

AUTO-REACTIVITY OF SERUM IMMUNOGLOBULIN TO
PERIODONTAL TISSUE COMPONENTS

By

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To my family, instructors, fellow residents, and friends who made this possible

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TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS	4
LIST OF TABLES	7
LIST OF FIGURES	8
ABSTRACT.....	9
CHAPTER	
1 INTRODUCTION	11
2 BACKGROUND	13
Healthy Periodontium	13
Periodontal Disease	15
Immune Response in the Gingival Lesion.....	18
B-1 Cells, Auto-immunity and Periodontitis.....	20
3 MATERIALS AND METHODS	28
Participant Cohort.....	28
Clinical Evaluation	28
Serum, Premolar and Gingival Tissue Sample Collections.....	29
Immunohistochemical Preparation and Examination.....	29
Protein Extraction	30
SDS Page and Western Blot Analysis	30
Protein Purification and Identification.....	31
Enzyme Linked ImmunoAssay (ELISA).....	32
Statistical Analysis.....	32
4 RESULTS	33
Clinical Characteristic of Participant Cohort.....	33
Immunohistochemical Evaluation of Auto-Reactivity	33
LAgP Serum Reactivity Differs in Quality and Quantity Compared to CP Serum Reactivity	34
Key Components of the Periodontal Structure are Putative Targets of Auto-reactivity	35
Auto-reactivity to collagen type I is more severe in localized aggressive periodontitis	35
5 DISCUSSION.....	42

LIST OF REFERENCES47

BIOGRAPHICAL SKETCH51

LIST OF TABLES

<u>Table</u>		<u>page</u>
4-1	Clinical Characteristics of Participant Cohort.	37
4-2	Putative Targets of Autoreactivity.....	38

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1 Anatomy of the Periodontium.....	25
2-2 Distribution of Cell Proportions in Periodontitis Lesions.....	26
2-3 Changes of Gingival Tissues during Development of Periodontal Disease.	27
4-1 Immunohistochemical Analysis of Auto-Reactivity.....	39
4-2 Auto-reactivity of Serum from Different Experimental Groups.....	40
4-3 Autoreactivity to Collagen Type I	41

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The normal gingival unit has virtually no inflammatory cells and is comprised of about 40-50% epithelial cells and the rest connective tissue. Upon the onset of disease, the gingival unit becomes infiltrated with a number of inflammatory cells, with plasma cells being the most abundant. Many studies have suggested that these plasma cells may potentially lead to auto-antibodies directed against host tissues, although the role of auto-antibodies in the disease progression has been poorly described. Therefore, this study was proposed to investigate an autoimmune basis in the pathogenesis of periodontal disease.

To accomplish this, peripheral blood samples were taken from taken from three groups with different periodontal conditions: localized aggressive periodontitis (LAgP), chronic periodontitis (CP), and periodontally healthy (PDH). These were tested for the presence or absence of auto-antibodies against proteins of the components of periodontal tissues using different analyses, such as immunohistochemistry, western blot and enzyme linked immunosorbant assay (ELISA). Our results showed a more exuberant level of auto-immune reaction in participants from the localized aggressive periodontitis group when compared to chronic periodontitis groups and periodontally healthy controls. Furthermore, multiple potential

targets of auto-immunity were identified, with unique targets identified to be reactive to localized aggressive periodontitis sera.

CHAPTER 1 INTRODUCTION

Periodontal diseases are a number of chronic inflammatory conditions that affect supporting structures around the teeth. It has been reported that periodontal disease may affect 30 – 40 % of the adult population with a prevalence of between 0.1% and 22 % in children.¹ Many factors which contribute to or are epidemiologically linked with periodontal diseases have been extensively studied, including age, gender, race/ethnicity, socioeconomic status, dental plaque, specific subgingival microbiota, cigarette smoking, diabetes mellitus, obesity, HIV infection, osteoporosis, psychosocial factors, and more recently, genetic factors.² While the initiation of periodontal disease requires a bacterial infection, the timing and severity is a result of the ensuing inflammatory response.

The normal gingival unit has virtually no inflammatory cells and is comprised of about 40-50% epithelial cells and the rest connective tissue.³ Upon the onset of disease, the gingival unit becomes infiltrated with a number of inflammatory cells, with plasma cells being the most abundant. Many studies have suggested that these plasma cells may potentially lead to auto-antibodies directed against host tissues, although the role of auto-antibodies in the disease progression has been poorly described. Therefore, this pilot study was proposed to investigate an autoimmune basis in the pathogenesis of periodontal disease.

We hypothesize that periodontal patients will present with auto-antibodies against components of the periodontal tissues. Therefore the aim of this study was to evaluate the presence or absence of circulating auto-antibodies in participants with localized aggressive periodontitis, chronic periodontitis, and periodontally healthy controls to various components in periodontal tissues. Our aim was accomplished using immunohistochemistry, western blot, and ELISA analysis.

Peripheral blood samples, taken from three groups with different periodontal conditions, were tested for the presence or absence of auto-antibodies against proteins of the components of periodontal tissues using different analyses. Our results showed a more exuberant level of auto-immune reaction in participants from the localized aggressive periodontitis group when compared to chronic periodontitis groups and periodontally healthy controls. Finally, multiple potential targets of auto-immunity were identified, with unique targets identified to be reactive to localized aggressive periodontitis sera.

CHAPTER 2 BACKGROUND

Healthy Periodontium

The periodontium refers to those structures that surround the teeth. These include the gingiva, cementum, bone and periodontal ligaments (PDL). The gingiva is mucosa that covers the alveolar process and surrounds the cervical portion of the teeth. Cementum is found on the surface of tooth roots and serves as anchorage for the principal fibers of the PDL. The bone can be further divided into alveolar bone proper and the alveolar process. The alveolar bone proper, which is commonly referred to as bundle bone, is part of the alveolar process and lines the sockets of teeth. PDL is comprised of connective tissue, which attaches the tooth to the alveolar bone. The main function of the periodontium, which is also known as the attachment apparatus, is to attach the teeth to the bone of the jaws and to maintain the integrity of the soft tissues of the oral cavity⁴.

The gingiva is comprised of an outer junctional epithelial layer and an underlying connective tissue layer known as the lamina propia. The epithelial layer is few layers thick and is made of non-differentiated, stratified, squamous cells, which attach to the tooth through hemidesmosomes.³ They comprise the first level of defense against invading bacteria. This layer is keratinized, stratified squamous epithelium which can be divided into four different cell layers contingent on degree of differentiation of keratin-producing cells. Starting at the basement membrane the layers are: basal layer (stratum germinativum), prickle cell layer (stratum spinosum), granular cell layer (stratum granulosum), and keratinized cell layer (stratum corneum).^{3,4}

In health, a small crevice, the gingival sulcus is formed adjacent to the tooth, extending from the crest of the gingiva to the junctional epithelial attachment. The PDL is richly vascular

and cellular tissue which surrounds the roots of teeth. It joins the cementum of teeth with the outer cortical bony layer of the socket wall through principal fibers known as Sharpey's fibers. The PDL consist of fibroblasts, osteoblasts, cementoblasts, osteoclasts, epithelial cells and nerve fibers.

Cementum is a specialized mineralized tissue that covers the roots of teeth. It consists mostly of hydroxyapatite, while it also contains collagen.⁴ Its functions include attaching the PDL to the root as well as contributing to repair after to root surface damage. Unlike bone, it does not have successive periods of resorption and deposition, but rather increases in thickness by constant deposition throughout life. This apposition causes some of the PDL fibers attaching to the cementum to become mineralized. There are three different forms of cementum found in human teeth. Acellular, extrinsic fiber cementum is found in the coronal and middle portions of the root. It contains mainly Sharpey's fibers and is an important part of the attachment apparatus, as it connects the tooth with the alveolar process proper⁴. Cellular, mixed stratified cementum occurs in the apical third of the roots and furcations. It contains cementocytes and both extrinsic and intrinsic fibers. Cellular, intrinsic fiber cementum is mostly found in the resorption lacunae.

The alveolar process is the extension of bone from both the maxilla and mandible that form and support the sockets of the teeth. The walls of the sockets are lined by a thick cortical bone, while the area between the sockets contain cancellous bone. The bone can be subdivided into alveolar process and alveolar bone proper. The former is not considered part of the attachment. The latter is responsible for the attachment of the tooth to the skeleton. Bone is a dynamic structure. Due to functional demands, alveolar bone must be renewed. Throughout life, teeth erupt and move mesially to compensate for wear. This movement requires constant

remodeling of bone. This process of remodeling consists of resorption and deposition, in which the bone is dissolved by cells known as osteoclasts and reformed by osteoblasts. Osteoclasts, which come from blood monocytes, accomplish their task by releasing acidic substances to dissolve the bone. Osteoblasts form bone by first depositing and organizing new bone matrix.

Periodontal Disease

Periodontal diseases are a number of inflammatory conditions, such as chronic periodontitis and aggressive periodontitis, that affect supporting structures around teeth. It has been reported that periodontal disease may affect 30 – 40 % of the adult population and 0.1 % to as high as 22 % in children.^{2,5} Periodontitis is initiated by oral bacteria that form around teeth and the associated gingiva. These bacteria will form a complex structure known as a biofilm that will lead to inflammation of the gingiva known as gingivitis. If left untreated, the bacteria may invade deeper structures and cause destruction of the underlying connective tissue attachment, PDL, cementum and bone known as periodontitis. Clinically, the sulcus depth increases and the JE begins to migrate apically as the underlying connective tissue and bone are destroyed, forming a periodontal pocket.

Chronic periodontitis is a slow, continuous progressive form of periodontal disease that has periods of acute exacerbation that causes attachment loss. It has been shown to be patient-related as well as site dependent. Most forms of periodontal disease are plaque-associated, which start out as the inflammation of the gingiva.⁶ It is interesting to note, that this inflammation will only progress to the bone in susceptible individuals and is largely influenced by differing host responses.⁷ For example, patients with uncontrolled diabetes mellitus will have a more severe destruction of these periodontal tissues. There is a significant variation in the pathogenic bacteria associated with chronic periodontitis, but most are often gram-negative, anaerobes. Some of the primary bacteria associated with periodontitis include *Porphyromonas*

gingivalis, *Prevotella intermedia*, *Bacteroides forsythus*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans* and *Treponema denticola*.⁸

Aggressive periodontitis is a group of rare, severe, rapidly progressing form of periodontitis usually characterize by an early age of disease, with no contributing medical history and a tendency for cases to aggregate in families.⁹ They usually have a more rapid attachment loss and bone destruction, as compared to those with chronic periodontitis.. The amount of microbial deposits are inconsistent with severity of periodontal destruction.¹⁰ There is usually an elevated proportion of *Aggregatibacter actinomycetemcomitans*, and in some populations *Porphyromonas gingivalis* may also be elevated.⁹ This disease can be divided into Localized aggressive periodontitis (LAP) and Generalized aggressive periodontitis (GAP). LAP is characterized by circumpubertal onset, localized first molar/incisor presentation with interproximal attachment loss on at least two permanent teeth, one of which is a first molar, and involving no more than two teeth other than first molars and incisors, and robust serum antibody response to infecting agents.⁹ GAP usually affects people under 30, but can occur in older individuals. This diseases is characterized by generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors, pronounced episodic nature of the destruction of attachment and alveolar bone, and poor serum antibody response to infecting agents.⁹

The development of gingivitis will cause cellular and structural changes in the periodontium. A study in dogs compared these changes during a 28 day challenge of plaque accumulation.¹¹ Initially healthy gingiva in dogs was allowed to accumulate plaque for 28 days. Biopsy samples were taken at various times during this period. Day 0 consists of a normal gingival unit with almost no inflammatory cells and is 40-45% epithelium and 55-60%¹¹

connective tissue. More than half of the connective tissue is comprised of collagen. During plaque accumulation, neutrophils and lymphocytes migrated into this area, increasing the volume. After 28 days, the connective tissue was made up of lymphocytes, plasma cells and macrophages, which remained in the tissue while the neutrophils continued to migrate into the gingival sulcus, which is now known as a pocket.¹¹ This extensive influx of leukocytes caused a significant reduction in the amount of collagen and fibroblasts, while the volume of residual tissue and small blood vessels increases.

The progression of gingival and periodontal inflammation has been divided into four phases: initial, early, established, and advanced lesions.¹² Within 24 hours of plaque accumulation on a tooth, inflammation will set in, giving rise to the initial lesion. As the microcirculatory vessels in the gingiva begin to dilate, their permeability increases, allowing fluids and proteins to exude into the tissues. This gingival crevicular fluid (GCF) will contain within it cellular elements such as neutrophils and lymphocytes, various protective proteins such as antibodies, complement and protease inhibitors, as well as bacteria and their noxious products.¹² The volume of exudates is proportional to the severity of the inflammation present and can be used as a marker for inflammation and, therefore, periodontal disease.

The early lesion follows one week of plaque accumulation.¹² It consists of an inflammatory, highly cellular state, with marked increase of fluid exudation and leukocyte migration into the tissues and gingival crevice. Lymphocytes and neutrophils make up the bulk of the infiltrating cells. Fibroblasts degenerate and collagen breaks down to make room for these cells. Few plasma B cells are seen at this stage.¹² Junctional epithelium starts to proliferate in an attempt to wall off the process. Some clinical signs of inflammation can be seen at this stage.

After an undetermined time, an established lesion will form.¹² The time it takes for the

lesion to reach this level is due susceptibility of the host. This lesion will have more overt inflammatory changes, such as increased edema, and is known as gingivitis.¹¹ There is continued white cell infiltrate at this stage, and at this time plasma cells become the dominant cell type. Collagen loss continues and the space is filled with this infiltrate.^{4,12} The junctional epithelium continues to proliferate and now becomes the pocket epithelium, which is less adherent to the tooth structure.¹² This pocket epithelium is now more permeable to the passage of substances in and out of the connective tissue, and it may be ulcerated in different places, resulting in bleeding. This lesion may remain stable and not progress for months or years. However, a more active type of disease may progress to a more destructive stage.

This leads to the final stage of this process known as the advanced lesion.¹² The junctional epithelium will continue its apical growth in response to the continued exposure to plaque. As the gingival pocket continues to deepen, plaque will follow this downward growth, creating an ideal environment for anaerobic bacteria. This stage shares all the features of the established lesion with several important additions: alveolar bone loss, fiber damage is severe, and junctional epithelium's apical migration extends beyond the cemento-enamel junction.^{4,12} Here there is a wide-spread manifestation of inflammatory and immunopathological tissue damage, with plasma cells being the dominant cell type.¹³ The lesion extends beyond the gingiva, as it and the inflammatory cell infiltrate continues to extend into the connective tissue in an apical and lateral direction.

Immune Response in the Gingival Lesion

The response of the body to the subgingival microbial biofilm will cause an inflammatory response which results in destruction of both hard and soft tissue.¹⁴ Leukocytes and their products are among the culprits which cause this destruction. They are regulated by immune mechanisms whose equilibrium or imbalance between the biofilm and inflammatory process will

determine the severity of the periodontal lesion. The proportion of immune cells in the various stages of the lesion has been reported. Plasma cells are the most common cell type and represent about 50% of all cells.¹⁵ Their proportion is larger than that of T cells, polymorphonuclear cells, and macrophages.¹⁵ The proportion of plasma cells and B cells is larger in more severe lesions of periodontitis than moderate or slight. Lesions in aggressive periodontitis share this composition.¹⁵ This high proportion suggests that special attention should be paid to the function and immune properties of these cell types.

Immune responses are divided up into innate and adaptive responses or immunity. Innate immunity combats a wide range of pathogens. It is non-specific and does not provide lasting immunity.¹⁶ It consists of primary barriers such as shedding epithelial cells and skin, as well as cells such as natural killer cells, neutrophils and macrophages. It also includes the complement system, which is made up of proteins that are cleaved in a series of events that leads to an activation cascade that will produce an amplification of the immune response. Complement is important for lysis of bacterial cells and/or viruses through opsonisation, triggering important cell functions in inflammation, immune regulation and immune clearance. This will allow different cell types, such as neutrophils and macrophages, to phagocytize and destroy invaders. Adaptive responses are developed to specific antigens from pathogens and produces memory cells which will provide immunity from future infections.¹⁶ Adaptive responses involve T cells and B cells, including plasma cells.

Some bacteria and parasites, as well as all viruses, replicate inside cells, where antibodies will not recognize them.¹⁶ T cells are responsible for their destruction through the cell-mediated immune responses of adaptive immunity. Two major types of T cells involved in activated immune responses are CD8 and CD4 T cells. CD8 or cytotoxic T cells are responsible for killing

host cells that are infected with a virus before viral replication has occurred and prevents new virus from being released. CD4 or helper T cells are either Th1 or Th2 and classified based on their cell surface receptors and cytokine production, giving them different effector functions.¹⁷ They are important for intracellular bacterial invasion as they activate infected macrophages to fuse their lysosomes with the vesicles containing the bacteria. They also help to stimulate the production of antibodies by their interaction with B cells.

Antigen will bind on different receptors of these cells. After antigen binding to a B-cell receptor on the B cell surface, they will proliferate into plasma cells.¹⁷ These are the cells that will produce antibodies, which are the secreted form of the B cell receptor.^{17, 16} They have identical antigen specificity, and causing the antigen to now become the target of future antibodies. They have three main functions in host defense. First they physically block the pathogens or their products by binding to them. This would block any access to the host cell by the invaders. This is known as neutralization and is important defense against viruses and bacterial toxins. Neutralization would prevent viruses from entering cells and replicating. Another method of defense is known as opsonization. It is the process of coating the pathogens and foreign bacteria by the antibodies that recognize them. Many bacteria can evade the innate immune system because they have an outer coat that is not recognized by phagocytic receptors. Antigens on the coat of bacteria can be recognized by antibodies.^{17,16} Receptors on phagocytes will recognize these antibodies bound to the bacteria. This will allow the different phagocytes to ingest and destroy the bacteria. The last function is complement activation.¹⁸ Antibodies bound to the bacterial surfaces form receptors for the first proteins in the complement leading the cascade. Therefore, once antibodies are produced complement activation increases.

B-1 Cells, Auto-immunity and Periodontitis

The antibodies that are produced by plasma cells are mostly directed at antigen found in

the subgingival biofilm of the gingival lesion. It is possible for the antibodies to be directed against the host tissue components. These are known as autoantibodies. In patients with chronic periodontitis, large number of B cells have been reported, out of which 30 % exhibit autoreactive characteristics.^{13,18} Autor-eactive B cells can serve as antigen presenting cells and develop an immune response to self antigen. This self antigen will become accessible to auto-antibodies causing activation of an immune response that can result in tissue damage.¹³ This damage can cause more self antigen to be accessible increasing disease progression leading to a chronic state. This can be seen in many autoimmune diseases such as diabetes type I, rheumatoid arthritis and systemic lupus erythematosus.

There are different subsets of among B cells. B-1 cells reside in specific anatomical compartments and are important in early antibody responses and are divided into B1a and B1b subtypes, where B-1a cells express the surface marker CD5. B-2 cells, which are also known as conventional B cells are involved in the traditional adaptive immune response, which yield memory and plasma cells.¹⁷

Plasma cells are the product of activated B cells responding to antigen.¹³ As previously stated, these plasma cells will secrete antibodies to specific foreign, as well as self antigen. Plasma cells also produce cytokines such as tissue necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-10, and transforming growth factor β (TGF- β).¹³ TNF- α regulates the turnover of extracellular matrix by inducing the expression of matrix metalloproteinases (MMP), while TGF- β will have the opposite effect by down-regulating their synthesis and and secretion, as well as producing different molecules that inhibit them.¹⁵ Plasma cells will also express vascular endothelial growth factor, which stimulate angiogenesis and activate MMP.¹⁷ Under typical healthy conditions, MMPs are important part of tissue development, remodeling and wound

healing.¹⁷

Inflammatory cell infiltrate is abundant with plasma cells in inflammatory diseases, such as periodontitis and rheumatoid arthritis.¹⁷ There is evidence to suggest that plasma cells take part in tissue destruction by expression of MMPs.¹⁸ Normally, amount and activity of MMPs are low, but can both rise considerably during pathologic conditions that cause tissue destruction. Their activity has been shown to increase during experimental gingivitis, and correlates with severity of disease.^{17,18} Conversely, their concentration has been shown to be reduced following periodontal treatment.¹⁸

B-1 cells require an immunoglobulin receptor-generated signal for their development, survival, expansion or phenotype expression. These cells have the ability to produce antibodies with different grades of affinity. They are found in high quantities in the sera of subjects with auto-immunity like rheumatoid arthritis¹⁹ (RA) and Sjogrens's syndrome²⁰ (SS). Upon activation of T cells, these B-1 cells have been shown to undergo class switching and mutations which produce IgG autoantibodies with high affinity.¹⁷ Other recent studies have shown that microbial DNA released during infections can intensify this autoimmune response by causing B cells to switch from IgM to a more pathogenic IgG independent of T cell stimulation.¹⁷

Recent findings demonstrate that auto-antibodies have been identified in patients with periodontitis. For example, using flow cytometry analysis of blood samples, Afar et al, showed significantly larger amounts of B-1a cells in patients with periodontitis when compared to healthy controls.²¹ Berglundh and co-workers reported five to six times greater proportion of these cells when comparing similar subjects.¹⁷

Other studies point to collagen type I as a main auto-antigen where they class switch from IgM to IgG.²² Indeed, Hirsch et al found that gingival lesions from patients with

periodontitis contained large amounts of cells that produced antibodies to collagen, the majority of which were IgG, IgA, and IgM.²³ Ftis et al found similar results in the peripheral blood of periodontitis patients, which harbored large percentage of auto-antibodies to type I collagen.²⁴ Sugawara et al looked at the gingival crevicular fluid (GCF), gingival biopsies and blood sample from periodontitis patients and healthy patients, where they found a significantly higher percentage of B cells bearing the CD5 surface marker in the gingiva than in the peripheral blood of subjects or controls and anti-collagen IgG antibody levels in the patient's GCF was higher than the healthy blood samples.²⁵ Berglundh et al looked blood samples and biopsies from patients with generalized chronic periodontitis, localized aggressive periodontitis, and periodontally healthy patients.^{15, 17} They found about 40-50% of the B cells in the blood samples of periodontal patients expressed markers for autoreactive features while less than 15 % of healthy patients circulating B cells did.¹⁵ At the same time, the gingival lesion in the chronic periodontitis patients contained a considerable amount of B cells 30% of which had autoreactive features.¹⁵

Components other than type-I collagen have also been shown to elicit an autoimmune response. Govze and Herzberg looked at blood GCF fluid and serum samples of periodontal healthy and diseased patients, where they found increased reaction of IgG autoantibodies to desmosomal proteins, desmoplakins and desmogleins, in the sera and GCF of diseased patients.²⁶ Novo et al looked at blood samples from subjects that had systemic lupus erythematosus or RA, half of which were periodontally healthy, and the other half had periodontal disease as well as otherwise healthy controls.²⁷ Here, they found a higher number of anti-neutrophil cytoplasmic antibody-positive sera in patients that had both systemic lupus erythematosus and periodontitis, while the rheumatoid arthritis patients did not differ from healthy controls.²⁷ Phospholipids have

also been shown to be targets of autoantibodies. Schenkein et al showed higher proportions of these antibodies in subjects with either chronic or generalized aggressive periodontitis patients than healthy subjects or patients with localized aggressive periodontitis.²⁸ Finally, periodontitis patients have also been found to have a higher frequency of seropositivity to heat-shock protein 60 than healthy controls. Antibodies to these proteins in the blood samples of these patients with chronic periodontitis were shown to be unchanged during treatment, which is suggestive that their synthesis was regulated independently during the course of periodontal infection.²⁹

This information suggests that there are several components in the periodontal lesions that can initiate an autoimmune reaction. Autoantibodies may be produced to tissues or specific cell products. The aim of this study was to compare and characterize the auto-reactivity of two distinct classification of periodontal disease, localized aggressive periodontitis (LAgP), and generalized chronic periodontitis (CP) and compare it to periodontally healthy controls.

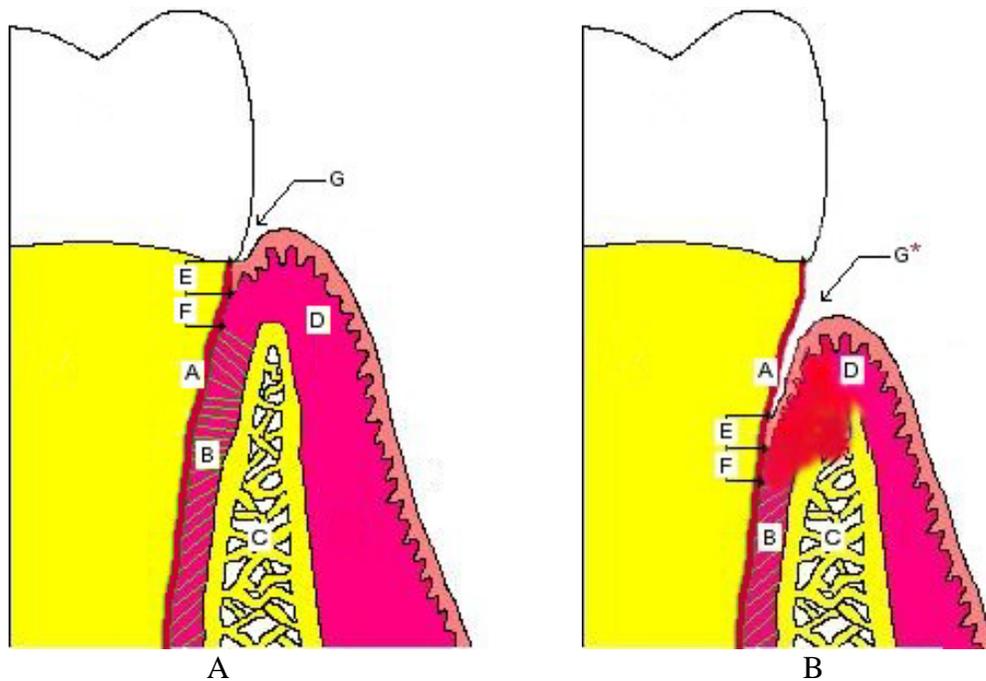


Figure 2-1. Anatomy of the Periodontium. A) Healthy Peridontium. B) Periodontal Disease. A. cementum. B. periodontal ligament . C. alveolar bone. D. gingival. E. junctional epithelium. F. connective tissue. Upon the induction of periodontal disease, apical migration of the junctional epithelium occurs as a bacterial induced inflammatory response destroys the underlying connective tissue attachment and bone (shown in red). The sulcus depth increases and a periodontal pocket (G*) is formed. Adapted from Master's Thesis of Ryan Mendro, University of Florida, College of Dentistry, 2008.

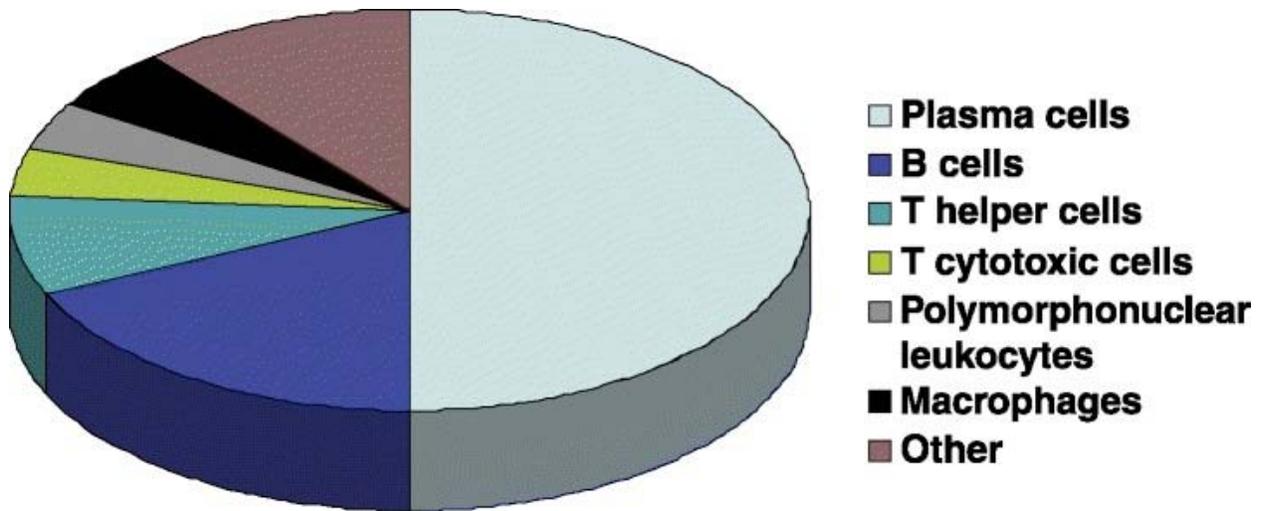


Figure 2-2. Distribution of Cell Proportions in Periodontitis Lesions. Plasma cells make up about half (50%) of all cells, while B cells make up about 18%. B cell population is greater than all T cells. T helper cells are more abundant than cytotoxic T cells. Polymorphonuclear cells (PMN) and macrophages make up less than 5% of all cells. Aggressive periodontitis and chronic periodontitis exhibit similar cellular composition. Adapted from Berglundh & Donati (2005).

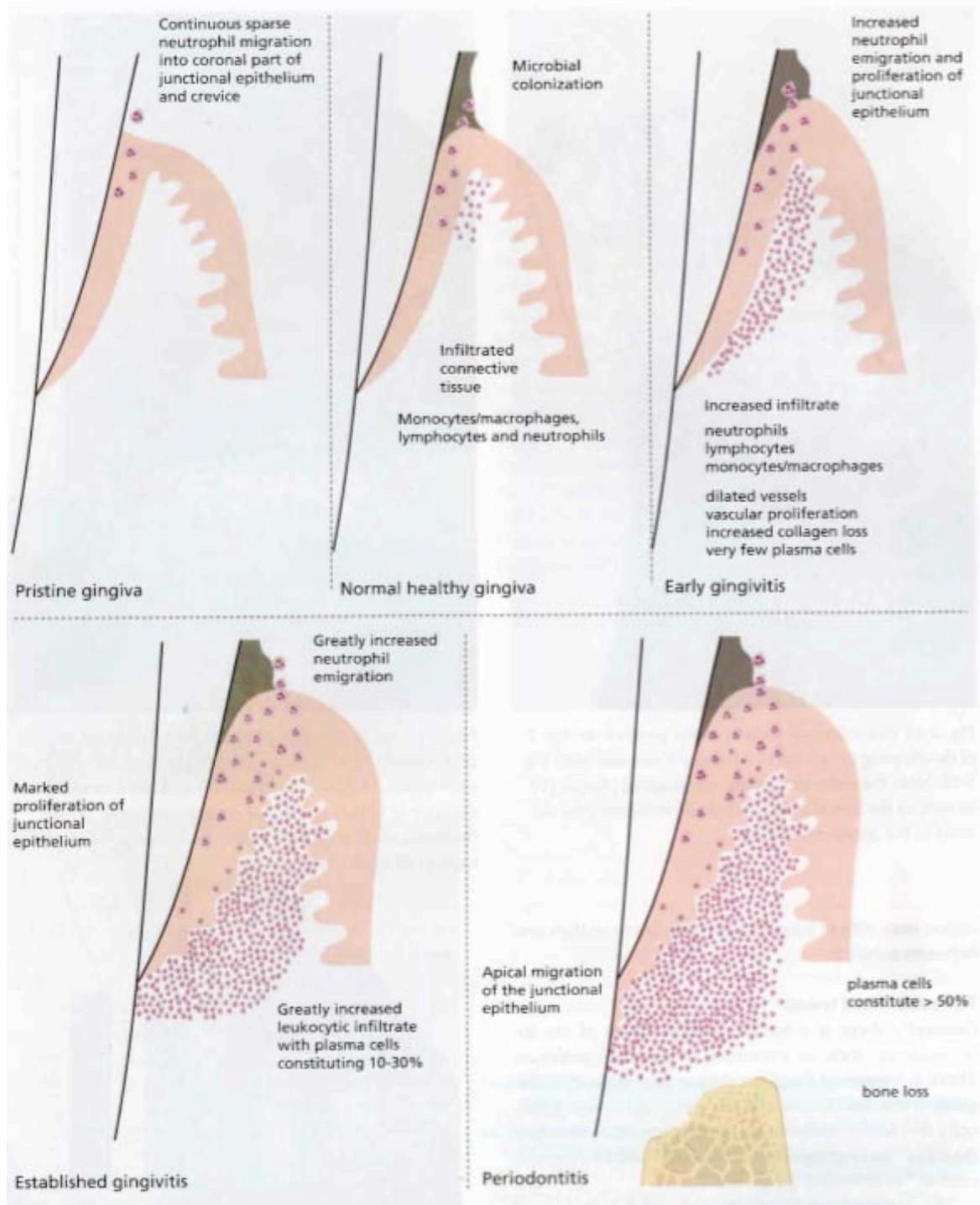


Figure 2-3. Changes of Gingival Tissues during Development of Periodontal Disease. As the lesion progresses from health to periodontitis, the extent and composition of inflammatory infiltrate, as well as the proliferation of the epithelium, increases significantly. Bone loss and apical migration of the epithelium are seen in periodontitis. Adapted from Clinical Periodontology and Implant Dentistry (Linhe et al, 2003)

CHAPTER 3 MATERIALS AND METHODS

Participant Cohort

This experiment was designed as a controlled pilot study, started in January 23, 2007 until the present. Three groups of patients were included and grouped according to their periodontal diagnosis. These groups were chronic periodontitis (CP), local aggressive periodontitis (LAgP) and periodontally healthy patient (PDH). Diagnosis of periodontal disease was given according to the American Academy of Periodontology Classification of 1999 (Armitage). Six periodontally healthy patients undergoing orthodontic treatment, requiring premolar teeth extractions as part of their treatment, were also recruited. Their teeth and associated tissues were used as targets of auto-reactivity. Twelve teeth and surrounding tissues were extracted. Patients from the CP and PDH groups were recruited from the University of Florida College of Dentistry Periodontology Clinic, Gainesville, FL. Patients of the LAgP group were recruited from the Leon County Health Department, Tallahassee, FL. Patients from whom teeth were extracted, undergoing orthodontic therapy were recruited from the University of Florida College of Dentistry Orthodontics Clinics. The protocol was approved by the Institutional Review Board for protection of human subjects at the University of Florida. All samples and data were obtained after reviewing and obtaining informed consent.

Clinical Evaluation

Patients of the CP, LAgP and PDH groups were examined with respect to probing depth (PD), location of the gingival margin in relation to the CEJ (GM), clinical attachment level (CAL) and bleeding on probing (BOP). PD was measured to the closest half millimeter with a manual periodontal probe. CAL was calculated as PD + GM. BOP was dichotomously recorded as presence/absence of bleeding within 15 seconds following pocket probing. The probing

assessments were recorded at six sites for all teeth except for third molars. For patients undergoing orthodontic therapy, the periodontal health confirmed from data available from periodontal examinations as part of their patient care program (data not shown).

Serum, Premolar and Gingival Tissue Sample Collections

10 ml of peripheral blood was collected by venipuncture from five participants in each experimental group (localized aggressive periodontitis (LAgP), chronic periodontitis (CP) and periodontally healthy (PH)), after which the serum was then separated by centrifugation and stored at -80° C until assays were performed.

2-4 premolar teeth were extracted from six periodontally healthy patients undergoing orthodontic treatment, as part of their normal standard of care. Here, patients received local anesthesia followed by surgical extraction of the teeth and 1-2mm of associated marginal tissue. Some specimens were placed in formalin for immunohistochemical analysis (n = 4), while others were placed in cell extraction buffer (Biosource) for protein extract analysis (n = 8).

Immunohistochemical Preparation and Examination

Formalin preserved specimens were paraffin embedded and 5 micron sections were mounted to microscope slides. Mounted slides were deparaffinized and rehydrated (xylene 25 min, 100% ethanol (ETOH) 9 min, 95% ETOH 9 min, 70% ETOH 9 min) and rinsed in distilled water followed by phosphate buffered saline (PBS). Any endogenous peroxidase activity of the equilibrated sections was quenched by incubation in 0.5% hydrogen peroxide for 30 minutes and rinsed in PBS. Sections were either probed with pooled serum (1:500) from one of the three experimental groups or incubated with PBS and allowed to incubate overnight at 4° C. Probed sections were then rinsed three times with PBS, after which peroxidase conjugated goat anti-human Ig (1:500) (Vector Labs) was allowed to incubate for 1 hour at room temperature (RT). The sections were developed using 3,3'-diaminobenzidine (DAB) (Vector Labs). and

counterstained in hemotoxylin (Fisher Scientific). Afterwhcih the slides were washed in tap water, dehydrated (95% ETOH,9 min., 100% ETOH 9 min, and xylene 9 min), and coverslipped with permount mounting media. Slides were visualized using a light microscope (Zeiss) and images captured using CapturePro software (Zeiss).

Protien Extraction

5 mls of cell extraction buffer (10mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1mM EGTA, 1mM NaF, 20 mM Na₄P₂O₇, 2mM Na₃VO₄, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate) (Biosource), supplemented with phenylmethanesulphonylfluoride or phenylmethylsulphonyl fluoride (PMSF) and a protease inhibitor cocktain (Sigma) was added to extracted teeth and associated tissues. Cementum was removed into the buffer using a no.15 scapel, afterwhcih all extracts were incubated in ice, vortexing every 30-60 secs every 10 minutes for one hour. The lysates were centrifuged at 14,000 rpm for 10 minutes at 4° C, after which the supernatant was transferred to a new tubes. A bicinchoninic acid assay (BCA) (Pierce) was then used along with a standard curve to determine a final protein concentration, according to the manufacture's instructions.

SDS Page and Western Blot Analysis

The protein extracts were combined with 4 x sample buffer (BioRad) supplemented with Dimethyl sulfoxide (DMSO) and dentatured at 70°C for 15 minutes. A running buffer was made by mixing 950 mL MilliQH20 with 50 mL of running buffer (BioRad 15 uL of each denatured protein extract were added to a 10 well mini-cell (BioRad) gradient gel (7-15%) along with a broad range ladder (BioRad). The gel was electrophoresised using NuPage MOPS SDS running buffer (Invitrogen) at 100 V and 50 mA for 45 min. The SDS Page gel was then transfered to a nitrocellulose membrane in transfer buffer (850 mL ddH₂O, 100 mL methanol, and 50 mL of 20 x transfer buffer (BioRad). for 60 minutes at 30 V and 140 mA

In order to detect reactivity of autoantibodies, transferred proteins were probed with pooled (n=5) or individual serum from each experimental group overnight at 4C . After which reactivity was detected using anti-human Ig conjugated to peroxidase (Jackson Labs). Reactivity was developed using ECL reagents (Pierce), xray film (Kodak). Reactivity was semiquantified using densitometric analysis and CapturePro software (BioRad).

Protein Purification and Identification

Database searching. Tandem mass spectra were extracted, charge state de-convoluted and de-isotoped by ABI analyst version 1.1. All MS/MS samples were analyzed using Mascot version 2.2.0 and X! Tandem. X! Tandem and Mascot was set up to search a subset of PIP_human_20070808 database also assuming trypsin. Mascot and X! Tandem were searched with a fragment ion mass tolerance of 0.30 Da and a parent ion tolerance of 0.30 Da. Lodoacetamide derivative of cysteine was specified in Mascot and X!Tandem as a fixed modification. S-carbamoylmethylcysteine cyclization (N-terminus) of the n-terminus, deamidation of asparagines and glutamine and oxidation of methionine were specified in Mascot and X! Tandem as variable modifications. *Criteria for Identification.* Scaffold was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability as specified by the Peptide Prophet algorithm (reference from article). Protein identifications were accepted if they could be established greater than 99.0 % probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm. (reference article) Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principle of parsimony.

Enzyme Linked ImmunoAssay (ELISA)

20 micrograms per well of collagen type I was coated on a 96 well plate in a carbonate coating buffer overnight at 4°C on rotator. Following washing three times with PBS/Tween, wells were blocked with PBS/Tween(0.05%)/BSA (0.5%) for 1 hour. After washing, 25ul of serially diluted (1:500-1:256000) individual patient sera were used for probing overnight at 4°C on rotator. Following washing three times, probed wells were reacted with 25ul of a 1:5000 dilution of goat ant-human Ig conjugated with alkaline phosphatase (AP) (Vector Labs), after which 100ul of p-nitro phenyl substrate reagent (BioRad) was used for development. The reaction was stopped using 100ul of a 3M NaOH and development read at on OD of 405nm.

Statistical Analysis

For the description of clinical date, mean values and standard deviation were calculated. For the semi-quantitative band densities and antibody titres to collagen type I, *p values* were calculated using ANNOVA and Student's T test with Welch's correction making the following comparisons: LAgP vs CP, LAgP vs. PDH and CP vs. PDH. The unpaired t test assumes that the two population have the same variances (same standard deviations). A modification of the t test (developed by Welch) can be used when you are unwilling to make assumptions. With Welch's t test, the degrees of freedom are calculated from a complicated equation and the number is not obviously related to sample size. *p value* < 0.05 was considered significant.

CHAPTER 4 RESULTS

Clinical Characteristic of Participant Cohort

Chronic and aggressive periodontal diseases follow different rates, timing of onsets, and disease progression. Because of this fact, it was of interest to determine if auto-reactivity to periodontal lesions would play a similar role in both diseases. The characteristics of the experimental and control groups are summarized in Table 1. The chronic periodontitis (CP) group, which consisted of 2 males and 3 females, age range 51-60 years, exhibited 35.6 % (± 17.1) of their sites with probing pocket depth (PPD) ≥ 4 mm and 34.6% (± 10.0) of their sites with clinical attachment level (CAL) ≥ 5 mm. The local aggressive periodontitis (LAgP) group, which consisted of 3 male, 2 female, aged 12-19 years, exhibited 13.7 % (± 7) of their sites exhibited PPD ≥ 4 mm and 6.5 % (± 3.1) CAL ≥ 5 mm. The majority of the attachment loss in the patients of the LAgP group was at the permanent first molars and incisors. The periodontally healthy (PDH) group had 5 periodontally healthy participants, which consisted of 1 male, 4 female, aged 21-28 years, with no sites exhibiting PPD ≥ 5 mm, no sites exhibiting CAL ≥ 1 mm and no more than 10 % of sites with bleeding on probing (BOP).

Immunohistochemical Evaluation of Auto-Reactivity

In order to determine serum auto-reactivity to components of the periodontal structures, immunohistochemical analyses was done. Extracted teeth and associated tissues, such as the periodontal ligament and gingival tissues were sectioned and mounted. These teeth were extracted from 6 periodontally healthy patients undergoing orthodontic treatment. as part of the over-all treatment plan for these patients. After mounting, these sections were probed with serum from each of the experimental groups. Reactivity to the components of the periodontal structures was detected in both the LAgP (Figure 4-1 B) and CP participants (Figure 4-1 A).

There was no reactivity detected in serum from the PDH controls (Figure 4-1 C). It was interesting to note the greater amount of auto-reactivity seen in LAgP group as compared to the reactivity of the CP group (Figure 4-1). This can be seen by the greater amount of brown tissue representing the reaction. From this data, we have shown that antibody mediated auto-reactivity is present in both CP and LAgP, although greater in the LAgP group, while no reactivity was present in PDH controls.

LAgP Serum Reactivity Differs in Quality and Quantity Compared to CP Serum Reactivity

It was of interest to determine what types of periodontal components these antibodies were reactive to. To accomplish this, extracts of cementum and associated tissues of extracted premolars, including the periodontal ligament and gingival tissues were probed with pools of serum (n=5) from each of our experimental groups, using a western blot technique. Serum from LAgP participants reacted to several bands of proteins (Figure 4-2 A), while serum from CP participants reacted to only one of these bands of proteins (Figure 4-2 A, Band 1). Serum from PDH participants did not demonstrate any reactivity (Figure 4-2 A). Since this analysis was originally done on pooled sera, there was a possibility that this reactivity was from high reactivity in one subject rather than an average reactivity of all subjects. To assure that the observed reactivity was representative of all members of the cohort, the same analysis was performed on individual samples from all subjects within all experimental groups. From this analysis, consistently higher reactivity to all bands analyzed (Bands I, II, III) were detected from individual LAgP serum when compared to individual CP and/or PDH serum (*p value* = 0.001) (Figure 4-2B). Additionally, individual serums of participants with CP were consistently reactive with only Band I of the tissue extracts (Figure 4-2A). As expected, individual PDH participants demonstrated little or no reactivity (Figure 4-2). From this information, we can clearly recognize the presence of auto-reactivity in participants of both LAgP and CP, and the

lack of it in the healthy group. It also suggests that this auto-reactivity is greater in intensity in the LAgP group.

Key Components of the Periodontal Structure are Putative Targets of Auto-reactivity

It was important to determine exactly which of the protein components incited the auto-immune reactions. In order to define them, the three reactive bands of proteins were excised and analyzed for peptide contents using tandem mass-spectrometry. Several peptides were identified in each band and are summarized in Table 2. Many of these peptides have been described before in association with auto-reactivity as targets and include those associated with collagen, HSP, lipoproteins and aggregated IgG (Table 4-2). In addition to these, other antibody targets which are important proteins for periodontal stability, but in which this auto-reactivity has not been previously seen were also detected. These include vimentin, spectrin, filamin, actin, lamin, keratin, and tubulin. Most of these proteins were extracted from bands II and III (Figure 4-2, Table 4-2), bands that were unique to LAgP reactivity. This indicates a lack of reactions from CP serum as there was no reaction from the serum in these bands. However, it is important to understand, that not all peptides identified in each band are going to be targets for auto-immunity. Further investigation will be necessary to determine which peptides are auto-immune targets and may also be involved in the progression of periodontal disease and which disease states they are associated with.

Auto-reactivity to collagen type I is more severe in localized aggressive periodontitis

Band I from CP participants serum was compared to that of LAgP participants serum. (Figure 4-3) It was noted that there was a significantly reduced amount of reactivity with CP participants., and the peptide analysis of Band I revealed several peptides of collagen to be present (Table 4-1). This information led us to further quantify the serum reactivity of these experimental groups to collagen. An ELISA analysis was therefore performed on serums from individual CP and

LAgP participants in order to determine an average collagen type I titer. Similar results as with the western blot analysis were achieved. While both experimental groups showed some reactivity, participants with CP presented with a significantly lower titer to collagen type I when compared to those with LAgP (p value = 0.027) (Figure 4-3). As expected, the PDH participants presented with no reactivity to collagen type I (Figure 4-3). This information further shows that reactivity to collagen type I is present in both experimental groups, with a stronger reaction in LAgP participants. These data suggest a role of auto-immunity in periodontal diseases, while its specific importance may differ based on type and/or stage of disease.

Table 4-1 Clinical Characteristics of Participant Cohort.

<i>Group</i>	<i>Age (years)</i>	<i>PD (mm)</i>	<i>CAL (mm)</i>	<i>BOP</i>	<i>% sites ≥ 4 mm</i>	<i>% sites ≥ 5mm</i>
CP (n=5)	55 \pm 4.2	3.5 \pm 1.6	4.0 \pm 2.0	49.4 \pm 7.2	35.6 \pm 17.1	34.6 \pm 10.0
LAgP (n=5)	16.2 \pm 2.9	2.9 \pm 1.4	1.5 \pm 1.4	54.3 \pm 9.5	13.7 \pm 7.0	6.5 \pm 3.1
PDH (n=5)	23.4 \pm 3.0	1.7 \pm 0.6	0.6 \pm 0.5	8.1 \pm 2.7	0	0

Table 4-2 Putative Targets of Autoreactivity.

	BAND		
	I	II	III
Previously Identified Classes of Targets in Periodontal Disease	Lipoproteins		
	Apolipoprotein A-I precursor	x	x
	Immunoglobulins		
	IGHA1		x
	IGHG2	x	x
	IGHG1	x	x
	IGHG1	x	x
	IGKV1	x	
	Heat Shock Proteins		
	HSP90	x	
	HSP70	x	
	HSP70		x
	Collagens		
	Collagen alpha-1(I) chain precursor	x	
	Collagen alpha-1(XII) chain precursor (isoform 4)	x	
	Collagen alpha-1(XIV) chain precursor (isoform 1)	x	
	Collagen alpha-2(I) chain precursor	x	
	Glycoproteins		
	Alpha-1-antitrypsin precursor	x	x
	Alpha-2-HS-glycoprotein precursor		x
Beta-2-glycoprotein 1 precursor		x	
Alpha-1B-glycoprotein precursor		x	
Fibrinogen precursor (gamma chain)	x	x	
Inter-alpha-trypsin inhibitor precursor (heavy chain H2)	x		
Blood Proteins			
Alpha-2-macroglobulin precursor	x		
C3 (187 kDa protein)	x		
Hemoglobin subunit alpha	x	x	
Hemoglobin subunit beta	x	x	
Hemopexin precursor		x	
Serum albumin precursor (isoform 1)	x	x	
Nuclear Proteins			
Elongation factor 1 (alpha 2)	x		
Putative Auto-Immune Targest of Interest in Periodontal Disease	Cell to Cell Interactions		
	Actin (cytoplasmic 1)	x	x
	Fibrinogen precursor (beta chain)		x
	Filamin (alpha)	x	
	Lamin (isoform A)	x	x
	Spectrin (isoform b)	x	
	Tubulin (beta chain)	x	
	Vimentin	x	x
	Proteoglycans		
	Lumican precursor		x
	Versican precursor (isoform V0)		x
	Epithelial Cell Associated Protiens		
	Keratin type I (cytoskeletal 10)	x	
	Keratin type II (cytoskeletal 1)	x	x
	Osteoblast Associated Proteins		
	Periostin precursor (isoform 1)	x	x
	Auto-immune Associated Proteins		
	Alpha-enolase		x
	Calreticulin precursor		x
	Enzymes		
Aldehyde dehydrogenase (member A1)		x	
Glyceraldehyde-3-phosphate dehydrogenase	x		
Peroxi-redoxin-2	x	x	
Protein disulfide-isomerase precursor		x	
Binding Protiens			
Anion transport protein	x		
Selenium-binding protein 1		x	
Serotransferrin precursor		x	
vitamin D-binding protein precursor		x	
Proteins of Unknown Function			
Uncharacterized protein SPTA1	x		



Figure 4-1. Immunohistochemical Analysis of Auto-Reactivity. Five micron sections of healthy teeth and surrounding tissue extracted for orthodontic treatment were probed with 1:500 dilution pooled serum (n=5) from different groups. A) chronic periodontitis (CP), B) localized aggressive periodontitis(LAgP), C) Peridontaly Healthy (PDH).3,3'-diaminobenzidine was used to detect autoreactivity indicated by the brown color. Note the more intense reaction in B indicating greater auto-reactivity in LAgP.

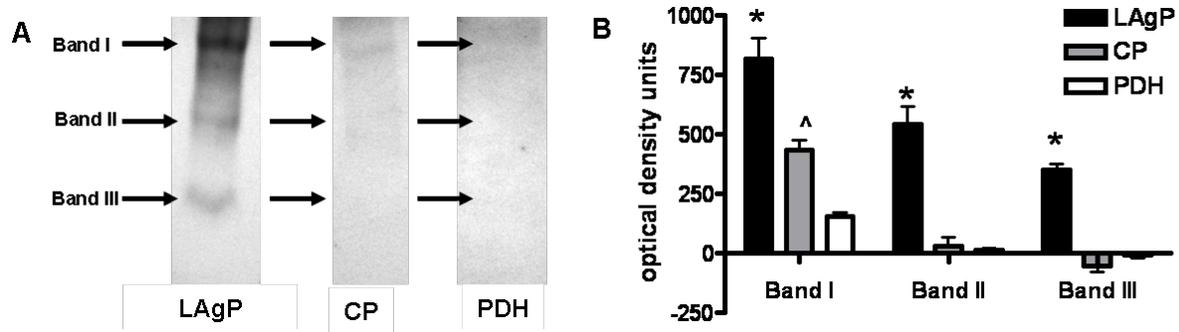


Figure 4-2. Auto-reactivity of Serum from Different Experimental Groups. A) Extracts from cementum scrapings and surrounding tissues from 8 healthy premolar teeth, extracted from orthodontic patients, were probed with 1:500 dilution of individual serum from each group using western blot protocol, B) A densitometer and Quality One software was used to semiquantitate individual serum (n=5) from each experimental group. * p value < 0.001 LAgP vs. CP and LAgP vs. PDH band I.; ^ p value = 0.001 CP vs PDH Band I; † p value < 0.002 LAgP vs CP and LAgP vs PDH Band II and Band III. Students T test *ANNOVA < 0 .0001.

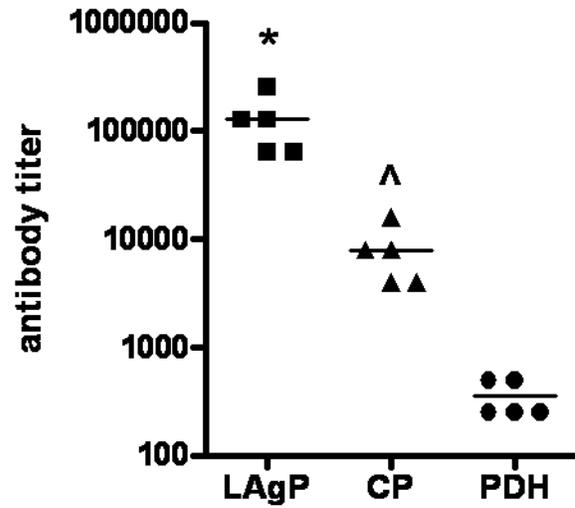


Figure 4-3 Autoreactivity to Collagen Type I. 20 micrograms of collagen Type I was probed with 25ul of serially diluted individual patient's serum (n=5) of each experimental group, localized aggressive periodontitis (LAgP), chronic periodontitis (CP), and periodontally healthy (PDH) using ELISA protocol. Auto-reactivity was more severe in LAgP group when compared to the CP and PDH groups. **p value* ≤ 0.027 LAgP vs. PDH and LAgP vs. CP, ^*p value* = 0.0251 CP vs PDH. Student's T test. Data are a compilation of three separate experiments performed in duplicate.

CHAPTER 5 DISCUSSION

The results of our study have the potential to have important implication in our understanding of the pathogenesis of various forms of periodontal disease. It has helped us to understand the role of auto-immunity in these diseases. From our studies we have seen that auto-reactivity is present in both participants with CP and patients from LAgP. It is interesting to note that when compared to PDH patients, no reactivity could be detected. This is a very important finding as it clearly shows a difference within the immune response among patients. Much interest has been given to the risk factor of host susceptibility in patients with periodontitis. These susceptibilities fall over a broad range of mechanisms although immune function is one of the most well studied. For instance, some reports suggest an unknown factor or deficiency in the immune system of patients is important for the development of this disease⁷. Different forms of genes are known as alleles and can produce variations in tissue structure, antibody response, and inflammatory mediators.¹⁶ Indeed, some have demonstrated that polymorphisms in multiple cytokine genes have been associated with periodontal disease. Specifically, these include polymorphic interleukin-1 (IL-1), IL-6, Fc gamma receptor , IL-10, and tissue necrosis factor α (TNF- α).^{30, 31, 32, 33, 34}

On the other hand, others have been unable to link cytokine polymorphisms with certain disease states of periodontal disease. For instance, contradictory findings have been found when IL-1 polymorphism in patients with aggressive periodontitis has been looked at. Hodge et al looked at IL-1a and IL-1b genetic polymorphism in Caucasians with generalized early-onset periodontitis and found no significant differences between patients and controls.³⁵ However, Parkhil et al. looked at a similar association of IL-1b and aggressive periodontitis and found a significant association in susceptibility to disease with a positive polymorphisms.³⁶ One of the

factors that aid in the diagnosis of patients with aggressive periodontitis is familial aggregation.¹⁰ These various genetic polymorphisms may be attributed to this phenomenon. One must be careful when considering this characteristic of disease, however, as it is difficult to differentiate between genetic links and environmental factors. While familial aggregation suggests a genetic predisposition, these familial patterns may reflect an exposure to common environmental factors within families.³⁷ Future studies with more advanced diagnostic aids are necessary to fully assess this issue.

Increased susceptibility has been seen in patients with auto-immune disorders such as diabetics, and patients with leukocyte deficiencies. With these findings, auto-immune reactivity potential in periodontal disease have recently developed some attention. Results from our study may contribute to information available regarding host susceptibility in the form of auto-reactivity as seen in both CP and LAgP groups (Figures 4-1, 4-2, 4-3). The more intense reactions seen in the LAgP group suggest that auto-immune reaction may play a greater role in patients with aggressive periodontitis patients when compared to those with chronic periodontitis. This would have significant implications in the way we view therapy for these patients. Recent studies regarding pathogenesis, as well as the rapid, severe destruction seen in aggressive periodontitis have suggested that a shift in the way this disease is viewed. More and more evidence is suggesting that these diseases may not be as closely related as once thought.³² Perhaps auto-immunity is one important factor that causes the difference between these two diseases.

Previous studies have found collagen to be a target of auto-reactivity in patients with periodontal disease. In 1986 Ftis et al. looked at 97 periodontal patients in comparison to 57 controls.²⁴ Using ELISA technique, he found higher levels of antibody to type I collagen in the

peripheral blood of the test group when compared to healthy controls. It has also been reported that the level of antibodies to collagen type I is higher in the gingival tissues when compared to peripheral blood.¹⁶ These studies corroborate our findings where auto-reactivity to collagen type I was present in CP and LAgP, and absent in the PDH group (Figure 4-2, 4-3). Collagen's role in the homeostasis of the periodontium is well known. It is critical in the connective tissue attachment and is a primary constituent of the extracellular matrix (ECM). It is contained in all aspects of the periodontium, such as bone, connective tissue, PDL, and cementum. Because of its important role, destruction of this tissue can have catastrophic results. Loss of any of these tissues will compromise the stability of the tooth and can lead to loss of its attachment and eventual tooth loss. In our study, band I, which represents autoreactivity to type I collagen, is the darkest band (Figure 4-2). It has stained significantly darker than the other bands containing putative auto-antigens. This indicates that the auto-reactivity is more intense in this group of proteins and therefore may play a more critical role in its pathogenesis. This information may help explain why this disease is so rapid in onset and so destructive.

In contrast to these studies, as well as our findings, other reports, found the levels of anti-collagen as well as the numbers of anti-collagen-producing cells in the peripheral blood to be low in patients with periodontitis, using enzyme-linked immunospot (ELISOT).²³ However, when they looked at analyses on dissociated gingival cells, the presence of high numbers of cells that secrete antibodies to type I collagen were found. The contradiction to our findings may be due to their technique of ELISOT, while we used ELISA.

Other components have also been described as targets for auto-immunity. Increased reactions of IgG auto-antibody with desmosomal proteins was seen in sera of periodontal patients when compared to healthy controls.²⁶ The same antibody retrieved from the GCF also

showed greater autoreactivity when compared to controls. Phospholipids have also been shown to be targets of autoantibodies. Higher proportion of these antibodies were found in patients with either chronic or generalized aggressive periodontitis when compared to healthy subjects or patients with localized aggressive periodontitis.²⁸ In our study, the reactive bands were excised and analyzed for peptide content (Table 4-2). Some of the peptides extracted have previously been classified as auto-immune targets, such as collagen, HSP and lipoproteins.^{28,29} Other targets, which are associated with the ECM and cell to cell interactions were also detected. These included vimentin, spectrin, filamin, actin, lamin, keratin, and tubulin.

Other putative targets found in the LAgP bands have also been associated with other auto-immune diseases. Two of these are alpha-enolase and calreticulin. Alpha-enolase has been identified as an auto-antigen in Hashimoto's encephalopathy³⁸, severe asthma³⁹, and Behcet's disease.⁴⁰ Calreticulin has been shown to bind to antibodies in sera of some Systemic Lupus Erythematosus and Sjogren's syndrome patients.^{41,42,43} Proteins associated with the structure of the periodontium, such as keratin, an important product of epithelial cells, and periostin, a product secreted by osteoblasts in the periosteum and PDL^{44,45}, were also detected to elicit autoreactivity. All these molecules were only found the LAgp serum, while the CP serum had no reaction. This indicates that the autoreactivity potential was only found in LAgP group and not in the CP group.

The extracts that were analyzed contained a list of different potential targets for auto-immunity in patients with LAgP (table 4-2). The presence of them and their implications need to be further studied using purified proteins and ELISA technology. Additional targets may also be identified in the future, as this is an ongoing area of research in our laboratory. This study was a preliminary step in identifying potential targets which will require additional investigation. It

has demonstrated that there is a role for auto-immunity in the pathogenesis of periodontal diseases. These reactions have been shown to be more exuberant and intense in patients with LAgP. While their validity needs to be confirmed, novel targets associated with the periodontium for autoimmune reactivity have been described.

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BIOGRAPHICAL SKETCH

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