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Immunofluorescent Study of the Distribution of Plasma Cells in Human Gingiva

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IMMUNOFLUORESCENT STUDY OF THE DISTRIBUTION OF PLASMA CELLS IN HUMAN GINGIVA

by
DENNIS JOHN ZBYLUT

A Thesis Submitted to the Faculty of the Graduate School Of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

June
1973
DEDICATION

To my wife, Barbara, whose patience, understanding, faith, and encouragement have been of immeasurable value.
ACKNOWLEDGEMENTS

I wish to thank the members of my advisory committee: Dr. Patrick Toto, Dr. Anthony Gargiulo, and Dr. Douglas Bowman for their help in organizing this project. I am particularly grateful to Dr. Toto for his continuing help and encouragement throughout the course of this study.

I would like to thank Mr. Muscavages for her help in the preparation of the microscopic materials.
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The two major disorders of an inflammatory nature involving the tooth supporting structures in most adults and many children are generally called periodontal disease. These are diseases that initially affect the marginal tissues of the periodontium, and if untreated, run a chronic and insidious course. As it progresses, periodontal disease leads primarily to disorganization of collagen bundles, resorption of bone, ulceration of the epithelium lining the gingival pockets, and loosening of the teeth.

Periodontal disease has, in the past, been implicated as the end product of systemic disease. The pathogenesis of periodontal disease is indeed very complex. The possibility exists that in many instances periodontal disease may originate from, or be aggravated by the very system that is supposed to be defending the body, namely the immunologic system.

The purpose of this study is to determine the relative distribution of immunoglobulins A, G, and M in plasma cells of the gingiva of patients with chronic periodontitis.
CHAPTER 2

LITERATURE REVIEW

The immune response is a defensive system of the body to protect it from foreign invaders. According to Weiser (1971), antibodies belong to a group of serum proteins called immunoglobulins. He further states that these globulins are designated as classes of immunoglobulins and are abbreviated as follows: IgG, IgM, IgA, IgD, and IgE. IgG comprises approximately 80% of serum antibodies directed toward antigens. IgM comprises between 5-10% of the total antibody protein in the serum. It has a larger molecular weight (900,000), and is most specific for lipopolysaccharide antigen of gram negative bacteria. IgA comprises 10% of gamma globulins in the human serum. These globulins are found in large quantities in the parotid saliva and tears. IgD comprises 3% of normal serum and its function is unknown. IgE is present in minute amounts in the serum and includes the cytophillic or reagin antibodies.

It is a well established fact that plasma cells and lymphocytes are the predominating inflammatory cells in chronic gingivitis and periodontitis. These plasma cells can be seen with light microscopy to be in varying stages

Tiselius (1936) by means of electrophoretic analysis recognized a distinct group of serum proteins as gamma globulins. The following year it was demonstrated that the antibodies found in the serum were present within the gamma globulin fraction (Tiselius and Kabot, 1937).

Fagraeus (1958) reported that antibody formation commences in undifferentiated cells. She theorized that reticulum cells are activated under direct or indirect stimulation of antigen and thus antibody formation is started. She stressed the fact that antibody production commences in young or undifferentiated cells not in mature plasma cells or mature lymphocytes present in the body before antigenic challenge. Askonas and White (1956) observed antibody concentration in tissues without concomittant serum titer increases could only result from local antibody synthesis. Askonas and Humphrey (1958) demonstrated antibody synthesis in nonlymphoid tissue chronically exposed to antigenic stimulation. They also found that tissues stimulated to produce specific antibody also produced increasing amounts of other gamma globulins not identified as specific antibody.

Toto (1961) reported that plasma cells arise directly from undifferentiated connective tissue reserve
cells, and not lymphocytes. At this time, he proposed that the local derivation of these cells were to serve a functional purpose, that of immunological defense. Dickler et al (1969) reported the frequency of plasma cells in chronic gingivitis. He found that the distribution of lymphocytes and plasma cells were not equal. Notably, the cellular content of thirty-nine gingival biopsies contained 80% or more plasma cells.

Toto et al (1964) suggested that the proliferation of loose connective tissue in the gingiva and alveolar bone is essential in defense mobilization during periodontitis. The destructive activity in the gingiva and bone is an accommodation for proliferation of loose connective tissue and differentiation of plasma cells. They suggested at this time that the function of the undifferentiated connective tissue cells was to replenish the plasma cells that had synthesized and released their gamma globulins and had ceased functioning.

Histochemical staining technique as demonstrated by Coons and Kaplan (1950) is by far the most specific method of studying antibody formation. This method employs: 1) reaction of antibody in tissue specimens with dilute antigens; 2) detection of those sites by means of a precipitin reaction carried out with
fluorescein isothiocyanate labeled antibody. Wherever antigen-antibody precipitates form, the fluorescein labeled antibody is fixed. Under a fluorescent microscope, the brilliant yellow-green light from deposited fluorescein - antibody reveals the presence and location of the homologous antigen.

In the clinical and histologic pictures of periodontitis and gingivitis, a characteristic feature that is seen is one of inflammation. Rosebury (1952) suggested that periodontitis was an endogenous destructive disease arising from a local imbalance of a host-parasite relationship. Socransky (1963) stated that gingival debris is composed almost entirely of bacteria and it lies in direct contact with the surrounding tissues. Therefore, on this basis, the amount of bacterial debris to which the gingiva is exposed is a measure of the bacterial dosage. Courant et al (1965) found no evidence that enhanced virulence of any particular bacterial type associated with the initiation of gingivitis. Brandtzaeg (1964) stated that the severity of periodontal disease was a linear function of the accumulation of gingival debris. Loe et al (1965) demonstrated that gingival inflammation results when the local bacterial numbers exceed certain levels. Therefore, the authors concluded that bacteria thus seem to represent
the single most important etiologic factor in periodontal disease.

It is generally accepted that most forms of periodontal disease are of microbial etiology. Genco et al (1969) described dental plaque as a source of antigens and endotoxins which seem to play an important part of the initiation of the inflammatory response seen in the pathogenesis of periodontal disease. However, the mechanisms by which micro-organisms induce destruction of the periodontium are still vague. Two general possibilities that have been postulated are: 1) direct initiation of the inflammatory response by injurious microbial metabolites; 2) initiation of periodontal inflammation by antigens of oral organisms setting immunopathologic processes into action (Genco, 1970). There is little doubt that bacterial enzymes and microbially derived cytotoxic substances are present in the gingival environment. Little proof has been demonstrated that these by-products are in fact involved in the pathogenesis of human periodontal disease.

Brandtzaeg (1966) stated: "Any foreign protein, several carbohydrates, as well as carbohydrates or lipids linked with proteins, may act as antigens and stimulate the immune system of the host..." He further states that the bacterial cell wall is made up of several
antigens and many bacterial products like enzymes and toxins, that also possess antigenic properties. Brill and Bjorn (1959) demonstrated that a continuous flow of fluid took place from the area between the gingiva and enamel of the tooth. It was shown later, by electrophoretic analysis that this fluid contained plasma proteins and immunoglobulins of the IgA, IgG, and IgM classes (Brandtzaeg, 1965). With the presence of immunoglobulins found in the gingival pocket fluid, the speculation proposed was that antibody-mediated destructive process may be occurring in the gingiva.
B. Hypersensitivity and The Immune Response

Hypersensitivity, simply defined, is the destructive effect of the immune reaction. Allergy and hypersensitivity are used in the same context to show altered reactivity due to previous exposure to an agent (Weiser, 1971). Gell and Coombs (1968), have suggested a simple classification of allergic reactions which are capable of inducing tissue damage. They are: 1) anaphylactic reactions; 2) cytotoxic reactions; 3) arthus type reactions; 4) delayed or cell mediated reaction.

In the anaphylactic type of reaction, antibodies fix strongly to cells. Upon subsequent challenge with antigen, potent substances such as histamine, serotonin, and bradykinin are released from the sensitized cells. These pharmacologically active substances induce immediate vascular and smooth muscle changes (Weiser, 1971).

Cytotoxic reactions are reactions characterized by antibodies reacting with antigens tightly bound to cell membrane. These antigens may be natural components of cell membrane or non-natural cell-associated antigens derived from bacteria altered tissue components. IgG and IgM are considered cytotoxic antibodies, i.e., involving complement fixation (Gell and Coombs, 1968).
Arthus reactions are dependent upon the formation of antigen-antibody complexes in slight antigen excess. In tissue spaces, the immune complexes form micro-precipitates in and around the blood vessels causing local cell damage (Weiser, 1971). In 1961, Mergenhagen and Rizzo described this histologic picture after repeated antigenic challenges of horse serum on the gingiva of rabbits. Terner (1965) induced arthus reactions on tongue, palate, buccal mucosa, and gingiva. She felt that local allergic manifestations may be of primary importance in the pathology of the human mouth. Mergenhagen et al (1970) stated that bacteria upon penetration of gingival epithelium can induce antibody activity in regional lymph nodes. These antibodies upon interaction with bacterial antigen are altered so as to activate an immunologic effector system (complement). This system, when activated, releases products that change vascular permeability and cause chemotaxis of neutrophils. In the presence of continuing challenge by the antigen, this process might be expected to become chronic and lead to changes seen in periodontal disease.

Delayed hypersensitivity is initiated by the reaction of specifically modified lymphocytes containing a substance or mechanism capable of responding specifically
to antigens deposited at a local site (Sell, 1972). The reaction is manifested by infiltration of cells, beginning with perivascular accumulation of lymphocytes and monocytes at the site of antigen deposition. In some manner the reaction of a few sensitized cells with the antigen in the tissue causes large numbers of cells to infiltrate with subsequent tissue destruction. The term delayed is applicable because time is measured in days versus hours for Arthus reaction. It differs from immediate allergic reaction in that there is no circulating humoral antibody and reactivity cannot be transferred by serum but only by cells (Sell, 1972).

Going (1965) suggested that periodontal disease may be a result of immediate and delayed hypersensitivity reaction. It was shown that guinea pigs sensitized with fusospirochetal antigen demonstrated dermal responses to cell-free extracts prepared from the exudate. This response indicated the presence of an Arthus-like reaction and the tuberculin type hypersensitivity. Dalbow et al (1969) reported varying degrees of patient sensitization to all bacterial antigens tested (α γ Streptococci, Nisseria, Micrococci, Bacteroides, Fusobacterium). Lymphoid sensitization was noted by cytotoxic effects on fibroblast monolayers in culture. Ranney and Zander (1970) demonstrated both clinically and histologically that the
First signs were suggestive of Arthus reaction, but the last induration, central necrosis and lymphocytic infiltration after three days is consistent with a delayed reaction. They concluded that it was probable that both Arthus and delayed hypersensitivity reactions were present in the immune-phenomena. Baram and Arnold (1971) suggested that there is likely to be a greater delayed hypersensitivity response to microorganisms than to soluble antigen. Upon subsequent challenge with the antigen in the gingiva, reaction to the antigen may be due to lymphocyte-antigen interaction (delayed) to antibody-antigen interaction or both.
C. Periodontal Disease and the Immune Response

The relationship of the immune response to periodontal disease was suggested by Orban (1940). He reported eosinophilic leukocytes in pulp and gingiva. He alluded to the possibility of the allergic reaction playing an important role in chemotaxis of the eosinophil. Sherp (1962) proposed the possibility of periodontal inflammation due indirectly to the immune response. He thought that the inflammatory response was due to allergy to the antigen of the crevicular flora. Mergenhagen and Rizzo (1961) demonstrated that the allergic reaction may play an important role in development of the pathologic lesions of periodontal disease. They showed rabbits sensitized for longer periods and challenged intramucosally gave lesions consistent with true hypersensitivity reactions, i.e., marked edema, necrosis, and plasmacytosis.

Thonard and Dalbow (1965) presented evidence that immunologically competent cells could be found in the gingival mucosa of rats and guinea pigs after local tissue sensitization with sheep erythrocytes. They concluded that locally deposited antigen is carried by the reticuloendothelial system elements to lymphoid centers with subsequent immunologic competence of local
tissue due to transfer of lymphoid elements back to the site of initial antigenic challenge.

Schneider et al (1966) demonstrated specific bacterial antibodies in inflamed human gingiva. They postulated that the antibody produced by the plasma cells within the gingiva could produce its effect either by liberation into the periodontal pocket and effecting control of the population of bacteria, or reacting within the connective tissue with the soluble antigens produced by the organism. Mergenhagen and Bergland (1969) observed that local stimulation of endotoxin (intra mucosal injection) produced specific antibodies in the regional lymph nodes along with a heavy infiltrate of lymphocytes and plasma cells appearing at the injection site. They demonstrated that specific antibody to endotoxin was being elaborated by certain cells in the local inflammatory response.

Bergland (1970) demonstrated that immunoglobulins in diseased gingiva manifested antibody specificity for bacterial antigens. His findings suggested gingival antibody was of serumal origin but that in some instances concentrations of tissue immunoglobulins were probably inadequate for detection (two patients). On the other hand, larger amounts of antibody activity were observed over some gingival specimens than was expected from the
magnitude of serum titers. He postulated that higher antibody concentrations in the tissues could have resulted from local antibody synthesis.

Hartzer et al (1971) noted that significant antibody activity against fragmented Streptococcus mitus cells occurs in clinically inflammed gingiva and in clinically normal gingiva in both pregnant and non-pregnant women. Inflammed gingiva showed significantly more antibody activity to Streptococcus mitus antigens than clinically normal gingiva in both pregnant and non-pregnant women. In clinically normal gingiva of pregnant women, there was apparently less antibody activity than that of non-pregnant women. He therefore suggested that the immune response was depressed to some degree, but not to the point where host resistance of the gingival tissues is lowered.

Platt et al (1970), stated that prolonged experience with a specific antigen will give rise to a state of hyperimmunity within the host. They felt that this defensive reaction took two forms: 1) serum mediated globulins; 2) delayed form of hypersensitivity. They postulated that the immediate reaction could be active in initial events contributing to tissue degeneration. The cellular type of hypersensitivity would most likely contribute to the perpetuation of the pathology through
mechanisms that influence normal repair of these tissues. They felt these mechanisms may be expressed as direct cytotoxicity or they may more subtly alter the tissue repair system and influence a degenerative course.

Nisengard and Beutner (1970) reported that seventy humans had antibodies to actinomyces. With increased periodontal inflammation increases in antibody titers were seen. They skin tested these same patients and found that there was a significant correlation between periodontal inflammation and hypersensitivity to actinomyces. Hence, they suggested that gingival bacteria stimulate several immune responses.

Rizzo (1970) concluded that antigen and endotoxin penetration required ulceration of gingival epithelium. He alluded to the possibility that these substances play an important role in disease progression rather than disease initiation.

Schwartz et al (1972) using tritiated endotoxin application on clinically healthy gingiva, demonstrated that endotoxin could penetrate intact crevicular epithelium. They found by autoradiography that within twenty-four hours the endotoxin had disseminated into the lamina propria and had increase concentrations around blood vessels. This appears to be in conflict with past findings of Rizzo (1970).
D. Immuno Fluorescent Studies

Brandtzaeg and Kraus (1965) using immunofluorescent techniques found that in clinically healthy gingiva, many plasma cells were producing IgG. They found that very few plasma cells demonstrated positive fluorescence to IgA and only rarely IgM. In clinically inflammed specimens, the number of IgA producing plasma cells drastically increased as compared to IgG. IgG and IgA immunoglobulins were seen in the connective tissue and the gingival epithelium.

Thonard (1966) reported evidence suggesting the presence of all major immune globulins in diseased gingiva. He demonstrated in the study that the IgM producing cells were the dominant fraction. IgA producing cells were found in smaller quantities and IgG fluorescing cells were rarely seen.

Platt et al (1970) reported that plasma type cells of the acute disease chiefly react with antisera to IgM and its fragments. IgG appeared frequently but IgA was reported as non-conclusive. In patients with severe periodontitis, again they found numerous large cells that reacted with anti-IgM antisera and found many cells now fluorescing with different types of anti-IgA conjugates. In the severe periodontitis they found
very few positive cells when stained with anti-IgG or its fragments.

Kraus et al (1972) found that sulcular application of C. histolyticum collagenase did not induce histopathologic changes or antibody formation in gingiva of non-immunized rabbits. He also found that it did not induce immune complex injury in immunized rabbits. Repeated injections in both groups induced gingival inflammation and production of specific antibody in gingiva and cervical lymph nodes. These were primarily of IgG class of immune globulins known to bind complements.

Dolby (1972) demonstrated that immune complex formation did not play a part in the pathogenesis of acute ulcerative gingivitis. Using immunofluorescent techniques he observed that IgA and IgG were in and around blood vessels.

Shillitoe and Lehner (1972) measured levels of IgG and IgA, IgM and complement (C'3) in crevicular fluid and serum. His findings in this experiment suggested that a complement dependent immune reaction in gingival crevice favoring IgG so that relative concentrations of IgG and C'3 are lower than those of IgM and IgA.

Cowley (1969) reported that the majority of gingival plasma cells have been found to contain large quantities of acid phosphatase, esterases, aryl sulphatase,
beta glucuronidase, and amino peptidases. He felt that the globulins produced by these plasma cells played an undetermined role but the large quantities of acid hydrolases were significant in the breakdown of the local tissues in the gingiva.

Evidence available today most certainly implicates the involvement of the immune system in the pathogenesis of periodontal disease. The point at which the host immune response ceases its defensive nature and begins its destructive action cannot be separated. The two actions are ubiquitous in the immunologic system.
CHAPTER 3
MATERIALS AND METHODS

Gingival tissue was obtained from ten patients at Loyola University School of Dentistry. The patients were undergoing routine periodontal therapy. The sites chosen for tissue selection were based on both clinical and radiographic examination. The tissue was obtained by internal bevel incision into the attached gingiva with subsequent removal of the entire papillae. Care was taken to secure the entire intact papilla to insure that the entire picture of chronic inflammation could be seen histologically.

Tissues obtained were fixed in cold acetone (4°C). Care was taken to maintain an approximate 6:1 volume ratio of acetone to tissue specimens. The specimens were embedded in paraffin blocks. Serial sections from each specimen block were sectioned at six microns and placed on a clear glass slide. One section from each block was stained with H and E to demonstrate, if any, the presence of chronic inflammatory infiltrate.

The remainder of the tissue sections were incubated (40°C) with goat anti-human IgA, IgG, IgM, bound to fluorescein isothiocyanate (Hyland Laboratory, Costa Mesa, California) for one hour. The slides were then washed in buffered saline for fifteen minutes and wiped dry, except
for the area of the sections. One drop of reagent glycerol containing one part in ten buffered saline was placed on the section (Coons and Kaplan, 1950). A clean cover slip was placed over and allowed to settle. Fluorescent plasma cells were identified and photographed immediately, using exposure values from 30-40 seconds at 1000 magnification.

Controls employed were twofold. The positive control utilized was non-inflamed human attached gingiva. Sites chosen for these tissue specimens were in edentulous ridge areas. Edentulous sites were chosen because of proven absence of inflammation as determined histologically. Interdental papillae in a clinically healthy periodontium can show histologic signs of inflammation and therefore was not used. These specimens were incubated for one hour with goat anti-human IgA, IgG, and IgM bound to fluorescein isothiocyanate. After incubation the sections were washed in glycerol buffered 0.85% saline, dried and covered with a glass slip. H and E sections were also prepared for light microscopy.

The second control employed was a negative or blocking control on the diseased gingival specimens. The ten specimens were incubated with goat anti-human IgA, IgG, and IgM not bound to fluorescein isothiocyanate for one hour. The slides were washed with buffered
saline. After drying, the slides were incubated with rabbit anti-goat antisera to bind antigenic reactive sites. Subsequent incubation with anti-human IgA, IgG, and IgM bound to fluorescein isothiocyanate then was performed.

The slides were inspected using a Reichert-Zetopan fluorescent microscope (American Optical Co., Rochester, New York) with the Binolux twin lamp used in fluorescent microscopy. The slides were examined under low and high power to establish qualification of the specific immune globulin indicated by areas of fluorescence.

In cytologic counting of the positive fluorescing cells in the specific class of immune globulins, extreme care was taken to fully identify the plasma cells before including it into the cell count. The morphology of the positive fluorescing cells were closely examined to ascertain: 1) nuclear size and shape; 2) position of the nucleus; 3) definite clarity of positive reactive cytoplasm; 4) relative cell size. Only cells that best fulfilled these requirements were included into cell counts.

Sections were viewed under low power to ascertain positive fluorescence of the specimen. Upon determining positive fluorescence, the microscope was adjusted to accommodate the oil immersion lens. Randomly selected
fields were viewed upon adjustment of oil immersion lens and positive fluorescing plasma cells were counted for IgA, IgG, and IgM antisera classes. Ten oil immersion fields were viewed in each of the sections. Ektakrome slides were taken of selected sections. Exposure times varied between 30-40 seconds.
CHAPTER 4
RESULTS

Non-Inflamed Mucosa

Normal tissue was obtained from edentulous ridge areas. The sections demonstrated a keratinized surface. Epithelial ridges were relatively shallow with a well defined basement membrane. The underlying lamina propria was composed of dense orderly arranged collagen bundles. The principal cell was the fibroblast with an occasional plasma cell seen near the basement membrane.

Inflamed Gingiva

The reaction to localized injury by the gingival tissue seen in H and E sections were first characterized by physical changes in the epithelium. The surface epithelium of this inflamed tissue section was keratinized. Furthermore, the prickle cell layer appeared atrophic with greatly widened interstitial spaces. In many sections, however, there was epithelial hyperplasia characterized by long, downward projections of the ridges into the underlying lamina propria accompanied by varying degrees of inter-cellular space widening caused by edema. Acanthosis was a
frequent finding in all sections. The epithelium lining the periodontal pockets was not keratinized and in some instances, micro ulcerations were clearly visible, histologically. Also, polymorphonuclear leukocyte infiltrate could be seen in these areas of ulceration.

An acute inflammatory response could easily be seen in the lamina propria. This was characterized by polymorphonuclear leukocytes. The gingival tissue beneath the areas of epithelial hyperplasia showed chronic inflammation characterized by both lymphocytes and plasma cells. However, the plasma cell was the most common cell type seen. In the majority of cases, the fibrous connective tissue beneath the pocket epithelium was replaced by the plasmacytic and lymphocytic cellular infiltrate. The collagen fibers, where present, appeared swollen and arranged in haphazard bundles. In some cases, the collagen was absent, especially around capillaries. Inflammatory cellular exudate could be seen between the collagen fiber bundles.

The chronic inflammatory infiltration primarily occurred close to the basement membrane of the epithelium lining the periodontal pocket. The cells were arranged in groups around capillaries. These groups were seen to be close to the gingival pocket and extending into
the deeper layers. In some sections, inflammatory cells were seen to be widely distributed throughout the connective tissue forming no specific patterns.

Fluorescent - Non-Inflamed Gingiva

In the gingiva, fluorescence was seen in the epithelium. While the fluorescence was present, as in the inflamed tissue, it was not as intense. The interstitial spaces appeared positively fluorescent with antisera IgA, IgG, and IgM. The connective tissue was extremely dense with collagen bundles showing positive fluorescence. This was evident for IgA, IgG, and IgM classes of immunoglobulins.

Immuno Fluorescence - Inflamed Gingiva

Immunoglobulins A, G, and M were disclosed by immunofluorescent staining techniques in the epithelium and connective tissue. The surface epithelium in the inflamed specimens showed intense and widespread positive fluorescent staining for all three classes of globulins (Plate 1, 2, 3). Also, intercellular staining was observed with the intensity of fluorescence increasing toward the surface of the epithelium. The intercellular
intensity was greater than normal.

The plasma cells were seen specifically containing the three classes of immune globulins. Such plasma cells were seen to be found in discrete groups around capillaries or widely distributed throughout the lamina propria, as seen with H and E light microscopy (Plates 4-8). These groups could be identified by such brilliant fluorescence sometimes eliminating cellular detail.

It was not uncommon to find some fibroblasts demonstrating fluorescence, although collagen reacted negatively. In a few sections, the collagen fiber bundles were so dense that cellular outlines of the plasma cells were lost and only fluorescent patches could be seen.

Of ten inflamed specimens of gingiva examined, nine showed an increase above normal controls in the mean cell counts of fluorescent plasma cells containing three classes of immune globulins (Fig. 1,2). The increases producing mean cell counts of plasma cells IgA, IgG, and IgM were shown to be higher ($P < .01$) when compared to mean cell counts of IgA, IgG, and IgM in the non-inflamed gingiva (Table 1,2).

Statistical analysis of the immune globulin plasma cell groups showed that within the inflamed specimen group, there was no significant difference between
IgA vs. IgG, and IgG vs. IgM. However, there was a significant difference between IgA vs. IgM (P < .05). In contrast, within the non-inflamed specimens, there was a significant difference between IgA and IgG, but similarly a difference was seen between IgA vs. IgM and IgG vs. IgM (P < .05).

In comparing cell counts of the non-inflamed gingiva with that of inflamed gingiva, there was a significant difference between the numbers of fluorescent plasma cells: IgA (inflamed) vs. IgA (non-inflamed), IgG (inflamed) vs. IgG (non-inflamed), and IgM (inflamed) vs. IgM (non-inflamed) all showed statistically significant difference (P < .01). (Table 2)

Ratios of mean plasma cell counts from IgA, IgG, and IgM immunoglobulin from non-inflamed and inflamed gingiva were contrasted to normal serum values for IgA, IgG, and IgM (Table 3). In comparing these ratio values, it was demonstrated that there was a significant difference between the ratios of IgA, IgG, and IgM in inflamed gingiva and the ratios of IgA, IgG, and IgM found in non-inflamed gingiva (P < .05). (Table 4) There was also a significant difference in the ratio of plasma cell counts in inflamed tissue where compared to normal serum ratios (P < .05). No difference could be demonstrated comparing ratios of non-inflamed fluorescing plasma.
cell counts to normal serum concentration ratios (Table 3,4).
Fig. 1

Mean number of plasma cells showing fluorescence
IgA, IgM, and IgG per oil immersion field.

Negative - Poor Section - Epithelium Only

PATIENT NUMBER

IgA  IgG  IgM  PATIENT NUMBER

AGM 2  AGM 3  AGM 4  AGM 5  AGM 6  AGM 7  AGM 8  AGM 9  AGM 10
Mean number of plasma cells showing fluorescence of IgA, IgG, and IgM per oil immersion field.
**TABLE 1**

Mean number of plasma cells showing fluorescence for IgA, IgG, and IgM per oil immersion field.

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## TABLE 2

### Significance of Intergroup Statistical Analysis

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<td>IgA non-infl. vs. IgM non-infl.</td>
<td>3.649</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>IgG non-infl. vs. IgM non-infl.</td>
<td>4.261</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>IgA infl. vs. IgA non-infl.</td>
<td>16.0</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>IgG infl. vs. IgG non-infl.</td>
<td>12.219</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>IgM infl. vs. IgM non-infl.</td>
<td>7.55</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>
TABLE 3

Relative Ratios of Plasma Cells Containing IgA, IgG, IgM.

Inflamed Gingival Specimens

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>2.80</td>
</tr>
<tr>
<td>IgG</td>
<td>2.14</td>
</tr>
<tr>
<td>IgM</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Non-Inflamed Gingival Specimens

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>3.67</td>
</tr>
<tr>
<td>IgG</td>
<td>12.22</td>
</tr>
<tr>
<td>IgM</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Average Serum Concentrations of Human Immunoglobulins (Mg/ml)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>3.9</td>
</tr>
<tr>
<td>IgG</td>
<td>12.4</td>
</tr>
<tr>
<td>IgM</td>
<td>1.2</td>
</tr>
</tbody>
</table>

TABLE 4

Significant Differences of Intergroup Ratios Determined by Chi Square

<table>
<thead>
<tr>
<th></th>
<th>$x^2$ Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflamed Specimens vs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Inflamed Specimens</td>
<td>8.673</td>
<td>P. &lt; .05</td>
</tr>
<tr>
<td>Inflamed Specimens vs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Serum Concentrations</td>
<td>9.546</td>
<td>P. &lt; .05</td>
</tr>
<tr>
<td>Non-Inflamed vs. Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Concentrations</td>
<td>.197</td>
<td>P. &gt; .99</td>
</tr>
</tbody>
</table>
Plasma cells containing IgA, IgG, and IgM are present in human inflamed gingiva. There was a significant difference between the number of fluorescing plasma cells producing IgA, IgG, and IgM in the inflamed tissues as compared to the plasma cells producing IgA, IgG, and IgM in the non-inflamed gingiva. (P < .01)

Plasma cell counts showed a marked increase in the number of positive reacting cells in the inflamed gingiva. There was a significant quantitative increase in the numbers of fluorescent plasma cells between IgA and IgM class of immunoglobulins. No significant difference was demonstrated between IgA and IgG and IgG and IgM class of immunoglobulins.

Plasma cells also were seen in non-inflamed gingiva, but in lesser numbers. No doubt, because of the edentulous ridge site chosen, the degree of inflammation was greatly minimized. However, the fact that plasma cells were present indicates that these tissues do have the capabilities of local production of immunocytes. In this way, it is suggested that these tissues can maintain a relatively constant defense against the bacterial flora present within the oral cavity.

The ratio of plasma cells seen in non-inflamed
tissues closely approximated the 12:4:1 ratio of IgG to IgA to IgM found in the serum (Fahey and McKelvey, 1965). In the inflamed gingiva, the ratios seen did not correlate with ratios of normal serum or non-inflamed gingiva. The approximate ratios of plasma cells in the inflamed gingiva was observed as 3:2:1, IgA to IgG to IgM. Hence, the increases in positive fluorescing cells were not demonstrated to follow normal serum ratios. The most dramatic increase of cells were found in the IgA and IgM class of immunoglobulins.

In reviewing the results of the study, records indicated that patients number 5, 7, and 9 were diagnosed as having chronic localized periodontitis with minimal radiographic bone loss. Clinically, the cases in question were treated with gingivectomy procedures for pocket elimination. The tissues in these cases exhibited a comparatively high number of IgM producing plasma cells. The significance of this has not been established, but it could suggest an order of appearance of the specific immunoglobulin class with IgM being found in the initial stages and IgA following as the inflammation becomes more chronic.

This has been observed on experimental models where IgM antibodies are first to appear in the serum following antigenization. These antibodies are usually followed
by the appearance of IgG antibodies. The serum level of
a particular class of immunoglobulins is probably governed
by the number of cells engaged in the production of the
particular antibody. The disappearance of IgM antibodies
in the serum is most likely the result of specific feedback
suppression by IgG antibodies (Weiser, 1971). On the
other hand, polysaccharide antigen may cause continued
IgM production without appreciable IgG antibody increases
in the serum (Weiser, 1971). If data from patients 5, 7,
and 9 were eliminated, then mean observed plasma cells
would decrease to $\bar{x}=1.3$. The ratios of immunoglobulins
would then have shifted to 5.4:4:1. This still indicates
that the greatest shift in plasma cells would be in the
IgA series of immunoglobulins. The levels of cells
from patients 5, 7, and 9 could suggest either an
alteration in the chronicity of inflammation or possibly
a shift in the bacterial flora. The cell wall of gram
negative species of bacteria contains the endotoxin of
the cell, a complex of lipid and protein with a
polysaccharide which serves as a specific determinant
of the dominant somatic antigen (Burnett and Sherp, 1962).
The higher mean observed plasma cell values seen in
patients 5, 7, and 9 could, in fact, be due to specific
alteration of bacterial flora at the surgical site.

The remainder of the patients in this study were
clinically diagnosed as having chronic generalized periodontitis. The lesions providing the tissue specimens for this study were infrabody defects of approximately 7 mm. in depth. In these patients, the common factor determined was the chronic periodontal lesion. In eight of nine patients, the most common finding was a higher number of plasma cells in the IgA class of immunoglobulins. Immunofluorescence studies have shown that IgA containing plasma cells outnumber IgG containing cells in the local immune response of the intestinal mucosa (Crabbe et al, 1965). This is contrary to the humoral response. It may well be, that IgA is particularly suited for protecting mucous membranes and therefore produced in larger quantities when challenged by antigen from external environment (Brandtzaeg, 1966). It has been suggested that IgA might be the ancestral type of immune globulin, formerly associated and presently restricted to production sites close to the epithelial surfaces by which the organism establishes contact with the septic environment of the outside world (Heremans et al, 1966).

Tomasi (1967) states that the IgA class of immune globulin includes antibody against some of the nuclear factors involved with collagen diseases as well as a host of other pathogens. Also, studies showing an individual with good serum antibody titers but no
detectable local antibody response, would become ill when challenged with a respiratory virus. On the other hand, the patient with good local antibody responses but little serum antibody would ward off the infection without manifesting any overt clinical signs. It should be emphasized that local production of IgA does not necessarily indicate a hypersensitivity response.

The literature has shown conflicting reports concerning immunoglobulin producing plasma cells in inflamed gingiva. Brandtzaeg and Kraus (1965) found in clinically inflamed tissues the number of IgA containing cells greatly increased compared to IgG containing cells. Only rarely was an IgM producing cell seen. In this study, the authors suggested that IgA and IgG were extracellular and that intercellular transport of these globulins occurred through widened intercellular spaces into the gingival pocket. Platt et al (1970) reported that plasma type cells of the acute disease reacted chiefly with antisera to IgM. It was also reported that tissues taken from patients with severe periodontitis showed large amounts of IgM producing cells, but many cells reacting with IgA were being found. Very few cells were found to have reacted with IgG antisera. In their study, no reference was made to clinical impressions as to the degree of inflammation or radiographic observation of the
patients in question. The results of this study demonstrated an increase of plasma cells in IgA, IgG, and IgM with the most significant change in ratio values in the IgA class of immunoglobulin. Antibodies of the IgA, IgG, and IgM classes of immunoglobulins can be specific to identical antigens. It is not known why the same antigen should elicit so many responses, nor is it known why there is a wide variation in the individual response (Tomasi, 1967).

In the non-inflamed gingiva, it must be noted that in five patients, no positively fluorescing cells could be demonstrated to the IgM class of immunoglobulin. The sites chosen were from clinically non-inflamed edentulous ridge areas. None of the ten control patients wore prosthetic appliances, which could have influenced the control. The absence of IgM producing cells could suggest relative freedom of inflammation. One control patient showed a higher IgM value. This may be interpreted as an initial immunologic reaction where antigenic challenge had stimulated local immunocyte production.

As suggested by the literature, immune induction does occur following exposure to antigenic stimuli in the gingival tissue. Investigations presently indicate that immunoglobulins from inflamed gingiva originate from cells in the inflamed tissue as well as from the serum (Bergland,
1970). Immunofluorescent studies have indicated that immune globulins are present around bacteria in the oral cavity, suggesting antigen-antibody reactions (Brandtzaeg, 1966). Other studies have shown specific bacterial antibodies within inflamed tissues. Plasma cells were shown to be a site of bacterial attraction (Schneider et al, 1966). Clinically, periodontitis is seen as a chronic inflammatory disease involving the supporting structures of the teeth. The antigenic challenges in and near the sulcus produce the effects we see clinically and histologically. It is reasonable to suggest that the plasma cell was specifically differentiated to aid in combatting injury to the gingiva. Antigen-antibody complexes involving IgG and IgM class of immunoglobulins can fix complement with resultant production of vasoactive mediators of the inflammatory response.

Periodontitis has been described in the past as a proliferative phenomenon (Toto et al, 1964). Cowley (1969) has described presence of acid phosphatases, esterases, hydrolases, etc. within plasma cells. These catabolic enzymes are present in the lysosomes and, in effect, prepare the area for the differentiation of additional plasma cells. This preparation or accommodation for differentiating reticulum cells is on one hand beneficial to defending the integrity of the tissues, and destructive,
in that it adds to the local tissue breakdown.

It seems reasonable to suggest that in the more chronic periodontal defect, IgA would be the most ideal immunoglobulin. IgA has not been shown to bind complement and therefore minimizes the presence of any acute mediators of inflammation. In 8 of 9 patients, all demonstrated definite increases in IgA plasma cell numbers. This may be suggestive of the biologic response to the antigenic stimulus that would most efficiently bind the irritants and at the same time minimize local damage induced by the acute inflammatory response.
CHAPTER 6
SUMMARY AND CONCLUSIONS

Gingival biopsies of ten inflamed and ten clinically normal areas were taken from twenty adult patients. The biopsy specimens were immediately fixed in cold acetone and embedded in paraffin. Specific fluorescein conjugated antisera was reacted with sections of each specimen to show the presence of plasma cells producing gamma globulins in the tissue. Cell counts were performed on each specimen in the IgA, IgG, and IgM class of immune globulins.

Results indicate that plasma cells are found in human gingiva. These plasma cells are producing globulins of the IgA, IgG, and IgM class of immunoglobulins. In the inflamed gingival tissues, significant increases of mean plasma cell counts were demonstrated in the IgA, IgG, and IgM classes of immunoglobulin when compared to mean cell counts of the IgA, IgG, and IgM classes (P < .01).

Ratios of the classes of immunoglobulins were compared demonstrating that the ratio of plasma cells producing IgA, IgG, and IgM in the non-inflamed were no different than values reported for serum concentrations. Ratios of plasma cells producing IgA, IgG, and IgM in the inflamed gingiva were shown to be significantly different (P < .05) than ratios in the non-inflamed gingiva or
normal serum.

In the non-inflamed gingival biopsies, fluorescence was demonstrated with IgA, IgG, and IgM. The fluorescence was minimal but nonetheless, fluorescence was demonstrable. There was a significant difference between mean cell counts of IgA, IgG, and IgM in the non-inflamed gingiva.

In the inflamed gingival specimens, there was a significant difference between the number of IgA and IgM producing plasma cells. Differences between IgA and IgG, and IgG and IgM could not be demonstrated. Increased mean plasma cell counts occurred in all three classes of immunoglobulins with the most dramatic in the IgA class of globulins.
Immunofluorescent (low power) photomicrograph of anti-IgA antisera. Note patches of fluorescence within epithelium and widespread fluorescence in connective tissue.
PLATE 2

Low power photomicrograph of fluorescence seen with IgG fluorescence in connective tissue. Shows a great deal of variation in the intensity.
Low power photomicrograph showing fluorescence with anti-IgM antisera. Demonstrable fluorescence can be noted.
Plasma cell aggregation in loose connective tissue surrounding capillary. These specific plasma cells were stained with anti-IgA antisera.
Plasma cells positive to IgG antisera found beneath basement membrane. Note differences in maturity of these cells.
Plasma cells positive to IgM antisera. Note number of distinguishable plasma cells within the cluster. Also note the number of positive cells and morphology.
Plasma cells demonstrating positive fluorescence to IgA antisera. Note differences of intensities and morphology.
Plasma cells beneath basement membrane positive to IgA. Note disorganization of collagen bundles with random distribution of the plasma cells.
Photomicrograph showing fluorescent cells in vicinity of blood vessel for IgM antisera.
Positively fluorescent plasma cells to IgG antisera.
Photomicrograph showing positive fluorescence of plasma cells to IgG antisera. Note different morphologic characteristics and absence of dense connective tissue.
Photomicrograph showing positive fluorescence in non-inflamed gingiva to IgM antisera.
CHAPTER 8

BIBLIOGRAPHY


Going, D., Hypersensitivity in Guinea Pigs to Oral Fusospirochetal Organisms, J.D.R., 44:1358, 1965.


Orban, B., Eosinophilic Leukocytes in the Pulp and Gingiva, J.D.R., 19:537, 1940.


APPROVAL SHEET

The thesis submitted by Dennis J. Zbylut, D.D.S., has been read and approved by a committee from the Department of Oral Biology.

The final copies have been examined and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 21, 1973
DATE

Signature of Advisor