

Oral Defence Machanism

Pradeep Christopher¹, H.C. Girish², Sanjay Margod²,
Shymala Karnam² and Poorna. R Devadoss³

¹Department of OMFS, TMDCH, Chennai, India.

²Department of Oral Pathology, RRDCH, Bangalore, India.

³Department of Orthodontics, RRDCH, Bangalore, India.

doi: <http://dx.doi.org/10.13005/bbra/1561>

(Received: 25 September 2014; accepted: 16 November 2014)

The human mouth is one of the main routes of entry into the body for foreign microorganisms. During the usual course of daily living the host may be invaded by microbes possessing various harmful qualities, or the host may acquire breaks in its defenses, or may undergo operative procedures. In these conditions, the microorganisms find themselves in inadequately protected tissues because of break in local barrier. As tissues are injured and microbes increase, a variety of signals in the host brings about mobilization and local accumulation of protective factors and these are generally sufficient to contain the pathogens, prevent their dissemination, and allow healing to proceed promptly. The purpose of this review is to study both innate and immunologically-mediated defense systems in the human mouth and to review extensively the functions of these various defense mechanisms in protecting the host from colonization with microorganisms and cancerous cells with particular emphasis on the oral cavity and its immediate surroundings.

Key words: Microorganisms, local barrier, pathogens, Immunologically mediated defense, colonization.

Health is not a static condition, it is a dynamic state in which the living and functioning organism as tissue remains in balance with a constantly changing environment. These changes in the environment provoke corresponding alterations in tissue activity so that normal function can continue. This constant process of readjustment to maintain normal tissue activity, normal function and ultimately the continuity of life is known as *homeostasis*. If an environmental change is so great that homeostasis cannot be maintained, the activity of the tissues becomes abnormal. Normal function cannot be continued and the change in tissue activity is perceived as disease.¹

Human beings are subjected to various infections, some mild, others severe. Although infections are often self-limiting, many require the attention of the clinician. In establishing an infection, there is interaction among three factors: the host, the environment and the organism. In a state of homeostasis, a balance exists among these three; disease occurs when an imbalance exists.²

It has been well established that host defense mechanisms are the major factor in determining the outcome of an infection, the environment and the microbe playing important but usually secondary roles².

Oral cavity in particular faces a lot of challenges from external environment. A number of mechanisms operate to protect the oral cavity from attack by foreign bodies and toxins, including microorganisms. These protective mechanisms have been discussed in this review.

* To whom all correspondence should be addressed.
Mob.: 98417 59105;
E-mail: p_ortho@yahoo.com

Review of literature

Bacteria constitute an important part of our environment, indeed life without bacteria would be impossible. Usually all external surfaces in nature including those of living tissues are covered by bacteria. The skin and gut are no exceptions and the oral mucosa as part of the gut is covered by many species of bacteria, the oral flora. Bacteria attach themselves to surfaces by a number of means, by the microscopic roughness of the surface, by the hair like extensions on the surface of the bacteria and by natural glues made up of proteins and polysaccharides as in the glycoprotein.¹

Various mechanisms protect the oral cavity from attack by foreign bodies and toxins, including microorganisms. These protective mechanisms have been discussed in this review under following titles¹:

Non specific mechanisms

- a. Bacterial balance.
- b. Surface integrity.
- c. Surface fluids and enzymes.
- d. Phagocytic cells and the complement system.

Specific protective mechanisms

- a. Humoral immunity
- b. Cell mediated immunity.
- c. Hyper sensitivity reactions.

Though many or most of these defense mechanisms are interdependent, they have been reviewed under specific headings to permit ease of explanation and clarity of understanding with particular emphasis on the role of saliva and gingival crevicular fluid.

Non specific protection mechanisms

Bacterial balance

The mouth as a whole and various zones in the mouth, including what has been called the crevicular domain can be viewed as ecosystems in which a balance exists between the different species of micro-organisms and between the flora and tissues. Most of the mechanisms involved in microbial interference are not well defined but include interference of microbial binding to epithelial cells, competition for nutrients and release of by products that are toxic to other microbes.¹

Surface integrity

The surface integrity of skin and mucous

membrane barriers, including the gingival, is maintained by the continuing renewal of the epithelium from its base and desquamation of the surface layers which maintains the constant thickness of epithelium. The efficiency of the surface barrier is enhanced by keratinization and parakeratinization and by the secretory and drainage capabilities.¹

Surface fluids and enzymes

All vital surfaces are washed by fluids which are products of surface glands and which contain enzymes capable of attaching foreign material. In addition to mechanical lavage, the saliva functions as a part of the oral immune system which contains both specific immune components such as secretory iga and non specific immune components such as lysozyme, peroxidase and lactoferrin.³

The crevicular fluid contains immunoglobulins such as IgG, IgG and IgG in addition to complement components such as C3, C4, C5 and C3 pro-activator. It also contains glycoproteins, lipoproteins in addition to lysosomal enzymes, proteases, lysozyme and hyaluronidase. Crevicular fluid also contains macrophages, and t and b cells which migrate from the underlying blood vessels.³

Phagocytic cells and the complement system

Certain cells in the blood stream and in the tissues are capable of engulfing and digesting foreign material. The two most important phagocytic cells are polymorphonuclear leukocyte and the macrophage.³

The polymorphonuclear leukocyte protects the body against acute invasion and has the ability for amoeboid movement and can pass through capillaries and through tissues, including the gingival connective tissue and epithelium. The direction of their movement is determined by tissue damage products which are chemotactic.³

Macrophages are cells which start life as a monocyte and swells in the tissue to become an efficient phagocyte which is capable of digesting large foreign particles. The macrophages also take up antigens in the circulating fluid for presentation to the lymphocytes.³

Phagocytosis is aided by a battery of nine related proteins known as complement which act by immobilizing the bacteria or toxins, so that phagocytes can act more effectively in disposing

foreign matter.³

Specific protective mechanisms

This includes the immune system which can protect the body from bacteria, viruses and even cancer cells. This system has characters which can distinguish between self and nonself so that it does not attack parts of itself. The immune system contains elements specific against antigens.³

This is possible because each antigen bears on its surface specific chemicals which the immune system uses to recognize non self. The immune system on first contact with antigen produces a primary response in which uneducated lymphocytes proliferate and mature, and the antigen is memorized so that further contact provokes an immediate response.³

The specific immune system has two basic components

- a) Humoral immunity
- b) Cell mediated immunity which is separate but interdependent.

Humoral immunity is mediated through b-lymphocyte which has receptors on its surface which can recognize a specific antigen and promotes b-cell proliferation to become plasma cells which in turn produces large quantities of special proteins called immunoglobulins which act as anti-foreign bodies or antibodies. There are five types of immunoglobulins - IgA, IgD, IgE, IgG And IgM.

Cell mediated immunity is mediated by T-lymphocytes which is in circulation constantly in blood and lymph. This takes up organisms already active in the body and which have been taken by surface of a macrophage. They can produce special chemicals called lymphokines which can inhibit the activity of macrophages or activate them.³

The term 'human system' could be used to mean the systemic circulation, the various organ systems and viscera excluding the skin surface, its appendages and the body tracts and apertures that are usually colonized by commensal microorganisms. Colonization of this sterile human system by microorganisms, toxins or cancer cells constitutes a part of the spectrum of disease.

The defenses that the body lays down against such an invasion depend on the following factors.

1. Integrity of the skin and mucous membranes [both structural and functional]

2. Quantity and composition of saliva, gingival crevicular fluid, sweat, tears, mucosal discharges and other body fluids.
3. Lymphoid aggregates acting as traps at strategic locations in the body [for e.g. Inner and outer Waldeyer's ring]
4. Blood components towards specific and nonspecific immune reactions and the process of inflammation.
5. Specialized host cells to recognize, trap and inactivate foreign microorganisms and cells.

Role of saliva

The recognition of the protective function of saliva had its basis in the discovery of a variety of antimicrobial properties in its secretion. The mucosal secretions share a general function in the protection of epithelial tissue against harmful effects. Some of the functions are:⁴

Protective functions:

- 1) Tissue coating
- 2) Lubrication
- 3) Humidification
- 4) Remineralization of the teeth

Host defense functions

- 1) Immunological activity
- 2) Anti-bacterial activity
- 3) Anti-viral activity
- 4) Anti-fungal activity

Digestion

- 1) Digestive enzymes
- 2) Bolus formation

Taste

Human whole saliva contains a number of antimicrobial agents which are either synthesized in the salivary glands or leak into the mouth from blood, usually via gingival crevices. The glandular antimicrobial factors include secretory IgA, salivary peroxidase and histidine rich polypeptides where as lysozyme, lactoferrin and IgM may originate from both saliva and gingival fluid. Salivary IgG is almost of crevicular origin.^{5,6,7} Along with these antimicrobial agents phagocytic cells, mainly polymorphonuclear leukocytes enter the oral cavity through gingival crevices and can release considerable amounts of myeloperoxidase, lysozyme and lactoferrin.^{7,8,9,10}

Non immunoglobulins, innate factors

- 1) Lysozyme
- 2) Lactoferrin

Salivary peroxidase system [enzyme-SCN-H₂O₂]

Myeloperoxidase system [enzyme-SCN-/Halide- H_2O_2]

Agglutinins [parotid saliva glycoproteins, mucins, sialic lysozyme, H_2O_2 -microglobulin, fibronectin]

Histidine-rich polypeptides

Anionic antimicrobial proteins

Phagocytic cells

Immunoglobulins, acquired factors

- 1) Secretory IgA
- 2) IgG
- 3) IgM

Salivary components have been shown to interact selectively with bacteria to form a salivary-bacterial pellicle. Bacteria, when they enter the mouth are immediately coated with a number of specific salivary proteins.¹¹ This prevents the microbial adhesion to host surfaces.^{12, 13}

These bacteria are clumped and are easily swept away from the oral cavity by swallowing. The conservation of a salivary component binding to a bacterium depends not only on the affinity of the molecule for the bacterial surface but also for its abundance in saliva.¹²

These form an effective antibacterial system for the regulation of oral bacterial colonization.¹¹

Immunoglobulins, acquired factors

Immunoglobulins in saliva

The immunoglobulins such as IgG And IgM are usually of crevicular origin.^{5,6}

Secretory IgA is the predominant immunoglobulin found in saliva secreted by salivary glands and by specific cells, the B lymphocytes.⁵

The S IgA is made up of two four chain units of IgA, one secretory component and one joining chain where as serum IgA exists largely as a monomer.¹³ this immunoglobulin provides protection to the mucosal surfaces of the oral cavity from microbial colonization and penetration, primarily by replication of IgA secreting cells lining these sites.¹ IgG is the most important circulating antibody and forms up to 80% of circulating antibodies found in serum. IgG binds to cell surface receptors of polymorphonuclear leukocytes, monocytes and lymphocytes via the fc region, in addition to complement fixation.¹

IgM is the first antibody to appear as the primary response to an antigenic stimulus. IgM is

active in complement fixation and a potent agglutinator of particulate antigens and cellular antigens and also binds to surface receptors.

These immunoglobulins neutralize antigens from viruses, toxins and enzymes and interact with other innate immune factors of saliva and help in disposal of toxins and antigens.

Non immunoglobulins, innate factors

Lysozyme and lactoferrin

Lysozyme also known as muramidase with a molecular weight of 14 Kda was first recognized by Fleming in 1922 for its antibacterial effect.¹³ It is a widely occurring enzyme in many human secretions such as tears, nasal secretions, saliva, and gastric secretions as well as in many invertebrates to form the primitive defense system.¹³ Lysozyme is able to cleave β [1-4]-Glycosidic bonds between muramic acid and n-Acetyl Glucosamine residue in the peptidoglycan of the bacterial cell wall. Major part of the lysozyme is known to be derived from oral leukocytes which migrate from gingival crevices.^{5, 6, 7, 9}

Several theories have been proposed to explain the non-enzymatic bacteriological activity of lysozyme which includes binding of lysozyme to bacterial cell wall which may activate bacterial autolysins, inhibitors of bacterial adherence, aggregation and metabolism.¹³ Lactoferrin has a molecular weight of 75kda and is synthesized by glandular acinar and epithelial as well as inflammatory cells. It is a glycoprotein possessing two sialic acid containing n-linked oligosaccharide units per molecule. Lactoferrin binds two atoms of iron per molecule, with the simultaneous binding of two molecules of bicarbonate. Lactoferrin acts as an antimicrobial agent with its ability to sequester iron. In addition few lactoferrin [apolactoferrin] may also possess a direct, iron dependent, bactericidal effect. A variety of gram positive and gram negative bacteria are susceptible to actions of apolactoferrin which requires direct binding of the protein to the bacterial surface.^{12, 14}

Myeloperoxidase

Myeloperoxidase which is found in saliva has its origin in polymorphonuclear cells which are found in substantial numbers in the oral cavity. These cells migrate from the gingival crevice and through the oral mucosa. Leukocytes are rich in myeloperoxidase and lysed cells release active enzyme into the saliva.^{7, 9}

Myeloperoxidase is an abundant protein making up about 5% of the total protein. It might utilize hydrogen peroxides to generate toxic oxidized halide derivatives or it might play an important protective role against oxygen free radicals.

Sialic acid

Sialic acid is an important structural component of salivary glycoproteins, having an essential role, as in enhancing bacterial agglutination. Sialic acid containing glycoproteins are also important structural components of the acquired pellicle and of dental plaque.⁶

Salivary peroxidases

The salivary peroxidases consist of the peroxidase enzyme, the thiocyanate ion [SCN^-] and hydrogen peroxide. The enzyme is found in secretions such as tears, milk and saliva and is able to inhibit bacterial growth, catalyzes the oxidation of SCN^- by H_2O_2 generating highly reactive, oxidized forms of thiocyanate such as OSCN^- . These products have a direct toxic action on a variety of microorganisms. Salivary peroxidases can also neutralize the deleterious effects of hydrogen peroxide produced by a number of oral microorganisms, reduce acid production by glucose stimulated dental plaque and inhibit glucose uptake by *S. mutans*.^{6,7}

Salivary peroxidase system also interacts with other defense factors such as lysozyme, lactoferrin. It has also been demonstrated that peroxidase system can also bind with IgA, IgG and IgM antibodies but the combination of IgA-peroxidase has more enhanced antimicrobial action than other combinations.

Salivary agglutinins

Saliva in mediating agglutination, gathers up unattached bacteria to quicken their clearance from the oral cavity. Several studies have suggested that saliva induced bacterial agglutination is calcium dependent while others have shown no dependency for divalent cations. It seems that parotid agglutination requires calcium, while the submandibular-sublingual agglutinins do not. There is no doubt that multiple components in saliva can agglutinate bacteria. It is important to point out however that the salivary molecules that agglutinate bacteria may also serve as receptors of bacterial adhesion to host surface. It has also been suggested that agglutinins may influence

pathogenesis of dental caries. Submandibular saliva from caries resistant subjects was found to be more capable of agglutination of *S. sanguis*, while the same saliva was less capable of promoting bacterial adhesion to saliva-coated hydroxyapatite [SHA]. Thus the saliva of caries-resistant individuals may be more capable of cleaning bacteria from the oral cavity and as a result, these subjects may be less prone to plaque formation.¹²

Saliva from subjects with high levels of indigenous *mutans streptococci* did not aggregate these bacteria or foster as much adhesion to *mutans streptococci* to hydroxyapatite as the saliva from those with low level of these bacteria. Saliva from subjects with low levels of *mutans streptococci* better aggregate the bacteria, suggesting a protective role for salivary agglutinins.¹²

Histatins

Histatins are a family of small basic peptides characterized by a high content of histidine. At least seven members one of which is phosphorylated have been identified in human saliva; these vary in size from 3 to 5 Kda. These molecules, which are present in acquired enamel pellicle and inhibit the precipitation of calcium phosphate salts, have been shown recently to display bactericidal and fungicidal activities. Histatins can inhibit the development of *Candida albicans* from the noninfective to the infective germinated form.¹²

Histatins are secreted mainly in parotid and to a lesser extent in submandibular saliva. Twelve salivary histatins have been isolated from human saliva and their primary structure has been determined. Histatins possess antimicrobial properties against a few strains of *Streptococcus mutans* and inhibit hemagglutination of the periodontal pathogen *Porphyromonas gingivalis*. In addition to this, histatins also neutralize the endotoxic lipopolysaccharides located in the outer membranes of gram negative bacteria which may be an important part of the host defense. Histatins are also potent inhibitors of the growth and germination of *Candida albicans* and its efficiency could be comparable with synthetic antibiotics like imidazole and clotrimazole. Histatins are also involved in formation of acquired pellicle and participation in mineralization dynamics of oral fluids. Histatins are also capable of inhibiting release of histamine from mast cells suggesting

that they play a role in inflammation.¹³

Cystatins

Cystatins were first identified by immune-electrophoresis as the double component and later as cysteine containing phosphoproteins because of the presence of half cystine and O-linked phosphate now recognized to be members of the cystatin super family. At least seven cystatins are present in human saliva; they differ slightly in molecular weight [14-15 Kda] charge and degree of phosphorylation. Their ability to complex with mucins may serve to target cystatins to various surfaces, where they may play a role in remineralization, demineralization processes and suppress the growth and protease activity of oral pathogens.¹²

The levels of cystatins in saliva are comparable to plasma. Cystatins are one kind of endogenous proteinase inhibitors to regulate protein metabolism and to protect tissue from proteolytic attacks by bacteria or viruses. Cystatins may also regulate the activity of cathepsins liberated during inflammatory reactions. Cystatins are important in the inhibition of several viruses presumably by blocking necessary cysteine proteinases. Another function of cystatins is the control of the proliferation and invasion of human cells. Cystatins also bind to hydroxyapatite and therefore may play a role in acquired pellicle formation.¹³

Defensins

Defensins, a subfamily of homologous antimicrobial peptides constituting an important component of innate immunity found predominantly in vertebrates, are among the proteins expressed at the highest levels in the oral mucosa.¹⁵ Recent understanding of innate immunity indicates that in addition to providing a first-line of defense against invading organisms, innate immune mechanisms also trigger the adaptive immune response.¹⁶ defensins are expressed in gingiva, tongue, salivary glands, and mucosa.^{17, 18}

They are present in oral inflammatory conditions, oral carcinomas, and some cell lines derived from oral carcinomas.¹⁹

Salivary antioxidant system

The salivary antioxidant system has an essential anticarcinogenic role in the oral cavity, aimed at fighting ROS and reactive nitrogen species

(RNS) caused by smoking, alcoholic beverages, food, carbonated drinks, dental restorations and/or various other volatile sources freely entering the oral cavity through the body's largest open gate—the mouth.⁵

The salivary antioxidant system includes various molecules and enzymes. The most important are the uric acid molecule and the peroxidase enzyme; both are water-soluble. Uric acid contributes approximately 70% of the total salivary antioxidant capacity.⁶

An animal model showed the anticarcinogenic capability of saliva significantly inhibits the initiation and progression of oral cancer.⁷

In a study using the AMES test, saliva inhibited the mutagenicity of oral cancer inducers: cigarette smoke and 4-Nitroquinoline 1-Oxide (4nqO).⁸ Saliva also plays an important role in preventing cigarette-induced deoxyribonucleic acid (DNA) damage.⁹ This antioxidant capacity of saliva protects against oral cancers.¹⁰ However, in cases of periodontal disease formation and progression when normal cellular mechanisms are hampered, the oxidation process occurs due to an increased ROS production induced by other etiologic factors of periodontitis, such as bacterial plaque formation.²⁰

Gingival crevicular fluid

Cellular and humoral components of blood can reach the dental and epithelial surfaces of the mouth by the flow of fluid through the junctional epithelium of the gingiva.²¹ The structure and function of junctional epithelium is therefore essential in our understanding of the biological relationship between the vascular components and the periodontal structures. Junctional epithelium forms an organic attachment to the tooth and is continuous with the sulcular epithelium which extends to the gingival margin. Junctional epithelium differs from other epithelia in having two basal laminae; one attaching to the connective tissue and the other to the tooth. The epithelium lacks a differentiating pathway and has wider intercellular spaces.²¹

Recently monoclonal antibodies have been developed to a number of keratin polypeptides and these have revealed a remarkable differentiation between the junctional and sulcular epithelia. An antiKeratin antibody to stratified

epithelium reacts with the sulcular epithelium whereas another antiKeratin antibody to simple epithelium reacts almost exclusively with junctional epithelium. The difference in polypeptides between the keratins of junctional and sulcular epithelia may be related to important functional differences between the two epithelia.²¹

Studies have indicated that the flow of the fluid is secondary to the inflammation induced by microbial accumulation at the dento-gingival junction and is also argued that it is a continuous physiological process. It has been established that gingival crevicular fluid and leukocytes pass through junctional epithelium from the gingival capillaries to the tooth surfaces.²¹

Fluid components

In addition to IgG, IgA and IgM some components of complement C3, C4, C5 and C3 proactivator have been detected in gingival crevicular fluid. This suggests that both the classical and alternative complement pathway might be activated in the gingival crevice. C3 is found in converted form and the complement activation may have occurred in vivo. Crevicular fluid IgG contains specific antibodies to a number of oral microorganisms.²¹

The presence of antigens and corresponding antibodies may lead to formation of immune complexes which will activate the classical complement pathway of C1q and then C3 to release C3a, C3b and C5a. The alternative pathway of complement can also be activated in the absence of antibody, by plaque or some of its constituents. C3a and C5a initiate vascular permeability which is an essential step in passage of large sized proteins and leukocytes from the capillaries into the lamina propria and also induce chemotactic factors for neutrophils and monocytes.²¹

There are a number of other components in crevicular fluid including albumin, transferrin, haptoglobulins, glycoproteins and lipoproteins.²¹

Cellular components

Studies have revealed that neutrophils constitute about 92% of cells. The remaining cells are mono nuclear, consisting of macrophages and T & B lymphocytes. These cells constantly migrate from blood through the junctional epithelium.²¹

Studies in changes in nature of the cellular infiltrate suggest that human periodontal disease

is not mediated by a particular cell but actually by various lymphoid cell types which infiltrate at different stages of disease. Studies show that gingival tissue associated with inflammatory periodontal disease contains substantial number of lymphocytes and plasma cells which appear to become numerous with the increasing severity of disease. Lymphocytes appear to be the predominant cell infiltrate approximately five times as plasma cells.²²

Host production of cytokines and immunoglobulins in response to bacterial infection may trigger the periodontal disease progression. Elevated levels of prostaglandins and leukotrienes are detected in patients with periodontitis. These mediators are generally associated with destructive inflammation. Prostaglandins have been implicated in bone resorption in vitro. Previous studies have demonstrated that B lymphocytes and plasma cells are dominant cell types in establishing periodontitis. Presence of IgA has also been documented which have all been implicated in the destructive aspects of the disease.²³

Cytokines such as IL-1 which has been suggested to be a product of macrophage in gingival tissue is necessary for cellular differentiations and co-operation. IL-2 has also been reported in GCF suggesting activation of T lymphocytes. IL-6 which has many functional attributes of IL-1 as well as affecting B cell maturation has been shown to be elevated in periodontitis patients when compared to healthy samples.²³

Polymorphonuclear leukocytes are predominant phagocytic cells in defense against bacterial infection. Their emigration through vascular endothelium into an inflammatory site is a critical step in their protective function. Polymorphonuclear leukocytes also pass through the junctional epithelium where they enter the gingival crevice. The passage of polymorphonuclear leukocytes into the oral cavity is considered physiologic but their numbers increase with the degree of gingival inflammation. Gingival crevicular neutrophils are functionally intact and in many respects comparable with their circulating counterparts. Crevicular cells can respond to chemotactic substances, phagocytose microbes and generate superoxide radicals. Most of these activities are diminished when compared

to blood polymorphonuclear leukocytes.²⁴

Normal blood neutrophils are spherical and show limited mobility. In the presence of chemotactic substances, these spherical cells change shape, become actively mobile and assume a polarized configuration with a knoblike tail at the rear end and extensive membrane ruffling at the leading front of the cell.²⁴

CONCLUSION

The normal flora of the oral cavity presents the greatest variety of microorganisms to be found on or in the body, and it is likely that more potentially pathogenic bacteria contact the human body in the oral cavity than any other similar-sized region. These transient pathogenic organisms include the etiologic agents of most bacterial and viral diseases, yet the oral cavity survives these occasional contacts, because it is the site of many of the body's strongest defense systems against infectious disease.

Profound knowledge as to how these host defense systems operate provides a basis for clinical applications, i.e. How to combat caries causing microorganisms to obtain caries free mouth, how to enhance saliva-mediated protection against oral diseases as well as other orally transmitted infections.

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