Periodontics

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HOST-PARASITE INTERACTIONS

Periodontal disease is initiated and sustained by factors (substances) produced by the subgingival microbiota (the biofilm). Some of these substances can directly injure host cells and tissues. Other microbial constituents may activate inflammatory or cellular and humoral immune systems that cause damage to the periodontal tissues. It is the latter pathway which accounts for most injury to the periodontal tissues.

Microbial invasion

The invasion process is considered a common strategy shared between various microbial pathogens and is thought to have evolved in order to access nutrients and shelter from host defences. *P. gingivalis* has the ability to bind and subsequently invade a range of eukaryotic cells other than oral epithelial cells, including fibroblasts, endothelial cells, and multiple cell lines, although it is not clear whether the mechanisms involved are the same for all cell types. The establishment of microbial species within an intracellular environment represents a challenging issue for extracellular and intracellular host defence mechanisms. The occupation of such a privileged niche within host cells is considered to be advantageous since the intracellular environment is not exposed to normal host immune defence mechanisms or many therapeutic agents, which represents a feature of a number of virulent organisms. It has been shown that *Aggregatebacter actinomycetemcomitans* can penetrate human epithelial cells by switching its morphological phenotype during the invasion process. Also, *Staphylococcus aureus and*

Pseudomonas aeruginosa has been reported to be exist in a high proportion of epithelial cells from periodontitis samples compared to healthy individuals, and that *E. faecalis* was the most predominant species discovered inside the oral epithelial cells of periodontitis samples.

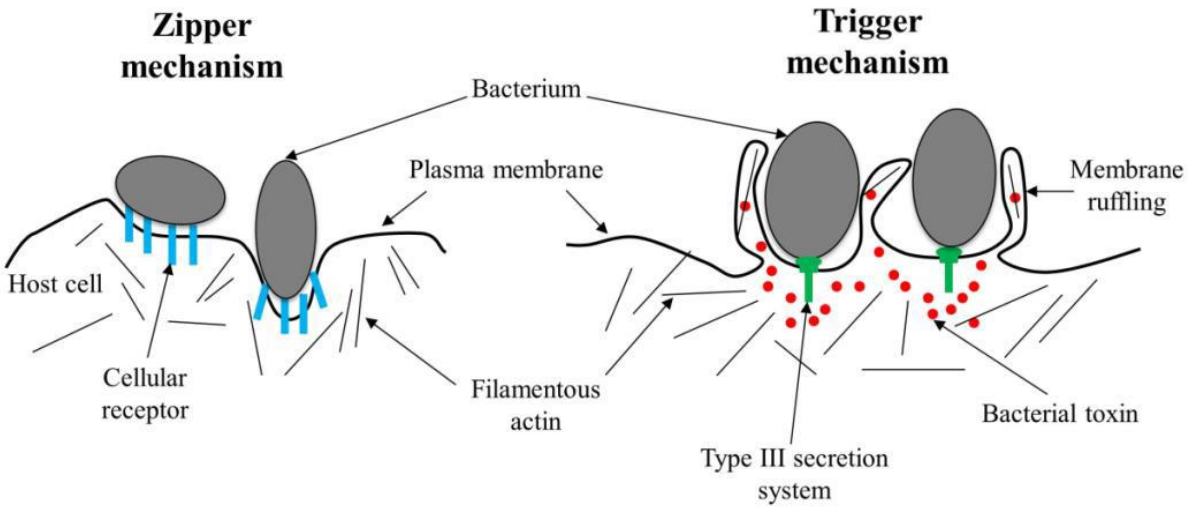
In addition, the long standing viability of *P. gingivalis* within epithelial cells and its capability for inter-cellular spreading has been attributed to its capability to invade variety of host cells including oral epithelial cells. The highly nutrient rich intracellular environment would be expected to favour bacterial survival and consequent reduction in expression of aggressive features of the bacteria should ensure host cell integrity and thus survival for a prolonged period.

The biological relevance of epithelial invasion is not completely understood. Although a higher number of internalized *P. gingivalis* has been found in crevicular and buccal cells associated with increased periodontal destruction sites, a number of those internalized bacteria were found inside crevicular cells at healthy periodontal sites.

Bacterial invasion is thought to occur by two major mechanisms; the zipper and trigger mechanisms. The **zipper** process is mediated by specific binding between certain microbial ligands and host cell surface associated receptors, which lead to clustering of the involved receptors at the membrane surface and subsequent "*phagocytic cup*" formation. Invasion is completed following activation of intracellular signalling pathways leading to actin remodelling and polymerization followed by microbial engulfment. Some microbial species are thought to use this mechanism for host cell invasion, such as *Yersinia pseudotuberculosis, Listeria monocytogenes,* and *Neisseria gonorrhoeae.*

The **trigger** mechanism uses the type 3 secretion system (T3SS) to deliver certain bacterial-associated virulence factors inside the host cell following contact with the cell surface. This results in the host-cell membrane ruffling due to direct stimulation of intracellular cytoskeletal

proteins and consequent microbial entry. *Shigella flexneri* and *Salmonella typhimurium* are thought to use this phenomenon to achieve their cellular internalization. However, it have proposed that it is unlikely that *P. gingivalis* utilizes the trigger mechanism for host-cellular entry, as it does not have a T3SS



Nevertheless, different invasion mechanisms, such as lipid raft mediated or clathrin-mediated endocytosis have been suggested as *P. gingivalis* invasion strategies rather than depending on a single mechanism of invasion.

Enzymes

Microorganisms produce a variety of soluble enzymes that may digest extracellular host proteins and other molecules and there by produce nutrients for bacterial growth. In addition to enzymes, bacteria also release numerous, harmful metabolic waste products, such as ammonia, indole, hydrogen sulfide, and butyric acid.

Amongst the enzymes released by bacteria in the biofilm, **proteases (proteinases)** are capable of digesting collagen, elastin, fibronectin, fibrin, and various other components of the intercellular matrix of both epithelial and connective tissues.

Endotoxin

Lipopolysaccharides (LPSs) of Gram-negative microorganisms are capable of triggering both the inflammatory and immune responses as they interact with host cells. Many of the functions attributed to LPS are associated with their ability to stimulate the production of cytokines. LPS also has profound effects on blood coagulation and on the complement system. The properties of LPS, as well as of lipoteichoic acids (LTAs) of Gram-positive bacteria, are numerous and may be influenced by many other molecules that interact with these outer membrane structures. LPS and LTA are produced and released from micro organisms present in the subgingival biofilm and cause release of chemical mediators of inflammation to produce vascular permeability and encourage, through chemotactic actions, inflammatory cells to move into and accumulate in the gingival tissues. Furthermore, leukocytes are stimulated to release pro inflammatory agents and cytokines.

Summary: Microbes are capable of producing a variety of substances which either directly or indirectly harm the host. The main detrimental effect may be the host's own innate, inflammatory, and immune response to the foreign molecules and antigens of the microbe.

Proteinases (proteases)

Periodontal disease results in tissue degradation, and thus proteases, derived both from the host and from bacteria, are central to the disease processes. Proteinases (collagenase, elastase-like and trypsin-like, as well as serine and cysteine proteinases) cleave proteins by hydrolyzing peptide bonds and may be classified into two major classes, endopeptidases and exopeptidases, depending on the location of activity of the enzyme on its substrate.

Proteinase inhibitors

Release of proteinases in the gingiva and the crevicular area promotes inflammatory reactions and contributes to connective tissue damage via several pathways. In contrast, proteinase inhibitors would dampen the inflammatory process. Among such inhibitors alpha-2 macroglobulin (A2-M) and alpha1 antitrypsin (A1-AT) must be recognized. In fact, gingival collagenase inhibition by A2-M has been demonstrated to occur in gingival tissues and poly morphonuclear leukocyte (PMN) collagenase is also inhibited by A1-AT.

Many host and microbial enzymes are likely to be present in the crevice at any one time. Realizing the potentially destructive features of such enzymes, consideration should be given to the source of these enzymes, their relative proportions and the inhibitory mechanisms available within the crevice. The main enzyme activity is host derived and specific and non-specific inhibitors are plentiful within the crevice and thus enzyme activity will be localized and short-lived.

Matrix metalloproteinases (MMP)

Matrix metalloproteinases (MMPs) hydrolyses components of the extracellular matrix. These proteinases play a central role in many biological processes, such as embryogenesis, normal tissue remodeling, wound healing, and angiogenesis, and in diseases such as atheroma, arthritis, cancer, and tissue ulceration. Currently 28 MMP genes have been identified in humans, and most are multidomain proteins.

The periodontium is structurally comprised of fibrous elements, including collagen, elastin, and glycoproteins (laminin, fibronectin, proteoglycans), minerals, lipids, water, and tissue-bound growth factors. In addition there are variety of extracellular matrix components, including tropocollagen, proteoglycans, and other proteins (elastin, osteocalcin, osteopontin, bone sialoprotein, osteonectin, and tenascin). All of these matrix components are constantly in a state of turnover and thus there is much matrix enzyme activity in health, disease, and tissue repair and remodelling. It is evident that the activity of MMPs and their inhibitors is associated with tissue turnover as well as with gingivitis, destructive periodontitis and with the healing of the periodontal tissues following therapy.

The periodontal ligament is one of the most metabolically active tissues in the body, and collagen metabolism represents most of this activity. The biological reason for this activity probably relates to its ability to adapt to occlusal forces generated during function. An important feature of connective tissues in general and the periodontal ligament in particular, is the process of constant renewal of the extracellular matrix components involving MMP.

The regulation of extracellular matrix (ECM) turnover is influenced by the action of both TNF-α and TGF-β, in that TNF-α can promote the ECM degradation by enhancing expression of MMPs. This action is balanced by TGF-β which down regulates the secretion of these MMPs and enhances the production of their inhibitors (tissue inhibitor of matrix metalloproteases (TIMPs)). This idea of immune dysregulation playing an important role in periodontal disease is confirmed when considering the activity of important MMPs. It has been shown that various MMPs are present at elevated levels within the GCF of periodontitis patients, which suggests an increased local proteolytic, tissue remodelling burden. Normal homeostasis is maintained when MMPs are in equilibrium with inhibitors, the TIMPs. However, TIMPs can be degraded by *P. gingivalis* which could contribute to such dysregulation.

Cytokines

Cytokines are soluble proteins, secreted by cells, which act as messenger molecules that transmit signals to other cells. They have numerous actions which include initiation and maintenance of immune and inflammatory responses and regulation of growth and differentiation of cells. The interleukins are important members of the cytokine group and are primarily involved in communication between leukocytes and other cells, such as epithelia, endothelia and fibroblasts, involved in both immune and inflammatory processes. These molecules are released in small amounts and have a variety of actions on cells which carry the specific receptor for the particular interleukin. Cytokines are numerous, many have overlapping functions and they are interlinked forming an active network which controls the host response.

Pro-inflammatory cytokines*:* Cytokines such as *interleukin (IL)-1α, IL-lβ* and *tumour necrosis factor (TNF)-α* stimulate bone resorption and inhibit bone formation. Studies on the mechanism of IL-1 action on fibroblasts, suggest that IL-1 can act on the fibroblasts to promote cellular matrix repair or destruction.

Chemotactic cytokines*:* A series of more than 20 molecules have been identified, among which the most famous and best characterized is interleukin 8 (IL-8), which has powerful chemotactic functions for leukocytes particulary for neutrophils but also for lymphocytes and macrophages.

Lymphocyte signaling cytokines*:* T helper cells are lymphocytes within the tissues which regulate both the humoral and cell mediated immune responses via cytokines. The humoral immune response is promoted by a T helper cell type 2 (TH-2) which produces characteristic cytokines namely IL-5, IL-10 and IL-13. The TH-1 lymphocytes release IL-2 and interferon (IFN)-γ which enhance cell mediated responses. These cytokines provide a precise mechanism for the control of the immune response so that a sufficient response is produced to deal with the offending pathogen.

Cytokines can influence the immune response through determining the class of immunoglobulin being produced, which may have quite a profound effect on antibody function. For example IgM molecules are more effective at bacteriolysis and IgG molecules are more effective at opsonization. The IgG antibodies exist as four distinct subclasses (IgG1, IgG2, IgG3 and IgG4) based on differences in the Fc portion of these molecules. The antibody subclass influences antibody function, IgG2 being less strong in binding antigen than IgG1. Several researchers have found IgG2 to be elevated over IgG1 in patients with severe periodontitis and propose that IgG subclass levels are important factors in susceptibility to periodontitis.

Prostaglandins

Prostaglandins are arachidonic acid derivatives which are important mediators of inflammation. The pro-inflammatory cytokines are capable of inducing macrophages and other cells to produce copious amounts of prostaglandins, particularly PGE2 which are potent vasodilators and inducers of cytokine production by various cells. PGE2 acts on fibroblasts and osteoclasts, together with cytokines, to induce MMP production, which is relevant to tissue turnover and in the destructive process in periodontitis. Many studies have examined the association of PGE2 with periodontal disease and suggest that its concentration in gingival crevicular fluid increases in gingivitis relative to health and is at very high concentrations during periods of periodontal disease progression.

Polymorphonuclear leukocytes (PMNs)

The PMN is the predominant leukocyte within the gingival crevice/pocket in both health and disease. PMNs from the circulation are attracted to the area via chemotactic stimuli elicited from microorganisms in the biofilm, and histologically PMNs can be seen traversing the gingival connective tissue in inflammation. Migration of leukocytes from the vessels into the gingival connective tissue, and through the junctional epithelium into the gingival crevice, is controlled via *adhesion molecules*. Cellular migration involves three main structures: the endothelial cells, the cell adhesion molecules and the extravasating cells. Adhesion of leukocytes appears to be essential in controlling cellular traffic into inflamed areas and it has been proposed that cytokines may play an important role in regulation of this traffic.

Host defense processes

Host–parasite reactions can be divided into innate (non-specific) and adaptive (specific) responses. Innate reactions include the inflammatory response and do not involve immunological mechanisms. Adaptive reactions that include immunological responses tend to be very effective as the host response is specifically "tailored" to the offending pathogen(s).

THE INNATE DEFENSE SYSTEMS

Innate immune mechanisms operate without any previous contact with the disease-causing microorganism. These mechanisms include *physical barriers* of the oral mucosal epithelial surfaces, *vascular* and *cellular* aspects of the *inflammatory responses*. The *epithelial surface is* the first region of the periodontium which comes into contact with and responds to bacteria attaching and colonizing the dento- gingival region. Prevention of attachment and colonization is important for the host defenses. The oral mucosa itself is not simply a barrier but has a chemical composition which may be detrimental to bacteria. Furthermore, the cells of the epithelium can respond to the bacteria by (1) producing and/or releasing cytokines and other molecules that kill the microbes and (2) releasing other molecules (such as IL-1) capable of inducing or enhancing the inflammatory reaction. The epithelium can also respond by increasing expression of surface molecules such as cell adhesion molecules which can function with cytokines and chemo attractants to bring leukocytes to the region.

The major functions of the innate immune system include:

- Acting as a physical and chemical barrier to infectious agents.
- Recruiting immune cells to sites of infection, through the production of chemical factors, including specialized chemical mediators, called cytokines
- Activation of the adaptive immune system through a process known as antigen presentation
- Activation of the complement cascade to identify bacteria, activate cells, and promote clearance of antibody complexes or dead cells
- Identification and removal of foreign substances present in organs, tissues, the blood and lymph, by specialized white blood cells

Adaptive immune system

The **adaptive immune system**, also known as the **acquired immune system** or, as the **specific immune system**, is a subsystem of the overall immune system that is composed of highly specialized, systemic cells and processes that eliminate or prevent pathogen growth. The adaptive immune system is one of the two main immune strategies found in vertebrates (the other being the innate immune system). Adaptive immunity creates immunological memory after an initial response to a specific pathogen, and leads to an enhanced response to subsequent encounters with that pathogen. This process of acquired immunity is the basis of vaccination. Like the innate system, the adaptive system includes both humoral immunity components and cell-mediated immunity components.

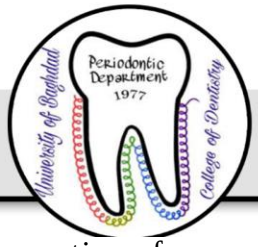
Unlike the innate immune system, the adaptive immune system is highly specific to a particular pathogen. Adaptive immunity can also provide long-lasting protection: for example; someone who recovers from measles is now protected against measles for their lifetime but in other cases it does not provide lifetime protection: for example; chickenpox. The adaptive system response destroys invading pathogens and any toxic molecules they produce.

Functions

Acquired immunity is triggered when a pathogen evades the innate immune system and (1) generates a threshold level of antigen and (2) generates "stranger" or "danger" signals activating dendritic cells.

The major functions of the acquired immune system include:

- Recognition of specific "non-self" antigens in the presence of "self", during the process of antigen presentation.



- Generation of responses that are tailored to maximally eliminate specific pathogens or pathogen-infected cells.
- Development of immunological memory, in which pathogens are "remembered" through memory B cells and memory T cells

The humoral immune response

Humoral immunity, also called the antibody-mediated beta cellularis immune system, is the aspect of immunity that is mediated by macromolecules (as opposed to cells) found in extracellular fluids such as secreted antibodies, complement proteins, and certain antimicrobial peptides. Humoral immunity is so named because it involves substances found in the humours, or body fluids.

Humoral immunity refers to antibody production and the accessory processes that accompany it, including: Th2 activation and cytokine production, germinal center formation and isotype switching, affinity maturation and memory cell generation. It also refers to the effector functions of antibodies, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination.

B cells

The principal function of B cells is to make antibodies against soluble antigens. B cell recognition of antigen is not the only element necessary for B cell activation (a combination of clonal proliferation and the terminal differentiation into plasma cells).

B cell activation depends on one of three mechanisms: *T cell-independent* (polyclonal) activation, *T cell-independent* activation (in which mature B cells respond to highly repetitive structures causing cross-linking of the B cell receptors on the surface of B cells), and *T cell-dependent activation*. During T cell-dependent activation, an antigen presenting cell (APC)

presents a processed antigen to a T helper cell (T_h), priming it. When a B cell processes and presents the *same* antigen to the *primed T_h cell*, the T cell releases cytokines that activate the B cell.

The large amounts of soluble and accessible antigens occurring in the periodontal environment require the involvement of host defense systems different from those involved in cell-mediated immunity. Specific antibodies (immunoglobulins), occurring in fluids such as plasma or gingival crevicular fluid, have the ability to bind to the antigen. This type of host defense is called *humoral immune response*. It is important to consider antibody function, i.e. the ability of an antibody to opsonize bacteria and to bind strongly to fimbriae and hereby prevent bacterial colonization. By the process of binding to the antigen, the antibody activates different effectors systems, e.g. *complement*. The activation of the complement system in turn mediates PMN and macrophage migration and phagocytosis. The process in which the antibody contributes to the elimination of antigens by enhancing phagocytosis is termed *opsonization*. Several studies suggest that assessments of the titer and avidity (the binding strength) of a patient's antibody to various microorganisms in the subgingival biofilm may be useful in the differential diagnosis and classification of periodontal diseases. Antibodies of different subclasses have different properties. Thus, IgG2 antibodies are effective against carbohydrate antigens (LPS) whereas the other subclasses are mainly directed against proteins. The proportions of plasma cells producing IgG and IgA subclasses were similar to the proportions of these immunoglobulin subclasses in serum. IgG1-producing plasma cells were predominant (mean 63%) in the gingival fluid; followed by IgG2-producing plasma cells (23%), while IgG3 and IgG4-producing cells were present in much smaller numbers (3% and 10% respectively).

The cell mediated immune response

Cell mediated immunity is an immune response that does not involve antibodies, but rather involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.

Cellular immunity protects the body by:

- T-cell mediated immunity or T-cell immunity : activating antigen-specific cytotoxic T cells that are able to induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;
- Activating macrophages and natural killer cells, enabling them to destroy pathogens.
- Stimulating cells to secrete a variety of cytokines that influence the function of other cell involved in adaptive immune responses and innate immune responses.

Cells of the adaptive immune system, e.g. T and B lymphocytes have also been implicated as effectors in the pathogenesis of periodontitis. Macrophages and dendritic cells secrete cytokines that aid in migration and activation of these lymphocytes. Upon activation by antigen presenting cells, T lymphocytes proliferate and differentiate into subsets, including T helper (Th) cells, cytotoxic T cells and regulatory T cells (Treg). Th cells can be further separated into Th1, which secrete interferon gamma (IFN-γ), IL-2, IL-12, TNF-α and TNF-β leading to the eradication of intracellular pathogens; Th2 which secrete IL-4, IL-5, IL-6, IL-9 and IL-13 stimulating antibody production by B cells and contributing to the eradication of extracellular pathogens and T17 cells which are pro-inflammatory and pro-resorptive. Treg cells secrete IL-10 and TGF-β, which are anti-inflammatory cytokines. Alterations in the Th cell population subsets may lead to disease progression. For example, a Th1 response has been reported to result in ‘stable’ periodontitis and a Th2 response may result in disease progression, possibly due to the activation of B cells. B cells and their differentiated subtypes, plasma cells, which secrete antibodies, have been found to be the most prominent immune cell type within periodontal lesions.