PATHOGENESIS OF PERIODONTAL DISEASE

INTRODUCTION:

Inflammatory and immune reactions to microbial plaque are the predominant features of gingivitis and periodontitis. The inflammatory reaction is visible both clinically and microscopically in the affected periodontium. Inflammatory and immune processes operate in the gingival tissues to protect against local microbial attack and prevent microorganisms or their damaging products from spreading into or invading the tissues. These host defense reactions are, however, also considered potentially harmful to the host in that inflammation can damage surrounding cells and connective tissue structures.

Furthermore, inflammatory and immune reactions that extends deep into the connective tissue beyond the cemento-enamel junction (CEJ) may include loss of connective tissue attachment to the tooth involved as well as loss of alveolar bone. These “defensive” processes could therefore paradoxically contribute to the tissue injury observed in gingivitis and periodontitis.

MECHANISMS OF PATHOGENICITY

For a periodontal pathogen to cause disease, it is essential that the pathogen be able to (1) colonize the subgingival area and (2) produce factors that either directly damage the host tissue or lead to the host tissue damaging itself.

To colonize subgingival sites, a species must be able to (1) attach to one or more of the available surfaces, (2) multiply, (3) compete successfully against other species, and (4) defend itself from host defense mechanisms.

Adhesions

To establish in a periodontal site, a species must be able to attach to one or more surfaces including the tooth, the sulcular or pocket epithelium or other bacterial species attached to these surfaces. Some of the adhesins that have

been identified on subgingival species include fimbriae and cell associated proteins. Receptors on tissue surfaces that species adhere to them include galactosyl residues and proline rich proteins.

Coaggregation

While many species attach directly to host surfaces, other species attach to bacteria attached to such surfaces. This phenomenon is called coaggregation, such as the coaggregation of Lachnoanaerobaculum saburreum, which is non-motile microbe, to T. denticola, which has the ability to migrate to the deeper periodontal tissue, the coaggregation of L. saburreum with T. denticola is characterized by "piggyback appearance". The whole process is highly mediated by P. gingivalis outer membrane vesicles.

Multiplication

The gingival crevice and/or periodontal pocket might be considered a lush area for microbial growth, but is in fact a rather stringent environment for a bacterial species to live. The mean temperature of the area averages about 35°C and ranges from 30°-38°C. The pH is rather restricted (pH 7. 0-8.5). Three sources of nutrient are available to subgingival organisms (diet, host and other subgingival species). Certain nutrients essential to some bacterial species must be formed by other species in that area. However, the precursors to such substances and certain specific growth factors such as hemin must be derived from the host. Gingival crevice fluid is not particularly rich in nutrients, creating a major competition for the small amounts available. In addition, nutrients delivered in relative abundance to the outer layers of plaque may not reach deeper layers.

Interbacterial relationships

Bacterial interactions play important roles in species survival. Some inter-species relationships are favorable, in that one species provide growth factors or facilitate attachment of another. Other relationships are antagonistic due to competition for nutrients and binding sites or to the production of substances which limit or prevent growth of a second species such as production of hydrogen peroxide by S. sanguis which suppress the

growth of A. actinomycetemcomitans. On the other hand, the growth of S. sanguis has been shown inhibited by a bacteriocin produced by A. actinomycetemcomitans.

Overcoming host defense mechanisms

Subgingival plaque microorganisms appear to overgrow and lead to severe disease in immune-compromised hosts, particularly those with neutrophil disorders. A bacterial species has a number of host-derived obstacles to overcome when colonizing a subgingival site. These include the flow of saliva and gingival crevice fluid and mechanical displacement by chewing and speaking. Substances in saliva and gingival crevice fluid may aid in the prevention of colonization by blocking the binding of bacterial cells to mammalian surfaces. Such factors include specific antibodies, salivary glycoproteins and mucins, which may act as non-specific blocking agents.

Once a bacterial cell has successfully attached to a surface in the subgingival area, other host mechanisms come into play. Desquamation of epithelial cells presents a new cleansing mechanism, which is overcome by certain species by their ability to bind to underlying epithelial cells. Other species are able to invade the epithelial cells and may multiply intracellularly and spread to adjacent cells. Specific antibody in the subgingival area could act by preventing bacterial attachment or, in some instances, by making the bacterial cell susceptible to various phagocytic or killing mechanisms.

A number of subgingival species have evolved mechanisms for evading the effect of specific antibody. Species including P. gingivalis, P. intermedia, P. melaninogenica and Capnocytophaga species possess IgG and IgA proteases that can destroy antibody. Other species are capable of evading antibody by changing their surface antigens or possibly by mimicking the host's antigens species. A number of bacterial mechanisms exist that might including the production of leukotoxin by A. actinomycetemcomitans and capsules by P. gingivalis and other species that inhibit phagocytosis. In addition, a number of species have developed strategies to interfere with the killing mechanisms of the polymorph nuclear leukocytes.

INITIATION OF PERIODONTAL DISEASE

The pathogenesis refers to biological and histological events that occur in the tissue during the process of conversion from the healthy state to diseased one.

Most normal subjects maintaining a high standard of oral hygiene are not likely to develop advanced periodontal disease. Experimental, short-term clinical studies have shown that microorganisms quickly start to colonize clean tooth surfaces once an individual abstains from mechanical tooth cleaning; within a few days microscopical and clinical signs of gingivitis are then apparent. These inflammatory alterations are resolved or reversed when adequate tooth-cleaning measures are resumed. The inflammatory changes may remain confined to the gingival area for several years, but at some sites gingivitis eventually shifts to destructive periodontal disease resulting in loss of connective tissue attachment and alveolar bone. Clearly some imbalance of the host-microbial relationship is occurring in the destructive lesions, which may be unique to that site and to periodontally susceptible individuals.

NORMAL "CLINICALLY HEALTHY" GINGIVA

Normal gingiva is characterized clinically by its pink color and firm consistency and the gingival margin exhibits a scalloped outline. The interdental papillae are firm, do not bleed on gentle probing and fill the space below the contact areas. The gingiva often exhibits a stippled appearance and there is a knife edge margin between tooth and soft tissue. Normal gingiva is theoretically free from histological inflammation, but this "ideal" healthy condition has two types: a super healthy or "pristine" state which histologically has little or no inflammatory infiltrate, and the "clinically healthy" gingiva which looks similar clinically but histologically features an inflammatory infiltrate.

In clinically healthy gingiva features an infiltrate of inflammatory cells, predominantly neutrophils associated with the junctional epithelium and

lymphocytes in the subjacent connective tissue. Exudative and transudative fluid and plasma proteins arrive in the gingival crevice region having left the vessels and travelled through the tissues to create the gingival crevicular fluid (GCF). The infiltrate at this stage may occupy as much as 5% of the connective tissue volume and is composed of monocytes, macrophages, lymphocytes and neutrophils. These cells are found in the junctional epithelium as well as in the connective tissue of clinically healthy gingiva.

Clinically healthy gingiva appears to deal with microbial challenges without progressing to a diseased state, probably because of several defensive factors which include:

1. Regular shedding of epithelial cells into the oral cavity.

2. Intact epithelial barrier.

3. Positive fluid flow of the gingival crevice which may remove non-attached microorganisms and noxious products.

4. Antimicrobial effect of antibodies.

5. Phagocytic function of neutrophils and macrophages.

6. Detrimental effect of complement on the microbiota.

HISTOPATHOLOGICAL FEATURES OF GINGIVAL INFLAMMATION

An experimental gingivitis study in dogs was done by Page and Schroeder had compared the cellular and structural composition of the affected area before and during the development of gingivitis over a period of 28 days. At Day 0 of this experiment the normal gingival unit has virtually no inflammatory cells and is comprised of approximately 40-45% epithelium and 55- 60% connective tissue. The connective tissue zone consists of collagen (60%), fibroblasts (13%), vessels (7%) and other tissue constituents, such as intercellular matrix and nerves (20%). Following plaque accumulation, neutrophils and mononuclear leukocytes readily migrate to this area and the connective tissue begins to form and increase in volume over the 28-day period. At this 28-day interval the connective tissue

is comprised of lymphocytes, plasma cells and macrophages which adhere to the collagen matrix and remain in the tissue, whereas neutrophils continue to migrate into the gingival sulcus. With the extensive influx of leukocytes, a marked reduction in the amount of collagen and fibroblasts occurs and the volume of residual tissue (intercellular matrix, degraded collagen, exudates material, degenerated or dead cells) and small blood vessels increases. Page and Schroeder classified the progression of gingival and periodontal inflammation on the basis of clinical and histopathological evidence into four phases: initial, early, established and advanced stages or lesions.

The initial lesion (clinically healthy gingiva)

Inflammation quickly develops as plaque is deposited on the tooth. Within 24 hours marked changes are evident in the microvascular plexus beneath the junctional epithelium as more blood is brought to the area. Dilation of the arterioles, capillaries and venules of the dentogingival plexus is evident histopathologically. Hydrostatic pressure within the microcirculation increases and intercellular gaps form between adjacent capillary endothelial cells. As the lesion enlarges, and gingival crevicular fluid flow increases, noxious substances from microbes will be diluted both in the tissue and the crevice. Bacteria and their products may thus be flushed from the sulcus. Plasma proteins escaping from the microcirculation include defensive proteins such as antibodies, complement and protease inhibitors and other macromolecules with numerous functions, probably within 2-4 days of plaque build-up the cellular response is well established and is helped by chemotactic substances originating from the plaque microbiota as well as from host cells and secretions. PMNs move through the connective tissue and the majority seem to accumulate in the junctional epithelium and gingival sulcus region.

The early lesion (early gingivitis)

The early gingival lesion occurs after approximately one week of plaque accumulation. Only an approximation of the time required can be given as marked subject variation occurs in humans although this may well be less variable in animal models. The variation seen amongst humans could be due

to differences in plaque accumulation, both at the site and subject level, or to differences between individuals in features such as hormonal levels.

The gingiva is erythematous in appearance as a result of proliferation of capillaries, opening up of microvascular beds, and continued vasodilatation. Increasing vascular permeability leads to increased GCF flow, and transmigrating neutrophils increase significantly in number. The predominant infiltrating cell types are neutrophils and lymphocytes (primarily thymic lymphocytes [T-cells]) and the neutrophils migrate through the tissues to the sulcus, and phagocytose bacteria. Fibroblasts degenerate, primarily via apoptosis (programmed cell death), which increases the space available for infiltrating leukocytes. Collagen destruction occurs, resulting in collagen depletion in the areas apical and lateral to the junctional and sulcular epithelium.

The basal cells of these epithelial structures begin to proliferate to maintain an intact barrier against the bacteria and their products, and as a result the epithelium can be seen proliferating into the collagen depleted areas of the connective tissues. As a result of edema of the gingival tissues, the gingiva may appear slightly swollen, and accordingly, the gingival sulcus becomes slightly deeper. The subgingival biofilm exploits this ecologic niche and proliferates apically (thereby rendering effective plaque control more difficult). The early gingival lesion may persist indefinitely or may progress further.

The established lesion (established gingivitis)

The progression from the early lesion to the established lesion depends on many factors, including the plaque challenge (composition and quantity of the biofilm), host susceptibility factors, and risk factors (both local and systemic). Generally there is a further enhancement of the inflammatory state as exposure to plaque continues. There is increased fluid exudation and leukocyte migration into the tissues and the gingival crevice. Clinically this lesion will exhibit more edematous swelling than the "early gingivitis”.

Plasma cells are seen situated primarily in the coronal connective tissues and the rete pegs extend deeper into the connective tissue in an attempt to

maintain epithelial integrity and a barrier to microbial entry. The junctional epithelium is changed and is no longer closely attached to the tooth surface. The pocket epithelium that now has formed has a heavy leukocyte infiltrate, predominantly of PMNs which eventually migrate across the epithelium into the gingival pocket. In comparison to the original junctional epithelium, the pocket epithelium is more permeable to the passage of substances into and out of the underlying connective tissues and may in places be temporarily ulcerated. The pocket epithelium is less able to resist the passage of the periodontal probe, so bleeding on probing is a common feature of chronic gingivitis.

It is important to remember that these inflammatory changes are still completely reversible if effective plaque control is reinstituted.

The advanced lesion (periodontitis)

The advanced lesion marks the transition from gingivitis to periodontitis. This transition is determined by many factors, the relative importance of which is, at present, unknown but includes the bacterial challenge (both the composition and the quantity of the biofilm), the host inflammatory response, and susceptibility factors, including environmental and genetic risk factors.

So the final stage in this process is known as the advanced lesion. As the pocket deepens, probably due to the epithelium spreading apically in response to plaque irritation, plaque continues it's apical down growth and flourishes in this anaerobic ecological niche. The lesion is no longer localized to the gingival, and the inflammatory cell infiltrate extends laterally and apically into the connective tissue of the true attachment apparatus. The advanced lesion has all the characteristics of the established lesion but differs importantly in that alveolar bone loss occurs, fiber damage is extensive, the junctional epithelium migrates apically from the cemento-enamel junction, and there are widespread manifestations of inflammatory and immunopathological tissue damage. It is generally accepted that plasma cells are the dominant cell type in the advanced lesion.

Inflammatory Responses in the Periodontium

Two main groups of molecules play a role in the pathogenesis of periodontitis:

microbial virulence factors : those derived from the subgingival microbiota and host-derived inflammatory mediators: those derived from the host immune–inflammatory response.

The subgingival biofilm functions:

1 initiates and continue inflammatory responses in the gingival and periodontal tissues.

2 contribute directly to tissue damage by the release of noxious substances .

3-activating immune–inflammatory responses (their primary importance in periodontal pathogenesis) that in turn, result in tissue damage .

Microbial Virulence Factors include:

1-Lipopolysaccharide

large molecules composed of a lipid component (lipid A) and a polysaccharide component. They are found in the outer membrane of gram-negative bacteria, they act as endotoxins (LPS is frequently referred to as endotoxin), and they trigger strong immune responses in animals.

A component of gram--positive cell walls, lipoteichoic acid, also stimulates immune responses, although less potently than LPS.

*Both LPS and lipoteichoic acid are released from the bacteria present in the biofilm and stimulate inflammatory responses in the tissues, thereby resulting in increased vasodilation and vascular permeability, the recruitment of inflammatory cells by chemotaxis, and the release of proinflammatory mediators by the leukocytes that are recruited to the area.

2-Bacterial Enzymes and Noxious Products

• Plaque bacteria produce several metabolic waste products that contribute directly to tissue damage. These include noxious agents such as ammonia (NH_3) and hydrogen sulfide (H_2S), as well as short--chain carboxylic acids such as butyric acid and propionic acid. These acids are detectable in GCF and are found in increasing concentrations as the severity of periodontal disease increases.

The short--chain fatty acids may aid P. gingivalis infection through:

tissue destruction, create a nutrient supply for the organism by increasing bleeding into the periodontal pocket, influence cytokine secretion by immune cells, and they may potentiate inflammatory responses after exposure to proinflammatory stimuli.

. 3-Microbial Invasion

• In histologic specimens, bacteria (including cocci, filaments, and rods) have been identified in the intercellular spaces of the epithelium

It has also been shown that Aggregatibacter actinomycetemcomitans can invade epithelial cells and persist intracellularly.

• Periodontal pathogens such as P. gingivalis and Aggregatibacter actinomycetemcomitans have been reported to invade the gingival tissues including the connective tissues.

• Fusobacterium nucleatum can invade oral epithelial cells, and bacteria that routinely invade host cells may facilitate the entry of noninvasive bacteria by coaggregating with them.

Fimbriae

The fimbriae of certain bacterial species, particularly P. gingivalis, may play a role in periodontal pathogenesis. P. gingivalis fimbriae stimulate immune responses.

Bacterial DNA

(DNA) stimulates immune cells through TLR-9.

Host-Derived Inflammatory Mediators:

The inflammatory and immune processes that develop in the periodontal tissues in response to the long-term presence of the subgingival biofilm are protective by intent but can result in considerable tissue damage, thereby leading to the clinical signs and symptoms of periodontal disease.

1--Cytokines:

Cytokines are produced by a large number of cell types, including infiltrating inflammatory cells (e.g., neutrophils, macrophages, lymphocytes), as well as resident cells in the periodontium (e.g., fibroblasts, epithelial cells). Effective in very low concentrations, they are produced transiently in the tissues, and they are act locally in the tissues in which they are primarily produced.

Cytokines bind to specific receptors on target cells and initiate intracellular signaling cascades that result in phenotypic changes in the cell by altered gene regulation.

2-Prostaglandins:

• group of lipid compounds derived from arachidonic acid, a polyunsaturated fatty acid found in the plasma membrane of most cells.

• PGE2 is produced by various types of cells and most significantly in the periodontium by macrophages and fibroblasts. PGE2 results in the induction of Matrix Metalloproteinases and osteoclastic bone resorption, and it has a major role in contributing to the tissue damage that characterizes periodontitis.

3-Matrix Metalloproteinases:

• MMPs are a family of proteolytic enzymes that degrade extracellular matrix molecules such as collagen, gelatin, and elastin. They are produced by a variety of cell types, including ,,neutrophils macrophages fibroblasts epithelial cells, osteoblasts and osteoclasts .