***Immunity of periodontal disease: Lec: Dr.Raghad Fadhil***

***Innate Immunity***

 Defenses against infection comprise a wide range of mechanical , chemical , and microbiologic barriers that prevent pathogens invading the cell s and tissues of the body.

The commensal microflora (e.g. , in dental plaque) may also be important in providing protection against infection by pathogenic microorganisms *through effective competition for resources and ecologic niches and also by stimulating protective immune responses.*  If these primary defenses are breached, then the cellular and molecular elements of the innate immune response are activated.

*Innate immune response*

 Innate immunity refers to the elements of the immune response that are determined by inherited factors (and therefore “innate”), *have limited specificity , and are “ fixed, ” in as much as they do not change or improve during an immune response or as the result of previous exposure to a pathogen* . Recognition of pathogenic microorganisms and recruitment of effector cell s (e.g. , neutrophils) and molecules (e.g. , the complement system) are central to effective innate immunity . **.**

Stimulation of innate immunity leads to a state of inflammation , and an important area of periodontal research is to understand the relationship between innate immunity and periodontal disease as a chronic inflammatory disorders.

If innate immune responses fail to eliminate infection (e.g. , in the susceptible host ), then the effector cells of adaptive immune response (lymphocytes) are activated.

*Barriers and molecules of innate immunity*

1. *Saliva*

ROLE OF SALIVA IN THE HOST DEFENCE

.1 A vehicle for swallowing bacteria

2. Inhibition of attachment of bacteria

3. Bactericidal action by the peroxidase system.

4.Bactericidal action by lysozyme, lactoferrin and other Factors

5. Saliva also contains specific immunoglobulin A (I gA) antibodies to periodontal pathogens that target specific antigens and inhibit bacterial adherence. Patients with periodontal disease have elevated levels of specific IgA, as well as IgG and IgM, antibodies to periodontal pathogen s

*#Salivary Peroxidase System*

Hydrogen peroxide is constantly secreted in low concentration by bacteria, neutrophils and other host cells and is used by peroxidase to oxidize the thiocyanate to hypothiocyanous acid, which kills bacteria.

*#Lactoferrin:*

It is secreted by salivary gland, which bind iron, an important growth factor or requirement for many micro-organisms. This action is bacteriostatic rather than bactericidal.

*#Lysozyme:*

It is an antimicrobial enzyme in the saliva secreted mainly by mucous salivary glands and it degrades mucopeptides in the cell wall of gram-positive bacteria weakening the wall and causing lysis.

***II.GINGIVAL EPITHELIUM***

Gingival epithelium has four functions:

1. Epithelial cells are tightly attached to each other.

2. Keratinization to resist trauma.

3. Presence of permeability barriers.

4. The oral mucosa itself is not simply a barrier but has a chemical composition that may be harmful to bacteria. the cells of the epithelium can respond to the bacteria by (1)increasing their proliferation, by altering their cell signaling events, and by changing the cell differentiation and cell death and altering tissue homeostasis. (2) releasing molecules, such asIL-1 beta, capable of inducing or enhancing the local inflammatory reaction, and (3) releasing IL-8, a chemokine which attracts host defense cells such as neutrophils and macrophages to reduce the microbial insult )4) producing antimicrobial peptides, including (α-defensins, β-defensins) that have been found in oral saliva, the epithelium and in neutrophils. these are present in the dentogingival junction region and kill microbes. The mechanism of action against microbes and pathogens is principally attributed to the disruption of the microbial cell membrane. In addition to their role played as antimicrobials, they also serve as effective biological molecules in immune activation, inflammation and wound healing, also they are expected in the future to be used as models for designing effective oral microbial antibiotics.

***III.GINGIVAL CREVICULAR FLUID***

Gingival crevicular fluid functions are

1.Washing non-adherent bacteria and their products out of

the crevice.

2. Reducing the diffusion of plaque products into the tissues.

 3. It also carries a steady supply of inflammatory protease inhibitors and host defense agents such as complement and antibody, into the crevice.

*#COMPLEMENT#*

The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promotes inflammation, and attacks the pathogen's cell membrane. It is part of the innate immune system. The complement system consists of a number of small proteins found in the blood, synthesized by the liver, and circulate as inactive precursors. When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The end result of this complement activation or complement fixation cascade is stimulation of phagocytes to clear foreign and damaged material, inflammation to attract additional phagocytes, and activation of the cell-killing membrane attack complex. Over 30 proteins and protein fragments make up the complement system, including serum proteins, and cell membrane receptors.

Three biochemical pathways activate the complement system: the classical complement pathway, the alternative complement pathway, and the lectin pathway

The functions of complement are:

a. Chemotaxis cellular activation: Complement products released in this reaction attracts phagocytes to the site of infection e.g. C3aand C5

b. Opsonization: Once they arrive at the site of infection the complement components coat the bacterial surface and allow the phagocyte to recognize bacteria and there by facilitating the bacterial phagocytosis,e.g. C3b.

c. Cytolysis: Damage to the plasma membranes of the cells can lead to lysis of the cell by membrane attack complex.

**Pathogen Recognition and Activation of Cellular Innate Responses**

I f plaque bacteria and their products penetrate the periodontal tissues, then specialized cell of the immune system can recognize their presence by certain receptors and signal protective immune responses. Thus macrophages and dendritic cells express a range of receptors (toll like receptors) that interact with specific molecular structures on microorganisms to signal immune responses.

Thus innate immune responses are activated that provide immediate protection , and adaptive immunity is also activated .Excessive and in appropriate immune responses lead to chronic inflammation and the concomitant tissue destruction associated with periodontal disease.

*Toll like receptors(TLR):*

TLRs are cell surface receptors that recognize pathogens. found on the surface of epithelial cell, dendritic cell, macrophage and mast cells. Among the 11 human toll-like receptors identified so far, toll-like receptor-2 and toll-like receptor-4 are the most defined members involved in periodontal disease. TLR-4 recognizes lipopolyscharride (LPS) from gram-negative bacteria and functions as part of a complex of cell surface molecules, including CD14 and MD-2. Interaction of these molecules ( CD14/TLR-4/MD-2 complex ) with LPS triggers a series of intracellular events, the net result of which is increased production of inflammatory mediators (most notably cytokines) and the differentiation of immune cell s (e.g. , dendritic cells) for the development of effective immune responses against the pathogens.

 Once LPS is released from the bacteria present in the biofilm and activate toll like receptor it will stimulate inflammatory responses in the tissues, resulting in increased vasodilation and vascular permeability , recruitment of inflammatory cell s by chemotaxis, and release of proinflammatory mediators by the leukocytes that are recruited to the area. LPS in particular is of key importance in initiating and sustaining inflammatory responses in the gingival and periodontal tissues.

*Pro inflammatory cytokines:*

A number of cytokines are particularly important in innate

immune signaling, The most important pro inflammatory cytokine is interleukin 1 beta ( I L-1β), which exerts its action directly by

1. by stimulating the synthesis and secretion of other, secondary mediators such as prostaglandin E2 (PGE2) a potent vasodilator and inducer of cytokine production by various cells. PGE2 acts on fibroblasts and osteoclasts to induce production of MMPs.

2. The effect of IL-1β i s amplified via a synergistic action with other cytokines such as tumor necrosis factor alpha (TNF-α).

3. Up regulation migration of neutrophils in to the periodontium.

4. IL-1β also stimulates the secretion of the chemokine IL8, which stimulates neutrophil chemotaxis .

5. I L-1β , TNF-α and PGE2 also activate matrix metalloproteinase (MMP ) secretion from fibroblasts and osteoclasts; this may facilitate the movement of neutrophils in the connective tissues(*by providing additional space for PMN due to destruction of collagen fiber and matrix by MMP)* and thus protective innate responses but may also ultimately contribute to tissue destruction associated with periodontal disease, along with MMPs from neutrophils.

Other cytokines up regulated as a result of activation of innate immune response include I L-6, which influences the development of a number of immune cells, including B cells and dendritic cells, as well as stimulating osteoclast differentiation.

*Cells of innate immunity*

 *Neutrophils#*

They are the initial leukocytes seen in the gingiva. They exit the circulation and migrate into the junctional epithelium and gingival crevice, where they provide the first cellular host mechanism to control periodontopathic bacteria. Polymorphonuclear neutrophils are short-lived cells, and also upon interaction with pathogens and its toxins, they die in great numbers at acute inflammatory periodontal sites. Therefore, the accumulation and massive death of polymorph nuclear neutrophils are among major causes for tissue breakdown in progressive periodontitis.

***Functions of Neutrophils***

Emigration , chemotaxis and phagocytosis

***# Emigration:*** Leukocytes normally travel along the center of the lumen of the blood vessel, but in inflamed tissues the blood flow is slowed by fluid exudation and they adhere more readily to endothelial cells, the mechanism is called rolling and “margination”. When the neutrophils migrate across the endothelium it is called diapedesis.

 Neutrophils migrate from the gingival plexus to the extravascular connective tissue and then in to the junctional epithelium . At the molecular level , the interaction of adhesion molecules on endothelial and epithelial cells with integrins on neutrophils facilitates neutrophil migration

***#Chemotaxis***:

It is the directed movement of a cell along a chemical gradient. The neutrophils are attracted by chemical signals from multiple sources, e.g. chemotaxins which include compounds such as:

\* 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 particularly for neutrophils also for lymphocytes and macrophages.

\* complement fragments C5a

\*Leukotriene B4 (LT B4):It is secreted by mast cells, neutrophils macrophages.

\*bacterial products including LPS and factors released by damaged tissues.

*#Phagocytosis*

Once they arrive at the site of inflammation, the phagocytes have to recognize the infectious agent. This can be enhanced if the organism has been coated by C3b or IgG (opsonization). Or they may attach to micro-organism via their non-specific cell surface receptors. After attachment the phagocytes proceed to engulf the micro-organism by extending pseudopodia around it. Once inside, lysozymes fuse with the phagosome to form a phagolysozyme and Various killing mechanisms are activated, they are :

***A.Oxidative mechanism:*** Stimulation of phagocytic cells leads to an increase in cellular consumption of molecular oxygen, and associated with the generation of various oxygen metabolites, which are injurious to many species of microorganisms.

***B.Non-oxidative mechanisms:*** It appears to be based on the various components of the cell. Neutrophils contain three types of granules:

1. Primary granules/azurophilic granules: lysozyme, acid phosphatase.

2. Secondary/specific granules: Lactoferrin, lysozyme

3. Tertiary granules:Alkaline phosphatase, collagenase and gelatinase.

***Macrophages***

Macrophages develop from blood monocytes, which emigrate into the tissues from the blood and are triggered to develop into mature macrophages by cytokines, other inflammatory mediators, and bacterial products such as endotoxins.

Functions of macrophages includes:

1.Phagocytose and kill bacteria.

2.Remove damaged host tissue during inflammation

3. Trap and present antigens to lymphocytes for induction of immune responses.

Most of the above mentioned functions are carried out through the secretion of inflammatory mediators, including cytokines, prostaglandins, leukotrienes and complement components.

Other cells such as mast cells, fibroblasts, endothelial cells, and epithelial cells are also seen in gingival connective tissue during inflammatory response.