the tissue destructive action of chronic periodontal disease: (1) direct initiation of the inflammatory response by injurious microbial metabolites and (2) initiation of periodontal inflammation by antigens of oral microorganisms setting immunopathologic processes into action. Genco (1970).

For years, researchers have suggested that immunologic mechanisms may play a role in some form of periodontal disease. Daley, F.H., Healy, J.C. and Sweet, M.H. (1936). Harkavy, J. (1948). Ellison, S.A. and Mandel, I.D. (1963). It has been shown that the Arthus reaction Terner, C. (1965) and chronic allergic inflammation Rizzo, A.A. and Mitchell, C.J. (1966) can induce inflammatory responses in the periodontal unit.

Furthermore, a number of different antigens have been shown to induce inflammation upon repeated injury to the periodontium of several species of animals. Also human studies have shown periodontally diseased patients can have circulating antibodies specific for oral bacteria.

Nisengard, R., Beutner, E.H. and Hazen, S.P. (1968) have reported evidence which points to hypersensitivity as an etiologic factor in

human inflammatory periodontal disease. Animal tests with a reagent prepared from actinomyces indicated that it was both reactive and safe for human use. Skin tests of humans with this reagent revealed a correlation between the immediate hypersensitivity and the presence of periodontal disease.

Terner, C. (1965), has shown an active Arthus phenomenon successfully provoked in the tongue, attached gingiva, buccal mucosa of the rabbit, guinea pig and rat. In the attached gingiva, atypical reactions without necrosis occurs as frequently as the typical reaction with necrosis and ulceration. Lighter animals display the typical reaction in a significantly higher proportion than heavier ones.

Rizzo, A.A. and Mitchell, C.J. (1966) exposed rabbits repeatedly to a protein antigen by triweekly insertion of pellets containing egg albumin (EA) into labial gingival pockets of mandibular incisors. In one control group pellets containing saline were inserted in animal pockets. In rabbits, whose pockets were treated with EA, the gingiva showed chronic inflammation with numerous plasma cells and sera containing intermediate levels of anti-EA antibody. In contrast saline treated rats showed normal histology and their sera did not contain antibody. Findings seem to indicate that antigen was absorbed into the gingiva and that it caused both a local and systemic immunologic response. Their findings support the concept that the gingival plasmocytosis of chronic periodontal disease is brought about by local absorption of bacterial antigens.

Mergenhagen, S.E. and Rizzo, A.A. (1961) injected 1.0 ml of horse

serum every other day subcutaneously in the intrascapular area of New Zealand rabbits. After 3,5, and 6 sensitizing injections - animals were challenged intramucosally in the gingiva and palatal mucosa. Rabbits which were sensitized with as few as three injections over a six day period showed, mucous with edema and plasma cell infiltration. Nonsensitized animals showed a minimal response. Authors concluded allergic type mechanisms may play an important role in the development of the pathologic lesion of periodontal disease.

Beutner, E.H., Trifthause, C., Nisengard, R. and Frinnan, A.J. (1966) sensitized guinea pigs, by systemic immunization with bovine serum albumin in complete Freunds adjuvant. Subsequently the animals were challenged by a intragingival injection with the same antigen. Following challenge, the teeth had increased mobility, osteoclastic resorption and gingiva appeared inflamed as compared with control animals. The pathologic changes observed in the gingiva of sensitized guinea pigs following gingival challenge with the sensitizing antigen resembles those seen in inflammatory types of human periodontal disease.

Mergenhagen, S.E., de Arajio, W.C. and Varah, E. (1965) utilizing a hemolysis procedure employing soluble antigen extracted from a strain of Leptotrichia buccalis, showed hemolytic antibody to L. buccalis in the sera of adult individuals. This experiment indicated that man may have the capability of developing antibodies to somatic antigens.

Evans, R.T., Spaeth, S. and Mergenhagen, S.E. (1966) demonstrated mice immunized intraperitoneally with V. alcalescens in complete Freund's adjuvant developed high titers of bactericidal antibody in both serum and

ascitic fluid. Immunization of rabbits with serum sensitive strains of
V. alcalescens produced high titers of specific bactericidal antibody but
did not affect the levels of this antibody to serum sensitive strains of
E. coli or Salmonella typhosa. Sera from adult patients with periodontal
disease when compared to normal adult sera were found to contain increas-
es in bactericidal antibody to F. polymorphum an organism associated with
periodontitis and Vincent's infection.

The potential for an immunologic mechanism to play a role in perio-
dontal pathology is clear. Antigens are abundant in dental plaque and
periodontal tissues can exhibit immunologic reactivity, moreover, ex-
perimental periodontal disease has been induced in animal systems by
immunologic processes. Genco (1970).

A commonly found mitogen-antigen in dental plaque has been shown
to be endotoxin, a macromolecular complex of lipopolysaccharide derived
from the outermost membrane of gram negative bacteria. Simon, B.I.,
Goldman, H.M., Ruben, M.P., Broitman, S., Baker, E. (1972). Mergenhagen,
W.E., Bladen, H.A. and Hsu, K.C. (1966).

The presence of lipopolysaccharides in dental plaque and periodon-
tal disease has been associated with the pathogenesis of periodontal
disease. Simon, B., Goldman, H., Ruben, M. and Baker, E. (1969). Simon,
B., Goldman, H., Ruben, M., Broitman, S., and Baker, E. (1972).

The relationship of endotoxin and the etiology of periodontal
disease becomes of interest when one notes that endotoxin is a potent in-
flammatory agent.

Bacterial lipopolysaccharides have the capacity to exhibit

biological effects on the immune system. Included in these effects are mitogenicity, a phenomenon restricted to B lymphocyte cell population; Gery, I., Kruger, J. and Spiessel, S.Z. (1972), Andersson, J., Moller, G. and Sjoberg, O. (1972), adjuvanticity capable of antibody formation Johnson, A.G., Gaines, S. and Landy, M. (1956), Neter, E. (1969), and high immunogenic potential, in that low concentrations will cause a specific antibody response in experimental animals. Landy, M. and Baker, P.J. (1966) Nossal, G.J.V. and Ada, G.L. (1971).

Surprisingly LPS has the capability of circumventing thymus derived T-cell helper function, which otherwise is required for initiation of antibody response to T-cell dependent antigens. Schmidtke, J. and Dixon, F.J. (1972).

The LPS macromolecule has been chemically characterized into three regions: Luderitz, O., Westphal, O., Staub, A.M. and Nikaido, H. (1971)., (1) the O polysaccharide which is linked to (2) the core polysaccharide which is in turn linked to (3) a lipid A by a trisaccharide of 2-Keto-3-deoxyoctanoic acid. It has been demonstrated the mitogenicity and adjuvanticity were actions attributed to the lipid region of the molecule; whereas, the antigenic specificity was related to the polysaccharide region. Luderitz, O., Westphal, O., Staub, A.M. and Nikaido, H. (1971).

It has been demonstrated that LPS acting independently of antibody has the capacity to trigger the alternate complement pathway of inflammation. See figure 1. Roitt, I.M. and Lehner, T. (1980).

Furthermore, characteristic of some chronic antigenic stimulating diseases is polyclonal B-cell lymphocyte activation. Freeman, R.R. and

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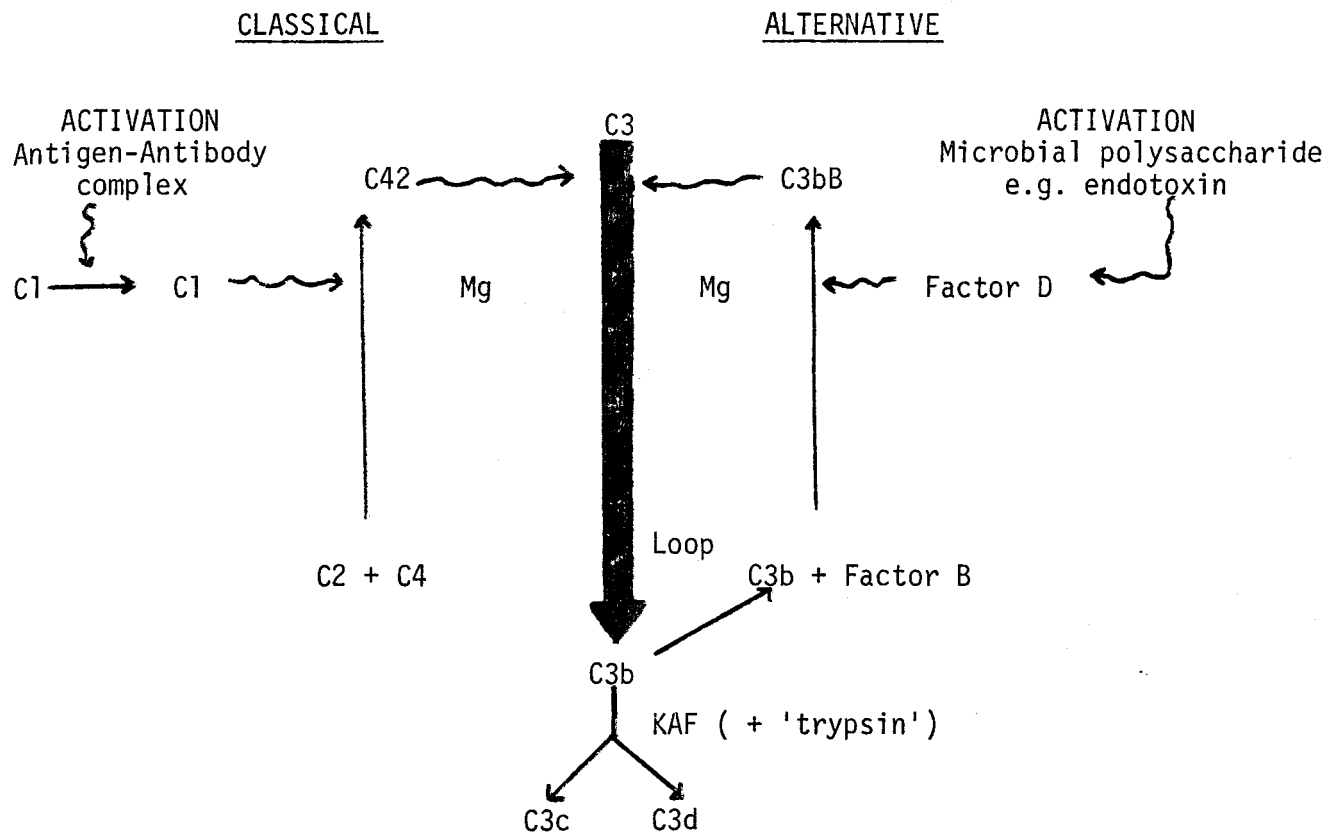
Figure 1. Similarity of Alternate and Classical Pathways of Inflammation.

Parish, C.R. (1978), found preparations of Plasmodium berghei K173 and Plasmodium yoelii 17X have activity in vivo, and that the treatment in mice with the mitogenic supernatant results in polyclonal B cell activation and non-specific IgM synthesis.

Greenwood, B.M. (1974), has hypothesized that the increased synthesis of immunoglobulin production seen in patients with African trypanosomiasis could be the consequence of, polyclonal B-cell lymphocyte activation.

A technique for the detection of polyclonal antibody production has been demonstrated by Cunningham. Cunningham, A.J. and Szenberg, A. (1968). Mice injected with B-cell mitogen lipopolysaccharide from Escherichia coli, showed the production of polyclonal B-cell activation measured by splenic plaque forming cells (PFC).

Kobayakawa, T., Louis, J., Izui, S. and Lambert, P.H. (1979), confirmed that the injection of Trypanosoma brucei (T. brucei) in mice leads to a nonspecific polyclonal B-cell lymphocyte activation. Their conclusions were based first on the observation that the number of lymphocytes in the spleens of infected mice increased fifteen times three weeks after the infection, and secondly, the cells producing antibodies against a variety of antigens appear in the spleen of infected mice. Furthermore these nonspecific PFC responses were composed of only direct PFC, suggesting that the secreted antibodies are of the IgM class. The data confirm the conclusion of Hudson, K.M., Byner, C., Freeman, J. and Terry, R.J. (1976), when an increase in the backround PFC directed against T. brucei in lymphoid tissues was demonstrated.

Kobayakawa further demonstrated that autoantibodies produced during the course of the parasitic infection, seemed to belong to the IgM class. Development of these autoantibodies is parallel to the growth of polyclonal antibody production. These findings suggest the formation of autoantibodies could be a consequence of the polyclonal B lymphocyte activation. Kobayakawa, T., Louis, J., Izui, S. and Lambert, P.H. (1979).

Similar immune reactions have been demonstrated to occur in antigenic challenge with bacterial lipopolysaccharides. Izui, S. (1979) demonstrated the production of rheumatoid factors (autoantibodies) in several strains of mice, including athymic nude mice. Significant IgM-rheumatoid factor activity was observed in seven eight week old female mice injected intraperitoneally with 50 μg of LPS from Salmonella typhimurium on days 0,7 and 14. Concentrations of IgM-RF increased continuously up to day 21, which was seven days after the last injection, but decreased rapidly after this time. Matched control mice receiving saline did not show significant changes in IgM-RF during the same period. The ability of LPS to induce IgM-RF was then studied in five other strains of mice and all responded to LPS injections by forming IgM-RF.

Gargiulo, A.V., Toto, P.D., Gargiulo, A.W. and Robinson, J. (unpublished) demonstrated, by latex slide agglutination, rheumatoid factor in peripheral blood of nine, 12 week old, male Sprague-Dawley rats. Rats were injected in the palatal gingiva with 50 μg and 100 μg doses of LPS from Salmonella typhimurium on day 0,9 and 16. Non-injected rat and vehicle control rat (injected with 0.085% sterile saline) demonstrated no rheumatoid factor in peripheral blood.

According to Kobayakawa, T., Louis, J., Izui, S. and Lambert, P.H.
(1979), the role of lipid A component of endotoxin in the genesis of
of polyclonal B-cell activation is a possibility.

Morrison, D.C., Betz, S.J. and Jacobs, D.M. (1976), have shown mice
lymphocytes to react mitogenically to the lipid A associated protein in
LPS.

Further evidence by Izui, S. (1979) demonstrates that the active
portion of the LPS molecule that is responsible for the RF induction
appears to be the phospho-lipid fraction containing lipid A; since the
highest levels of RF induced in mice correlated with injection of LPS
that contained the most lipid A per molecule.

Such a correlation has been demonstrated by Chiller, J.M., Skid-
more, B.J., Morrison, D.C. and Weigle, W.O. (1973), when induction of
DNA synthesis was measured to show lipid A was mitogenic for bone marrow
derived lymphocytes obtained from spleens of congenitally athymic mice.
Izui, S. (1979).

Lipid A has been shown to be structurally composed of a phosphory-
lated D-acetyl glucosamine to which fatty acids (lauric, myristic,
palmitic and 3-hydroxy myristic) are ester or amide linked. Rietschel,
E.T., Gottert, H., Luderitz, O. and Westphal, O. (1972) have demonstrated
removal of the ester-linked fatty acids by alkaline hydrolysis does
eliminate the B-cell lymphocyte mitogenic stimulus obtained from the
total molecule.

Since bacterial products, specifically lipopolysaccharides, have
been shown to induce polyclonal B-cell activation and enhance production

of IgM rheumatoid factor in chronic antigenic stimulating diseases; a review of rheumatoid factor and its association in chronic disorders is necessary.

Meyer, K. (1922) was the first to discover rheumatoid factor (RF), when he showed sensitized sheep red blood cells were agglutinated by sera of rheumatoid arthritis patients. Since, research in the field of rheumatoid arthritis and diseases of chronic antigenic stimulation associated with rheumatoid factor has been intensely and widely published.

Rheumatoid factors have been demonstrated to be antibodies (auto-antibodies) directed against altered or native gamma globulin. The most common rheumatoid factor occurs as IgM immunoglobulin, which has specificity for antigenic determinants of the Fc fragment of human or animal IgG. Taborn, J.D., and Walker, S.E. (1979). Williams, R.C. (1979). Immunoglobulin classes of IgG and IgA may also have specificity for IgG. Torrigiani, G. and Roitt, I.M. (1967).

Although the detection of rheumatoid factor in the serum may support a diagnosis of human rheumatoid arthritis, its presence may be associated with many other chronic antigenic stimulating diseases. (See Table I.)

Rheumatoid factors have a wide range of specificities for IgG. At least seven antigenic sites on the IgG molecule react with rheumatoid factor. Taborn, J.D. and Walker, S.E. (1979). Rheumatoid factor reacts most commonly with antigenic groupings of IgG that have been buried in the native molecule. Stage, D.E., and Mannik, M. (1973).The so-called "hidden determinants" may be revealed by unfolding of the IgG molecule

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TABLE I

CHRONIC ANTIGENIC STIMULATING DISEASES

ASSOCIATED WITH RHEUMATOID FACTOR

Normal persons (2 to 4% of the population)

Rheumatoid arthritis

Relatives of rheumatoid arthritis patients

Aging (10 to 20% positive over age 65)

Systemic lupus erythematosus

Sjögren's Syndrome

Scleroderma

Dermatomyositis

Sarcoidosis

Leprosy

Dysproteinemias and paraproteinemias

Liver disease (infectious hepatitis, biliary cirrhosis)

Syphilis

Idiopathic pulmonary fibrosis

Pneumoconiosis

Acute pulmonary tuberculosis

Acute viral infections

Parasitic diseases

Following prophylactic vaccinations

Subacute bacterial endocarditis

Diabetes mellitus patients on insulin therapy

Psychiatric disorders (schizophrenia, endogenous depression)

Following multiple blood transfusions

Parenteral narcotic addiction

in denaturation or by the reaction of IgG with unknown antigen. Taborn, J.D. and Walker, S.E. (1979).

The biologic properties of rheumatoid factor is unclear. Production of RF may be caused by an unknown process which changes the patients IgG and makes it autoimmunogenic. This process may be a chronic infection, an acquired abnormality in the IgG causing it to aggregate spontaneously and thereby becoming immunogenic. Taborn, J.D. and Walker, S.E. (1979). There may be an unknown factor in rheumatoid arthritis which breaks down IgG, thereby forming fragments which stimulate formation of anti-IgG antibody. More recently it has been shown in vitro, that B-cells programmed to synthesize RF are present in both normal and rheumatoid arthritis B-cell lymphocyte repertoires, but are preferentially expressed in rheumatoid arthritis. Koopman, W.J. and Schrohenloher, R.E. (1980).

The screening technique most commonly employed to identify the presence of rheumatoid factor is a latex slide agglutination test, first described by Singer, J.M. and Plotz, C.M. (1956).

The principle of the latex slide agglutination method for the demonstration of rheumatoid factor involves the use of an indicator system containing a large number of IgG molecules. The IgG is either bound to carrier particles, such as latex, bentonite, erythrocytes or the IgG molecules are simply aggregated by heat denaturation or other means. The presence of rheumatoid factor in serum is recognized by agglutination, precipitation or flocculation of the respective IgG containing indicator system. Waller, M.V. and Vaughn, J.H. (1956). Serial dilutions of the

serum samples provide an estimate of the quantity of the rheumatoid factor in terms of titer.

Of course, other methods for the detection of rheumatoid factor are available. Radioimmunoassay involves the binding of radioactively labelled antigen to a fixed amount of antibody which can be partially inhibited by addition of unlabelled antigen. The inhibition can then be used as a measure of the unlabelled material added. Sato, M., Kasukawa, R., Murai, T. and Yoshida, T. (1977) have demonstrated the detection of rheumatoid factors through solid-phase radioimmunoassay. Rheumatoid factors of IgG and IgM class were detected by means of solid-phase radio-immunoassay using formalinized sheep erythrocytes sensitized with rabbit anti-sheep erythrocyte serum and ^{125}I-labelled anti-human IgG or anti-human IgM antibody.

Another very sensitive and precise method for the detection of rheumatoid factor is the enzyme linked immunosorbent assay (ELISA). Voller, A., Bidwell, D. and Bartlett, A. (1977). The basis of the ELISA depends on two assumptions: 1) that antigen or antibody can be attached to a solid-phase support yet retain immunological activity and 2) that either antigen or antibody can be linked to an enzyme and the complex retain both immunological and enzymatic activity. Vejtrop, M., Hoier-Madsen, M. and Halberg, P. (1979), demonstrated that the ELISA was well suited for quantitative routine determination of IgM-RF. Gripenberg, M., Wafin, F., Isomaki, H. and Linder, E. (1979), have developed a simple enzyme linked immunosorbent assay, using human IgG as antigen, and found good agreement between the results and reproducibility.

Some investigators have suggested a protective role for rheumatoid factor. It has been postulated that rheumatoid arthritis has a viral etiology, and that rheumatoid factor may have antiviral properties in vitro. It is thought that viral neutralization may be enhanced by the addition of human IgG rheumatoid factor. Interestingly, Ashe, W.K. and Nothens (1971) have shown the addition of IgM rheumatoid factors facilitates neutralization of virus by antiviral antibody, blocking all antigenic sites in the virus available for cell penetration or fixation.

Rheumatoid factor also influences chemotactic activity and phagocytosis. The addition of rheumatoid factor, aggregated IgG and complement to a suspension of neutrophils, produced increased chemotactic activity compared to aggregated IgG and serum. Borel, J.F., Sorkin, E. and Loewi, G. (1971).

However, in another in vitro system phagocytosis of bacteria was inhibited by rheumatoid factor. Messner, R.P. (1968). Further, it was shown white blood cells which have ingested rheumatoid factor IgG complex have reduced ability to phagocytose bacteria.

Animal studies have shown, that rheumatoid factor can be induced with the use of prolonged intravenous hyperimmunization with coliform bacteria. Christian, C.L. (1963) or immunization with autologous gamma globulins. Catsoubi, E.A. (1965).

Rabbits immunized with autologous gamma globulin produced anti-gammaglobulin antibody with primary specificity for human rather than rabbit IgG. Similarly, injection of a rabbit with autologous immunoglobulin complexes in complete Freund's adjuvant stimulated production of

an anti-gammaglobulin reaction with human IgG and rabbit IgG. Rabbit rheumatoid factors react specifically with the Fc fragment of IgG and pepsin digested IgG. The above phenomenon was found in serum from rabbits receiving long term immunization of cell wall carbohydrate derived from streptococci. Osterland, C.K. (1966).

Restifo, R.A. (1965) demonstrated the induction of acute arthritis by intra-articular injection of autologous IgG in rheumatoid arthritis patients, who have rheumatoid factor; while the same injection in seronegative rheumatoid arthritic patients did not cause synovitis. In a similar study, prolonged intravenous infusion of rheumatoid factor had no adverse effect on normal subjects.

The proposed mechanism of tissue destruction in rheumatoid arthritis is the fixation of complement by rheumatoid factor-IgG complex which is engulfed by polymorphonuclear leukocytes. The leukocytes then indirectly release the tissue destroying enzymes.

In Sjogren's Syndrome with or without rheumatoid arthritis, the incidence of serum rheumatoid factor approaches one hundred percent. Bunim, J.J., Buchanan, W.W., Wertlake, P.T., Sokoloff, L., Block, K.J., Beck, J.S. and Alepa, F.B. (1964). No other population of patients, including those with rheumatoid arthritis, have such a high frequency of this autoantibody. Anderson, L.G., Cummings, N.A., Aosfsky, R., Hylton, M.B., Tarpley, T.M., Tomasi, T.B., Wolf, R.O., Schall, G.L. and Talal, N. (1972). In vitro studies utilizing lip biopsy specimens, suggest lymphoid cells infiltrating the salivary glands in Sjogren's Syndrome, synthesize large quantities of IgM and IgG, some with an anti IgG or rheumatoid factor

activity. Clinically, macroglobulin, a characteristic of Waldenstrom Syndrome appears to play a prominent role in the lymphoproliferative process in some patients with Sjogren's Syndrome. The above report supports the coexisting Sjogren's Syndrome and Waldenstrom's macroglobulinemia reported in the literature. Pinkus, G.S. and Dekker, A. (1970). Whitehouse, A.C., Buckley, C.E. III, Nagaya, H. and McCarter, J. (1967). Malsville, J., Heid, F., Rousselot, P., Bergoend, H., Grosshans, E. and Araujo, A. (1967).

Chronic antigenic stimulation is characteristic of both periodontal disease and rheumatic diseases. The method of tissue destruction by polymorphonuclear leukocytes associated with both of these chronic disorders is similar. Furthermore, the association between rheumatic diseases, disorders of chronic antigenic stimulation, and rheumatoid factor has well been established in the medical literature. Is there an association between periodontal disease, a disease of chronic antigenic stimulation, and rheumatoid factor? This is the purpose of the present investigation.

The association of periodontal disease with other diseases of chronic antigenic stimulation in which polyclonal antibodies are produced containing rheumatoid factor appears promising. It is the purpose of this study to determine the presence of rheumatoid factor in the gingival crevice of patients with moderate to advanced periodontitis.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Latex Human IgG Slide Agglutination Test-"Rheumanosticon" for the determination of rheumatoid factor, by Organon, West Orange, New Jersey.

   Kit Contents:

   a. Latex reagent-particle size, 0.81 microns

   b. Glycine buffered concentrate (glycine buffered saline)

   c. Positive serum control

   d. Negative serum control

   e. Dispenstirs, designed to deliver 0.03 ml

   f. Black background slide

2. 12 mm x 75 mm sterile polystyrene tubes (Cole Parmer Instrument Company, Chicago, Il.)

3. Disposable 0.5 ml microcentrifuge tubes (Cole Parmer Instrument Company, Chicago, Il.

4. Centrifuge

5. Blood lancets (Scientific Products, McGaw Park, Il.)

6. 70% isopropyl alcohol

7. Non-containing IgG, or blank latex beads, 0.80 microns (Sigma Laboratories, St. Louis, Missouri)

8. The Colorimetric Determination of Albumin (Sigma Laboratories, St.
   Louis, Missouri

Human Subjects:

   Thirty Type III Periodontitis patients, selected by using the American
Dental Association Classification for Periodontal disease.

   Classification:

       Type I   - Gingivitis (shallow pockets, no bone loss)

       Type II   - Early periodontitis (moderate pockets,

                   minor to moderate bone loss,

                   satisfactory topography, usually no

                   tooth mobility)

       Type III - Moderate periodontitis (moderate to

                   deep pockets, moderate to severe bone

                   loss, unsatisfactory bone topography)

       Type IV   - Advanced periodontitis (deep pockets,

                   severe bone loss, advanced mobility

                   patterns, usually missing teeth, may

                   become prosthetic reconstruction case)

Methods

A. Subgingival plaque and fluid were tested for rheumatoid factor
   as described below:

       1. Subgingival contents were sampled from a periodontal

          pocket, 5-6 mm in depth, of a Type III periodontitis

          patient, with the use of a sterile curette.

2. The subgingival plaque sample was sonicated in 0.18 ml of glycine buffered slaine (GBS), contained in a disposable 0.5 ml microcentrifuge tube.

3. The microcentrifuge tube containing the sonicated sample was placed in a 12 mm x 75 mm polystyrene tube, and centrifuged at 3,675 rpm for ten minutes to remove particulate matter.

4. The initial plaque dilution was heat inactivated at 56°C for thirty minutes, in order to inactivate complement components.

5. 0.03 ml of the initial dilution was placed on the black background slide issued in the kit, and mixed in a figure eight configuration with 0.03 ml of latex containing -IgG for one minute at room temperature. Tests will be scored as positive on flocculation or negative in the absence of flocculation, according to the manufacture's direction. All initial dilutions yielding a positive test were then diluted 1:20 and retested in the same manner as described above. 1:20 dilutions were made by pipetting 0.1 ml of initial plaque dilution and placing it in 1.9 ml of GBS.

6. Positive and negative controls accompanied the above reactions as provided in the test kits.

Positive control - a dilution of human sera

containing rheumatoid factor

causing flocculation of IgG

adsorbed latex particles.

Negative control - dilution of human sera

containing no detectable

rheumatoid factor, which will not

flocculate latex particles

adsorbed with IgG.

Negative control - latex particles not containing

adsorbed IgG, that is blank particles,

which do not flocculate with sera

with or without rheumatoid factor.

B. Saliva was tested for rheumatoid factor as described below:

1. 0.25 ml of human saliva was drawn from pooled saliva

from the floor of the mouth. This sample was placed

in a 0.5 ml microcentrifuge tube.

2. The saliva sample was heat inactivated at 56°C for

thirty minutes and centrifuged at 3,675 rpm for

ten minutes.

3. 0.03 ml of the centrifuged saliva sample was placed

on the black background slide and assayed with 0.03 ml

of latex containing IgG and mixed in a figure eight

configuration. Any initial samples yielding a

positive result were diluted 1:10 and re-tested in

the same manner, and examined for evidence of

flocculation.

4. Tests were scored as in step 5 of subgingival plaque testing, and similar controls were utilized as described in step 6 under subgingival palque testing.

5. Stimulated pooled saliva was also obtained from ten full denture patients, wearing full denture prosthesis. These patients were utilized as controls.

C. Blood was tested as described below:

1. The patient's middle finger was wiped clean with a 70% isopropyl alcohol. A finger puncture was made with a blood lancet provided by Scientific-Products.

2. The blood was not milked from the finger but allowed to flow, and it was collected in a sterile dispenstir, provided in the test kits. 0.03 ml of blood was drawn through the dispenstir, which is approximately equal to one squeeze of the dispenstir between the thumb and forefinger.

3. One free-falling drop was diluted in 0.25 ml of GBS in a 12 mm x 75 mm test tube. This represents a 1:10 dilution.

4. 0.03 ml of the 1:10 dilution is assayed with 0.03 ml of IgG containing latex beads on the black background slide and mixed in a figure eight configuration, and examined for flocculation. Any positive tests are further diluted to 1:20 in GBS and retested for rheumatoid factor.

5. Scores and controls were made as previously described.

D. Albumin concentration control:

A Colorimetric Determination of Albumin (Sigma Labs. St. Louis, Missouri), was performed on all subgingival plaque samples to determine albumin concentrations. In order to standardize plaque samples through protein concentration, albumin can serve as an indicator of serum protein component of gingival plaque and fluid.

In the above colorimetric test, Rodkey (1965) determined bromocresol green (BCG) will bind to albumin and not to other proteins. The intensity of the color of the albumin-BCG complex is measured at 630± 5nm in a Coleman 6/35 spectrophotometer.

Procedure

1. Sterile test tubes 12 x 75 mm were labelled, blank, standard, subgingival plaque 1, subgingival plaque 2 ... subgingival plaque 30.

2. To all tubes 5.0 ml Albumin Color Reagent, Stock No. 630-2 was added.

3. To blank test tubes, 20 µl of 0.85% sodium chloride solution was added.

To the standard, 20µl Protein Standard Solution, Stock No. 540-10 was added.

To each tube labelled subgingival palque, 20 µl of the sub-gingival plaque dilution was added.

4. All tubes were vortexed for thirty seconds and allowed to

stand at room temperature for 10 min.

5. Absorbance of blank, standard and subgingival plaque was read
    at 630 nm., in a Coleman Spectrophotometer 6/30.

CHAPTER IV

RESULTS

In this investigation, the patient sample size numbered thirty. There were twenty males and ten females, whose ages ranged from 28-65 years. The mean age was 45.1 years.

All patients selected for this study were classified as having a Type III periodontal condition and without history of rheumatic diseases.

Table II describes the results obtained from subgingival plaque in 1.8 ml of GBS, and serum diluted in GBS 1:10 and 1:20, when assayed with IgG-containing latex beads and blank (non-containing IgG latex beads) as a parallel control, for the detection of rheumatoid factor.

Negative and positive serum controls all were negative or positive, respectively. Of the experimental subjects, 66.6% showed positive agglutinations with bacterial plaque; 83.3% with serum 1:10 and 6.6% with serum 1:20.

Table III describes the results obtained from stimulated saliva and saliva diluted in GBS 1:10, when assayed with IgG-containing latex beads and blank beads as a parallel control, for the detection of rheumatoid factor. Of the experimental subjects 100% of the stimulated undiluted whole saliva samples were positive for RF but only 40.0% were positive at 1:10 dilution. Control saliva obtained from ten edentulous denture patients wearing full denture prosthesis, ranging from 59-88 years of age,

was assayed as described above for the thirty experimental patients. 50% of the stimulated samples were positive for RF; however, at 1:10 dilution only one sample or 10% was positive.

TABLE II

Positive and Negative Reactions Tabulated
For Detection of Rheumatoid Factor

Patient No.-Age	Subgingival plaque/1.88cc GBS	Serum 1:10	Serum 1:20	Neg. Cont.	Pos. Cont.	Blank Beads
1 - 31	+	+	-	-	+	-
2 - 29	+	+	-	-	+	-
3 - 60	+	+	-	-	+	-
4 - 46	+	+	-	-	+	-
5 - 51	+	+	-	-	+	-
6 - 39	+	+	-	-	+	-
7 - 51	+	+	-	-	+	-
8 - 46	+	+	-	-	+	-
9 - 58	+	-	-	-	+	-
10 - 32	-	+	-	-	+	-
11 - 32	-	-	-	-	+	-
12 - 33	-	-	-	-	+	-
13 - 33	-	+	-	-	+	-
14 - 47	+	+	+	-	+	-
15 - 28	+	+	+	-	+	-
16 - 33	+	+	-	-	+	-
17 - 52	-	+	-	-	+	-
18 - 65	+	+	-	-	+	-
19 - 37	-	+	-	-	+	-
20 - 61	+	+	-	-	+	-
21 - 28	-	-	-	-	+	-
22 - 65	-	+	-	-	+	-
23 - 41	-	+	-	-	+	-
24 - 45	+	+	-	-	+	-
25 - 31	+	+	-	-	+	-
26 - 55	+	+	-	-	+	-
27 - 52	-	+	-	-	+	-
28 - 63	+	+	-	-	+	-
29 - 52	+	+	-	-	+	-
30 - 57	+	-	-	-	+	-
No. of Agglutinations	20	25	2	0	30	0
Percent Agglutination	66.6%	83.3%	6.6%	0%	100%	0%
Mean	.66	.83	.06	0.0	1.0	0.0
Standard Deviation	±.47	±.37	±.25	±0.0	±0.0	±0.0

TABLE III

Positive and Negative Reactions Tabulated
For Detection of Rheumatoid Factor

Patient No.-Age	Stimulated Saliva	Saliva 1:10	Negative Control	Positive Control	Blank Beads
1 - 31	+	+	-	+	-
2 - 29	+	-	-	+	-
3 - 60	+	-	-	+	-
4 - 46	+	-	-	+	-
5 - 51	+	+	-	+	-
6 - 39	+	-	-	+	-
7 - 51	+	-	-	+	-
8 - 46	+	-	-	+	-
9 - 58	+	-	-	+	-
10 - 32	+	+	-	+	-
11 - 32	+	-	-	+	-
12 - 33	+	-	-	+	-
13 - 33	+	-	-	+	-
14 - 47	+	+	-	+	-
15 - 28	+	-	-	+	-
16 - 33	+	-	-	+	-
17 - 52	+	-	-	+	-
18 - 65	+	+	-	+	-
19 - 37	+	+	-	+	-
20 - 61	+	-	-	+	-
21 - 28	+	+	-	+	-
22 - 65	+	-	-	+	-
23 - 41	+	+	-	+	-
24 - 45	+	-	-	+	-
25 - 31	+	+	-	+	-
26 - 55	+	+	-	+	-
27 - 52	+	+	-	+	-
28 - 63	+	-	-	+	-
29 - 52	+	-	-	+	-
30 - 57	+	+	-	+	-
No. of Agglutinations	30	12	0	3	0
Percent Positive Agglutinations	100%	40.0%	0%	100%	0%
Mean	1.0	.40	0.0	1.0	0.0
Standard Deviation	±0	±.49	±0.0	±0.0	±0.0

TABLE IV

Control Saliva
Ten Full Denture Patients

Patient No.-Age	Stimulated Saliva	Saliva 1:10	Negative Control	Positive Control	Blank Beads
1 - 88	+	+	-	+	-
2 - 65	+	-	-	+	-
3 - 67	+	-	-	+	-
4 - 78	-	-	-	+	-
5 - 59	-	-	-	+	-
6 - 63	-	-	-	+	-
7 - 61	+	-	-	+	-
8 - 64	+	-	-	+	-
9 - 66	-	-	-	+	-
10 - 68	-	-	-	+	-
No. of Agglutinations	5	1	0	10	0
Percent Positive Agglutinations	50%	10%	0%	100%	0%
Mean	.50	.10	0.0	0.0	0.0
Standard Deviation	±.52	±.31	±0.0	±0.0	±0.0

Albumin concentration in each subgingival
plaque dilution as determined by the Colorimetric
Determination of Albumin, are listed in Table V.
Concentrations were calculated as follows:

Albumin-subgingival   Absorbance of subgingival
plaque dilution (gm/100 ml) = plaque dilution
                                     Absorbance of Standard
                                     albumin

Concentration of the
Standard albumin (gm/100 ml)

For example: the calculated concentration of albumin

          in the subgingival plaque dilution of

          patient no. 19, was determined as follows:

Albumin-subgingival   .02
plaque dilution (gm/100 ml) = ——— x 5.0 gm/100 ml
                              .45

                = .22 gm/100 ml

TABLE V

Albumin Concentration Determined by Color-
metric Determination of Albumin In Diluted
Subgingival Plaque

Patient No.	Absorbance subgingival plaque dil.	Albumin conc. in subgingival plaque dil. (gm/100ml)
1	.015	.16
2	.05	.55
3	.05	.55
4	.01	.11
5	.015	.16
6	.05	.55
7	.015	.16
8	.05	.55
9	.015	.16
10	.012	.13
11	.01	.11
12	.05	.55
13	.012	.13
14	.01	.11
15	.08	.88
16	.013	.14
17	.016	.17
18	.015	.16
19	.02	.22
20	.018	.20
21	.021	.23
22	.015	.16
23	.06	.66
24	.025	.27
25	.018	.20
26	.031	.34
27	.013	.14
28	.01	.11
29	.015	.16
30	.02	.22

Mean albumin concentration = .27 gm/100ml

Standard deviation = ± .20 gm/100ml

CHAPTER V

DISCUSSION

Diluted subgingival plaque, sera and saliva of patients with Type III periodontal disease show evidence of rheumatoid factor by the macroscopic agglutination between anti-IgG globulins or rheumatoid factors and the IgG containing latex beads.

Blank latex beads failed to agglutinate when assayed with subgingival plaque, serum or saliva. This demonstrates the latex beads themselves did not non-specifically agglutinate to some moiety present in the test samples.

Concentration of albumin in diluted subgingival plaque samples ranges from 0.11 to 0.88 g/100m. Mean and standard deviation of albumin concentration was determined to be 0.27 ± 0.20 g/100 ml. A significant reduction of albumin in the subgingival plaque samples was evident when compared to the normal serum albumin levels ranging from 3.8-5.1 g/100 ml. In this investigation, even though the plaque samples were diluted, reduction of albumin levels in the gingival exudate as compared with serum levels seems to be in agreement with the findings of Brill and Bronnenstan (1960) and Schenkein and Genco (1977). The significance of the presence of albumin in diluted subgingival plaque samples, indicates the presence of serum proteins in crevicular fluid. For example, Shillitoe and Lehner (1972) have described the concentrations of IgG, IgA, IgM and C_3 in

gingival fluid from periodontally diseased patients to be well below their respective serum levels.

Furthermore, positive agglutination reactions in subgingival plaque corresponded to low concentrations of albumin, suggestive of rheumatoid factor production locally and not from factors entering the gingival crevice from the serum. See Figure 2.

In eighteen subjects with chronically inflamed gingiva Schenkein and Genco (1977) have shown IgG, IgM and IgA in decreased concentration in gingival fluid as compared to serum levels.

Experimental subjects with chronic periodontitis have rheumatoid factor in their pooled whole saliva. This may be of positive diagnostic significance as saliva of edentulous patients are positive only in 50% of the patients. Moreover, dilution of control saliva results in only 1 in 10 positive. Perhaps edentulous patients may have had chronic periodontitis prior to full denture prosthesis; and this is the reason for the residual dilute rheumatoid factor. Only through additional studies may this point be answered.

Blank latex beads were unreactive with control saliva; this corresponds well with the results obtained with diluted subgingival plaque.

In analyzing agglutination reactions between plaque samples and 1:10 serum samples, all subjects with periodontitis demonstrating positive reactions in plaque (20 of 30) also showed positive reactions in 1:10 serum except one individual. This seems interesting, as none of the subjects with periodontitis had rheumatoid arthritis. As a serum dilution of 1:20 demonstrating agglutination is essential in supporting a

Figure 2

Concentration of Albumin in

Diluted Subgingival Plaque

(g/100ml)
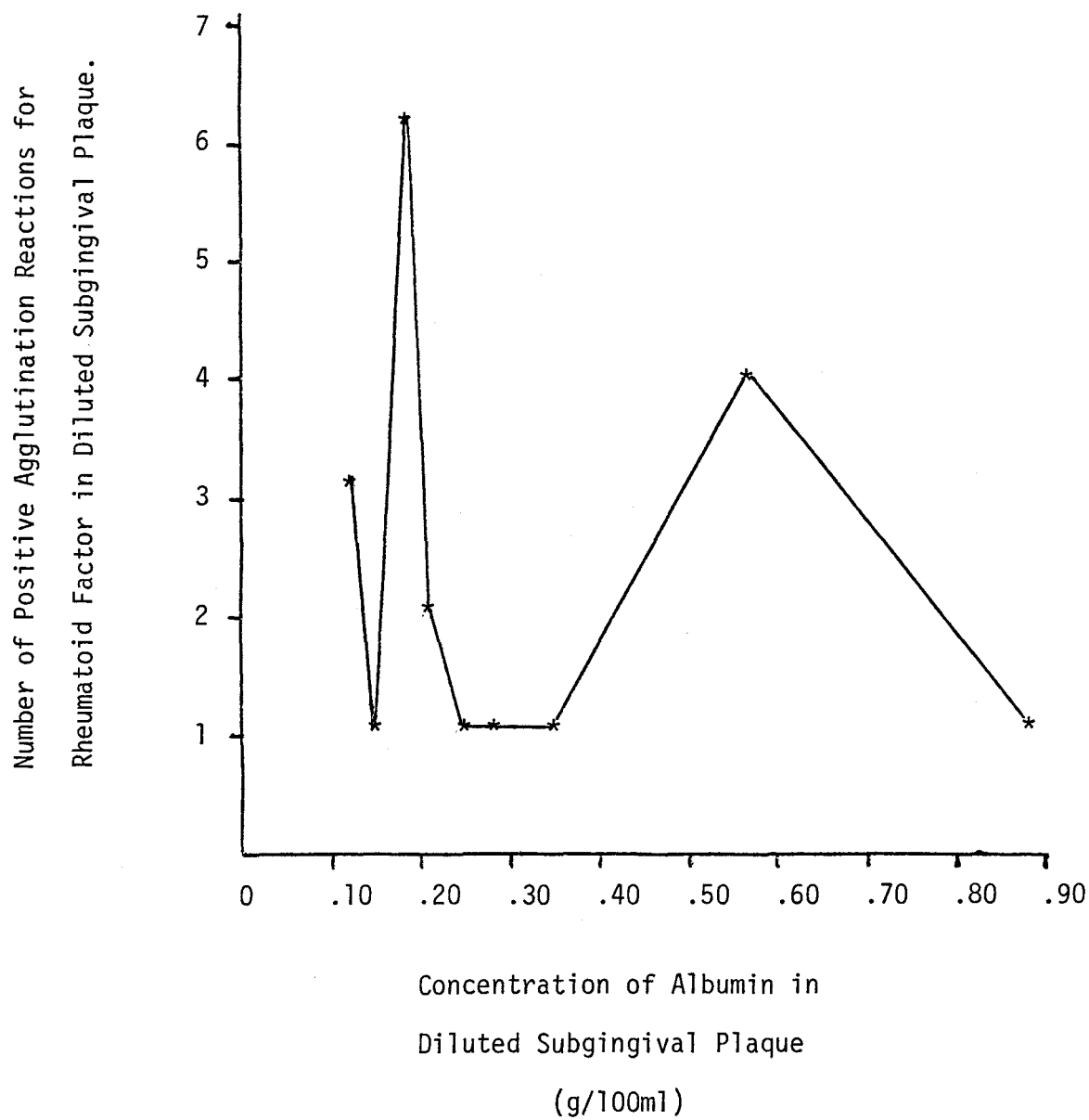
diagnosis of rheumatic disease. Substantiating this fact, is the observa-
tion of only two subjects of the experimental group demonstrating positive
agglutinations in serum diluted 1:20. Therefore, this is evidence that
the periodontal patients in this study did not have rheumatic disease; and
the two subjects with positive sera agglutination at 1:20, probably fall
into the category of normal persons with rheumatoid factor, as described
by Moskovitz (1975). Perhaps it is possible that agglutinations seen at
1:10 dilution in the absence of rheumatoid arthritis, is due to the pre-
sence of rheumatoid factor associated with the generalized chronicity of
antigenic-mitogenic stimulation characteristic of periodontal disease.

The potential etiology of rheumatoid factor production in periodon-
tal disease may possibly be two fold. First, rheumatoid factor may be
induced by the mitogenicity, adjuvanticity and immunogenicity of gram
negative bacterial lipopolysaccharides. Chiller, Skidmore, Morrison and
Weigle (1973) have demonstrated the mitogenic effect of lipopolysaccharide
could be related to a particular structural region of the molecule, namely
the lipid A region. Lipid A has been demonstrated in association with
gram negative bacteria, endotoxin and periodontal disease. Tanner, Haf-
fer, Brathall, Viscontis and Socransky (1979) have shown the subgingival
microbiota in advanced periodontal disease to be predominated by gram
negative rods. Slots (1979) determined the microflora of advanced adult
periodontitis was comprised mainly of gram negative anaerobic rods.
Schwartz, Stinson and Parker (1972) have shown endotoxin localized in
crevicular epithelium by autoradiography. Simon, Goldman, Reuben, Broit-
man and Baker (1972) have shown endotoxin in gingival exudate and with

increasing severity of gingival lesions associated with an increase in the numbers of gram negative bacteria. Endotoxin of gram negative bacteria has further been demonstrated in dental plaque by Mergenhagen, Bladen and Hsu (1966) and root surfaces of periodontally involved teeth by Jones and O'Leary (1978) and Aleo, DeRenzis, Farber and Varboncouer (1974). Therefore, the basis of gram negative bacterial induction of rheumatoid factor in periodontal disease by lipopolysaccharide appears substantially evident. It has been shown by Izui, Eisenberg and Dixon (1979) that bacteria associated with lipopolysaccharide causing infection when administered to rats may trigger the production of IgM-rheumatoid factor.

Secondly, immune complexes demonstrated by Berglund (1971) in periodontitis must be considered as a potential causitive agent of rheumatoid factor production. It has been shown that upon antigen combining with antibody the F_{ab} portions of the molecule assume a 180° angle in respect to each other. As the immune complex is formed an alteration in the tertiary structure of the antibody may occur revealing new or buried antigenic determinants in the F_c fragment of the IgG molecule. Vaughn (1972). Plasma cells might then produce autoantibodies or rheumatoid factors against these newly revealed antigenic determinants. This may be a factor in periodontitis but additional studies to determine the presence of Ag-Ab complexes are needed.

Subjects with positive reactions for rheumatoid factor in subgingival plaque samples also demonstrated corresponding positive reactions in samples of stimulated saliva. Theoretically, perhaps, chronic antigenic

stimulating substances, for example, lipopolysaccharides, may travel from gingival tissue to regional lymph nodes resulting in rheumatoid factor production by plasma cells in germinal centers. It has been shown by Mellors, Nowoslawski and Korngold (1961) that rheumatoid factor is produced in germinal centers of lymph nodes. Rheumatoid factors may then be transported to salivary gland tissue and secreted with saliva. Studies, are needed to determine if lipopolysaccharide is transported from gingiva to the lymphoid tissues in salivary glands. In this event, rheumatoid factor could be locally produced and secreted with saliva; secretory IgM has been reported in saliva by Brandtzaeg (1966).

The production of rheumatoid factor in periodontitis in the absence of rheumatoid arthritis possibly could occur, if the patient has genetically programmed B-cells for rheumatoid factor production. Rheumatoid factor can be produced by patients without rheumatoid arthritis as Koopman and Schrohenloher (1980) have shown B-cells from non-rheumatoid patients, stimulated by poke weed mitogen, were capable of producing IgM-rheumatoid factor.

It has been suggested that rheumatoid factors have a protective role in the body's defense. Vaughn (1972) have shown that rheumatoid factors have the ability to combine with soluble antigen-antibody complexes and thereby render them less soluble and more easily cleared by the reticuloendothelial system.

The presence of IgM-rheumatoid factor has been reported to inhibit some in vitro complement dependent systems, and this has given rise to a concept that rheumatoid factor could be protective, although in vivo

analogues are lacking, according to Johnson and Faulk (1976).

Supportive evidence of the complement fixing properties of rheuma-
toid factor is reported by Goodman (1961) and Franklin (1961) who demon-
strate an antigenic site on gamma globulin for rheumatoid factor binding;
and Ishizaka, Ishizaka and Sugakara (1962) showing binding sites for com-
plement component C_1; both sites are located on the F_c fragment of IgG.
Therefore, if rheumatoid factor binds to and occupies the F_c fragment of
IgG, and blocks complement binding site, the rheumatoid factor molecule
must provide the only available site to bind complement, in such case.

Further support of the complement activation by rheumatoid factor
is reported by Ashe and Notkins (1971) who demonstrate enhanced neutrali-
zation of herpes simplex virus by rabbit and human antibody complement by
the addition of IgM-rheumatoid factor. Furthermore, McCormick, Day,
Morris and Hill (1969) showed more exaggerated complement associated le-
sions of experimental nephrotoxic nephritis induced in vivo, when IgM
rheumatoid factor was present.

Complement activation by either immune complexes or endotoxin
appears to be present in periodontal disease, also. Schenkein and Genco
(1977) and Attstrom, Laurel, Larsson and Sjoholm (1974) demonstrated a
marked decrease in C_3 and C_4 levels in gingival fluid as compared to
serum. The above findings are felt to be suggestive of complement ac-
tivation and consumption during periodontal inflammation.

Similarly, Hedberg (1964) and Pekin and Zvaifler (1964) have shown
reduced complement levels in synovial fluid due to antigen-antibody re-
actions, in rheumatoid arthritis.

Tesar and Schmid (1970) have shown IgM rheumatoid factor has the capability of transforming soluble complexes to complement fixing aggregates, making immune complexes more easily phagocytized. Therefore, rheumatoid factor may possibly act as another homeostatic mechanism in periodontal inflammation and also be partly responsible for complement activation and consumption.

It is an established phenomena, that rheumatoid factor in serum may act as a diagnostic and prognostic indicator in rheumatic diseases. For example, William and Kunkel (1962) have demonstrated that positive serum reaction for rheumatoid factor in patients with bacterial endocarditis, upon cure, revert to negative.

In this investigation, one may raise the question: can rheumatoid factor be used as an indicator for severity of disease, prognosis and possible cure? This may only be determined through additional research.

The following theory is based on the role of rheumatoid factor in the pathogenesis of rheumatoid arthritis described by Taborn and Walker (1979), and which is modified in relation to the pathogenesis of periodontal disease. Chronic antigenic stimulating substances, i.e. gram negative bacteria derived lipopolysaccharides found in crevicular fluid or gingival tissue stimulate production of antibody. Antigen-antibody complexes are formed which may produce alteration in the tertiary structure of the antibody revealing new antigenic determinants. Such antigenic determinants stimulate production of the autoantibody, rheumatoid factor.

At this point it must be stated that theoretically rheumatoid

factor production may have at least two origins. Either the IgG-globulin becomes immunogenic upon lipopolysaccharide binding as an antigen or lipopolysaccharides induce rheumatoid factor directly, in genetically programmed B-cells. The generation of a population of competent B-cells can provide the autoantibody or rheumatoid factor to react with IgG even in the absence of the original antigen, of course, subject to other immunologic control factors. One cannot discount the idea of both mechanisms operating simultaneously.

In periodontitis, if immune complexes bound by rheumatoid factor form in crevicular fluid, then they may fix and activate complement components thereby attracting polymorphonuclear leukocytes and macrophages. In the event of chronic antigenic stimulation, lymphocytes and plasma cells would continue to proliferate as the disease process becomes more chronic. In this case, it could explain why rheumatoid factor levels are sustained in crevicular fluid.

The etiology of rheumatoid factor production in rheumatic diseases still is unknown, however a number of theories have been postulated. In advanced cases of periodontitis, the association of gram negative bacteria and endotoxin found in dental plaque, root surfaces, gingival exudate and gingival tissues; may be implicated as the etiologic agent of rheumatoid factor production in periodontal disease. Bacterial lipopolysaccharides or endotoxins have been associated with inflammatory periodontal disease as reported by Snyderman, Gewurz and Mergenhagen (1968), and Snyderman (1972).

CHAPTER VI

SUMMARY

Rheumatoid factor was determined by latex slide agglutination, on subgingival plaque samples, stimulated pooled saliva and serum of thirty patients with Type III periodontitis.

Twenty of thirty patients demonstrated positive reactions for rheumatoid factor in subgingival plaque, diluted in 0.18 ml of GBS.

All thirty patients demonstrated positive reactions for rheumatoid factor in stimulated saliva.

Five of the ten full denture patients utilized for control saliva demonstrated positive reactions for rheumatoid factor.

Twenty five of thirty experimental subjects demonstrated positive reactions for rheumatoid factor in serum diluted 1:10.

Blank latex beads, utilized as a parallel control were unreactive, to subgingival plaque, saliva and serum.

Albumin was detected at a mean concentration of .27 g/100 ml.

The etiology of rheumatoid factor and homeostatic mechanism in association with periodontal diseases are discussed and may be due to the reported association of gram negative bacterial endotoxin and the chronic antigenic stimulating nature of periodontal disease.

CHAPTER VII

CONCLUSIONS

In the light of this investigation, periodontal disease may be placed in perspective with other chronic antigenic stimulating diseases associated with rheumatoid factor.

Further investigation dealing with the role and application of rheumatoid factor in chronic periodontal disease is essential and necessitates additional research.

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The thesis is therefore accepted in partial fulfillment of
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