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Oral Defense Mechanisms.

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ABSTRACT

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.

Keywords: Microorganisms, local barrier, pathogens, immunologically mediated defense, colonization.

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INTRODUCTION

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 roles[2].

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].

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 under following titles[1]:

1. Non specific mechanisms.
    a. Bacterial balance.
    b. Surface integrity.
    c. Surface fluids and enzymes.
    d. Phagocytic cells and the complement system.
2. Specific protective mechanisms.
    a. Humoral immunity
    b. Cell mediated immunity.
    c. Hyper sensitivity reactions.

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, IgA and IgM 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 and 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.

It is the purpose of this project to review 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.

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.

DISCUSSION WITH REVIEW OF LITERATURE:

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:

- Tissue coating
- Lubrication
- Humidification
- Remineralization of the teeth

Host defense functions:

- Immunological activity
- Anti-bacterial activity
- Anti-viral activity
- Anti-fungal activity

Digestion:

- Digestive enzymes
- 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[8,9,7,10]. Some of the antimicrobial agents found in human whole saliva are[7]:

Non immunoglobulins, Innate factors

- Lysozyme
- Lactoferrin
- Salivary peroxidase system [enzyme-SCN-H_2O_2]
- Myeloperoxidase system [enzyme-SCN-/halide- H_2O_2]
- Agglutinins [parotid saliva glycoproteins, mucins, SiGA lysozyme, β_2-microglobulin, fibronectin]
- Histidine-rich polypeptides
- Anionic antimicrobial proteins
- Phagocytic cells

Immunoglobulins, acquired factors

- Secretory IgA
- IgG
- 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 conservance 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 SIgA 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 glycosamine 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[5,6,9,7]. Several theories have been proposed

to explain the non-enzymate 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[14,12].

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[9,7]. 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 thiocynate 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 thiocynate 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 fromthe 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 agglutinations 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 periopathogen 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 immunoelectrophoresis 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] β 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 (4NQ0).[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 laminas; 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 C142 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, transferring, 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]

LANGERHANS CELLS

In 1868 Paul Langerhans, a medical student interested in the anatomy of skin nerves, described a dendritical shaped cell population located in squamous epithelia of the epidermis in the suprabasal layers by using a gold chloride impregnation technique. These cells were later identical in virtually all stratified squamous epithelia of mammals. The cells represent 4% of epidermal cells in man. They are involved in receptor mediated endocytosis. Langerhans cells are also present in oral epithelium located in the stratum spinosum of keratinized and non keratinized epithelia. [25]

Although Langerhans cells were discovered more than a century ago, it has only recently been realized that they belong to a group of cells referred to as dendritic cell system [DCS]. The term dendritic has been used to denote members of this family of cells having the same characteristic morphology and immunologic features. This series of cells includes Langerhans cells possessing Birbeck granules, Interdigitating cells [IDC] within T cell areas of lymphoid tissues and so called veiled cells found in afferent lymph vessels. [25]

Langerhans cells are derived from bone marrow and migrate into the epithelium. According to some authors, Langerhans cells might also migrate from the epidermis to the lymph nodes. Although it is widely believed that dendritic leukocytes are end cells and therefore incapable of cell division mitotic figures have been demonstrated in epidermal LC as well as labeling with 3H-thymidine injected intradermally. [25]

Under electron microscopy two types of Langerhans cells can be distinguished. Type I is highly dendritic with an electron lucent cytoplasm, numerous granules and is found in the suprabasal layer. Type II shows dense cytoplasm, fewer birbeck granules and is usually located in the basal layer. [25]

Immunolabelling has established lymphocyte function associated antigen LEA-1 [CD11a] and VLA cells [CDw39]. This antigen plays an important role in the activation of T cells, together with the T cell receptor and MHC class II antigens.

Langerhans cells have been shown to be able to migrate and to handle antigens in a manner similar to that of the so called antigen presenting dendritic cells. Like APCS, LC's express MHC class II antigens at their surface, and through these molecules can present the antigen to T cells. [25]

ORAL MUCOSA AND ITS STRUCTURAL COMPONENTS

The epithelial lining, the secretion and the drainage system, the microbial flora and local humoral and cellular defenses compose the local defenses[2].

Epithelial Lining

The lining epithelial cells physically hinder the penetration of surface bacteria into deeper tissues. The importance of this function can be best illustrated in burn patients who are at greater risk for serious infections which can pass on to the deeper tissues. The mechanical barrier is further enhanced by keratin of the mucosa and by secretory and drainage capabilities of the mucous membrane and the organs lined by it. The keratin layer allows the skin to become relatively dry without injury to the deeper tissues. Dryness also limits the growth of certain organisms and is one of the factors that has been shown to limit bacterial population on the skin surface. Adherence of bacteria to epithelial cells by way of specific receptors has an important bearing on the potential pathogenicity of microorganisms[2].

Epidermal cells, mainly keratinocytes and to a lesser extent melanocytes and Langerhans cells release a variety of regulatory proteins [cytokines] which may assist local defenses by recruitment and enhancement of phagocyte functions and promotion of wound healing. Cytokines produced by epidermal cells include colony stimulating factor, interleukins, transforming growth factor, tumor necrosis factor, epidermal growth factor and fibroblast factor to name a few[2].

Secretion and drainage system

The secretion and drainage system assists host defenses by physical and chemical action. The mucociliary activity, peristaltic motion, flushing action all result in drainage and mechanical removal of bacteria. Obstruction or impairment of drainage almost always results in infection. Movement of lips, cheeks and tongue may aid further in removal of bacteria.

The chemical properties of secretions and other products of mucocutaneous surfaces are also important. Growth medium pH for the growth of certain bacteria; consequently the maintenance of pH in certain bodily secretions may be important for the control of the bacterial population. The nature and function of most of the chemical constituents of secretions are not known but few that have been identified and characterized have shown to have selective bactericidal properties. Most secretions contain lysozyme and lactoferrin which are potentially microbicidal. Lysozyme can lyse bacteria by splitting sugar from the peptidoglycan polymers of the bacterial cell wall and lactoferrin deprives bacteria of one of their vital enzyme cofactor iron. In addition to this, a peroxide mediated antimicrobial system has been described in saliva where hydrogen peroxide produced in the presence of bacteria such as streptococcus viridians, inhibits or kills other potentially pathogenic bacteria[2].

Microbial Interference

This refers to the inhibitory effect extended by one microorganism on the growth and proliferation of another. Normal mucocutaneous microflora exerts a protective influence on the host by preventing colonization by potential pathogens. This protective influence can be demonstrated clinically when antibiotics are given which leads to change in microflora caused by suppression of susceptible bacteria and the subsequent proliferation of the resistant ones.

Most of the mechanisms involved are interference of microbial binding to epithelial cells, competition for nutrients and release of byproducts that are toxic to other microbes. Secretory immunoglobulins also play a role in inhibiting the attachment of bacteria and parasites to epithelial cells[2].

Mucosal Immune system

Beneath the basement membrane of epithelium, large numbers of immunocompetent mononuclear cells are found. The B lymphocytes and plasma cells with the help of certain T lymphocytes locally synthesize IgA, IgE and small amounts of IgG and IgM. In addition, the secretory piece is locally synthesized. This becomes covalently linked to IgA and the complex appears in the secretory fluid on the mucosal surfaces as secretory IgA. The immunoglobulins in secretions, together with other local protective factors, compose an immensely important component of the first line of defense. The manner in which antigens are brought into contact with the immunoreactive cells in the subepithelial regions is not obvious. Soluble substances such as drugs can penetrate the epithelium and be absorbed into the circulation.

An assay of gingival fluid and serum showed that IgG concentration in gingival fluid was 85% of serum concentration and IgA and IgM were 72% and 75% of serum concentration respectively. Although gingival fluid levels were 15-30 percent lower than serum levels, these concentrations were found sufficient to mount an immune response. In normal mucosa IgA producing lymphoid cells predominate and IgA is the major immunoglobulin in secretions. This predominance of IgA occurs only in normal mucous membrane, because of relative concentration of secretory immunoglobulins can change during inflammation and IgG may increase dramatically during infection. Because of the large surface area of the mucous membrane, the mucosal immune system is extensive and is of greater significance for the defence of the host.

Besides IgA, IgE is also present in large quantities. This predominance of IgE in mucous membrane along with mast cells explains the frequency of immediate hypersensitivity reactions manifested[2].

Human epithelial cells establish direct antifungal defense through TLR4(toll like receptor-4) mediated signaling. TLR recognition of pathogenic microbes at mucosal surfaces orchestrates innate immune responses through the induction of chemokines and inflammatory cytokines, which coordinate the recruitment of PMNs and activation of macrophages that in turn leads to direct killing of the invading pathogens, primarily by phagocytosis. Protection against fungal invasion and cell injury can also be achieved without PMN phagocytosis of invading pathogens and does not even require physical PMN–epithelial cell interactions. Rather, PMNs protect the oral mucosa indirectly through the upregulation of epithelial TLR4, which in turn directly orchestrates the protective response against fungal infection. [26]

NEUTROPHILS, EOSINOPHILS. MAST CELLS, LYMPHOCYTES AND MONOCYTES

Metchnikoff coined the terms ‘macrophage’ and ‘microphage’ for the two main varieties of phagocytes and believed that they had a more important role in protective immunity than Ehrlich’s serum factors [i. e. immunoglobulins]. However, in 1903 Almoroth Wright demonstrated that the effector function of phagocytes is triggered by immunoglobulins- a similar state of affairs to that already described for the interaction between complement and immunoglobulin molecules. The clearance function of phagocytic cells-studied largely by the use of vital dyes-was emphasized in the definition of the ‘reticuloendothelial’ system but in recent years this misleading term has been replaced by a return to a Metchnikovian division into mononuclear phagocytes and neutrophils polymorphs. Mononuclear phagocytes are given different names in different tissues and are often referred to, collectively, as the mononuclear phagocyte system. They differ in size and morphology from neutrophils and, in addition to their role as phagocytes, are also able to present antigen to T cells. When activated, they secrete many proteins. [27]

Macrophages

Macrophages are released from bone marrow as immature monocytes and mature in various tissue locations where they reside for weeks or years. They accumulate slowly at sites of infection, respond to a variety of stimuli [including cytokines] and have considerable potential for synthesis, secretion and regeneration. Azurophilic lysosomal granules, which are more evident in monocytes than in mature macrophages, contain lysozyme, myeloperoxidase and acid hydrolases. Macrophages also possess a non-specific esterase and produce various neutral proteases, e.g. collagenase, elastase and plasminogen activator. Other secreted products include many complement components [and inhibitors], coagulation factors, fibronectin, cytokines and prostaglandins, e.g. PGE_2 and PGF_2. [27]

Neutrophils

Polymorphonuclear neutrophil leucocytes mature and are stored in bone marrow and are released rapidly into the circulation in response to various stimuli, notably bacterial infection. Neutrophils are 'end-cells' and only remain in the circulation for a few hours before they migrate into tissues where they die within 1-2 days. The functions of the neutrophils are mostly directed toward the killing and degradation of bacteria and are the major constituent of what, in the preantibiotic era, was known as 'laudable pus'. Their primary azurophil lysosomal granules contain several cationic proteins with antibacterial properties [the defensins and seprocidins] in addition to lysozyme, myeloperoxidase and acid hydrolases. Unlike macrophages, they also possess secondary specific granules which contain lactoferrin [an iron binding protein] as well as lysozyme, histaminase and transcobalamin II [a vitamin B_{12} binding protein]. Neutrophil polymorphs can also produce cytokines and are a potent source of leukotrienes, e.g. LTB_4 a chemotactic agent for polymorphs and monocytes and LTC_4, LTD_4 and LTE_4 which together constitute slow reacting substance [SRS]. They also produce prostaglandins, e.g. PGE_2 and platelet activating factor [PAF]. [27]

Once in tissue, it is postulated that neutrophils move among cells using the ICAM receptor, following a concentration gradient of IL-8, a chemotactic cytokine for neutrophils. In the gingival pocket, active neutrophils attempt microbial elimination by phagocytosis. [28]

Common features of phagocyte responses

Both kinds of cell respond to infective stimuli with the following sequence of activities: chemotaxis, target recognition, ingestion, killing and degradation.

Chemotaxis

Phagocytes exhibit directed movement along concentration gradients of chemotactic agents, e.g. anaphylatoxins [C3a, C5a], leukotrienes B4, interleukin-8 and phospholipids and peptides derived from bacteria. Neutrophils respond rapidly to these inflammatory stimuli by marginating to the walls of blood vessels, adhering to endothelial cells and exiting into sites of inflammation. [27]

Target recognition

Phagocytes can interact with targets hydrophobically or via specific sugar residues e.g. mannose and glycan, or lipopolysaccharides for which they have receptors. However, target recognition is greatly enhanced when specific antibody of class IgG and/or C3b becomes fixed to the target surface, a process called opsonization. Both neutrophils and macrophages possess Fc receptors specific for IgG1 and IgG3, Human polymorphs have 20 times as many receptors as macrophages and these receptors are scarcer on the immature monocyte. Neutrophil polymorphs also possess receptors of lower affinity for the Fc of IgA. Both cells have receptors for C3b [CRI] and C3bi [CR3 and CR4] which mediate the immune adherence phenomenon. CR3 and CR4 are members of the family of leucocyte integrins. The effective binding of complexed IgG to Fc receptors on phagocytic cells is due to a co-operative effect between adjacent IgG molecules brought together in an immune complex or other aggregated form as well as a conformational change in the immunoglobulin Fc region with the expression of a new binding site. The C3 receptor, on the other hand, only reacts with the converted C3b or C3bi fragments. C3 receptors are particularly effective in promoting the attachment of phagocytes to targets whereas Fc receptor binding induces both Phagocytosis and the respiratory burst which generates toxic oxygen compounds. [27]

Ingestion

Phagocytosis is a form of localized endocytosis and contrasts with the exocytotic process by which mast cells degranulate. It is an energy dependent process in which the plasma membrane gradually envelops the ingested particle and buds off the surface membrane internally to form a phagosome. This then fuses with lysosomal granules to form the phagolysosome in which many of the processes take place which kill and degrade the ingested material.[27]

KILLING AND DEGRADATION

Oxygen-dependent mechanisms

Phagocytes contain both oxygen-dependent and oxygen-independent mechanisms for microbial attack. Phagocytosis is accompanied by a burst of respiratory activity initiated by a membrane oxidase which reduces molecular oxygen to the superoxide ion [O_2^-]. Most of the respiratory activity takes place within the hexose monophosphate shunt which provides NADPH as a fuel for the reduction of molecular oxygen. This process, which is initiated at the cell surface, continues on the inner surface of the phagolysosome. Superoxide is converted to hydrogen peroxide by spontaneous dismutation [predominantly at the cell surface] with the production of singlet oxygen [$^1O_2$] or by the action of superoxide dismutase [SOD] [present intracellularly] giving rise to molecular oxygen. Singlet oxygen is a highly reactive and unstable molecular species which emits light as it returns to ground state. This process can be measured by the technique of chemiluminescence. Hydrogen peroxide and superoxide also interact to form another extremely reactive species- the hydroxyl radical [OH]. A major source of microbicidal activity develops in the phagolysosome when hydrogen peroxide interacts with halide [Cl^- in the neutrophils and I^- in the macrophage] in the presence of myeloperoxidase [MPO] to form hypohalite and water. Hypohalite can then further react with hydrogen peroxide to form more singlet oxygen. [27]

A variety of toxic materials are produced, therefore, during the oxidative burst. Several processes limit the spread of these toxic effects. Catalase, largely present in peroxisomes, converts hydrogen peroxide to water and oxygen; superoxide dismutase converts singlet oxygen to hydrogen peroxide, and hydrogen peroxide is also broken down by the glutathione redox system involving glutathione peroxidase. The chief extra cellular antioxidant is ceruloplasmin, which has an equivalent role to that of superoxide dismutase within the cell. Ceruloplasmin is one of several acute phase proteins whose synthesis is considerably increased by the liver following the release of interleukin-1.

Nitric oxide is another cytotoxic compound for which oxygen is a substrate. It is generated by neutrophils and some tissue cells via the action of nitric oxide synthetase [NOS] and the cofactor tetrahydrobiopterin [THBT] on L-arginine and O_2. [27]

Oxygen-independent mechanisms

Ingested microbes become exposed to the contents of lysosomal granules when these fuse with the phagocytic vesicle. In the neutral to alkaline conditions of the newly formed phagolysosome, the most active components are the families of basic cationic proteins which act against both Gram-positive and Gram-negative organisms. The defensins are cyclic peptides of 29-43 amino acids which insert into the membranes of their targets. The serprocidins are elastase, cathepsins G, proteinase G and azurocidin. Some, but not all of them, are serine esterases and their antibacterial properties are not dependent upon their enzymatic activity.

The pH drops within 10-15 minutes of phagosome-lysosome fusion. This acidic environment is itself detrimental to many microorganisms, and lysosomal enzymes become active: lysosome hydrolyses the peptidoglycan of Gram-positive cell walls and acid hydrolases digest many constituents. Lactoferrin has a bacteriostatic effect due to its ability to bind iron strongly, thus making it unavailable to bacteria.

The killing activity of phagocytes is enhanced in the presence of certain cytokines. Interferon a product of activated T cells and large granular lymphocytes, is particularly active and tumor necrosis factor- is produced by activated T cells, but also by macrophages when stimulated by certain bacterial products, e.g. lipopolysachcharide. [27]

MAST CELLS, BASOPHILS AND EOSINOPHILS

The three kinds of granulocyte: mast cells, basophils and eosinophils are distinguished from neutrophils by the differential staining characteristics of their granules. In mast cells and basophils this is due to the presence of an acidic proteoglycan; in eosinophils the characteristic granules contain several basic proteins. The basophil is a circulating cell whereas the mast cell is sessile and present throughout the body but chiefly in perivascular connective tissue, epithelia and lymph nodes. There is heterogeneity within the mast

cell population and dye binding is considerably affected by the method of fixation as well as the individual stains used. In appropriately fixed sections, the granules of mucosal and connective tissue mast cell differ in their staining properties. Mucosal mast cells have some features in common with basophils [which contrast with connective tissue mast cells], i.e. they are smaller, short lived, have chondroitin sulphate as acid proteoglycan, are resistant to the inhibitory effect of sodium chromoglycate and require T cells for their growth and differentiation. Basophils have been identified in some forms of T cell mediated immune responses, e.g. Jones-Mote or cutaneous basophil hypersensitivity, and the vagaries of fixation and staining techniques have probably caused them to be overlooked in other situations. [27]

Mast cell degranulation has a general role in immunity by regulating the progression of inflammatory cells and molecules through endothelial tight junctions whenever a local inflammatory response is required to deal with a focus of infection. This increase in capillary permeability may be partly due to the contraction of endothelial cells similar to the effect on smooth muscle fibers elsewhere. It is likely that the remarkable variation in permeability that occurs in the postcapillary venule of the lymph node is regulated by a similar process of IgE or complement mediated mast cell degranulation as mast cells are found plentifully at the cortico-medullary junction of lymph nodes, when appropriate fixation and staining methods are used. By contrast, the inappropriate activation of mast cells is one of the principal causes of allergic inflammation. [27]

TRIGGERING OF MAST CELLS AND BASOPHILS

Mast cells and basophils possess surface Fc receptors with a high affinity for IgE [Fc_gRI]. Mast cells become activated either when surface bound IgE molecules become cross-linked by antigen [or experimentally by anti IgE] or following the local release of the anaphylatoxins C3a or C5a for which mast cells also bear receptors. In either case, a complex series of events follows in which various membrane enzymes are activated, calcium ions enter the cell, and granules and their preformed mediator contents are released by exocytose. New mediators generated from arachidonic acid metabolism are released over a longer time scale, and were traditionally referred to as slow reacting substance of anaphylaxis [SRS].

The initial step involves the activation of a serine esterase followed by the activation of methyl transferases acting on the one hand, and adenyl cyclase which generates an increase in intracellular cyclic AMP and protein kinase activity, on the other. Phospholipid methylation and the action of phospholipases also lead to protein kinase activation [through the generation of diacyl glyceryl] and are associated with three other important events: the opening of membrane calcium channels and the release of intracellular calcium [the latter occurring via the generation of inositol triphosphate]; the generation of fusagenic lipids which encourage the fusion of perigranular and cell surface membranes, and the production of a supply of arachidonic acid from which various newly synthesized mediators are derived. The activation of adenyl cyclase is critical for mediator release although its inhibition does not prevent phospholipid methylation. Once calcium enters the cell it is bound by calmodulin, which increases the activity of various enzymes [including protein kinases] and promotes the process by which cytoskeletal proteins cause the contraction of microfilaments, leading to the extrusion of the granules and their contents. The anti-allergic drug sodium cormoglycate blocks mast cell degranulation and is thought to act by preventing the transmembrane influx of calcium ions. [27]

EOSINOPHILS

Eosinophils are distinguished by the striking affinity of their granules for acid or aniline dyes. They form a small proportion of peripheral blood leukocytes [1-5 percent] but are more prevalent in tissues. They probably share a common precursor with the basophil and show a later differentiation stage in the blood comparable to macrophage activation. They become more plentiful [in blood and relevant tissues] in allergic and parasitic diseases and their functions can be divided into effects on parasites and the inflammatory process.

Various factors have been identified which promote eosinophil proliferation and differentiation, e.g. granulocyte-macrophage colony stimulating factor, interleukins 3 and 5 and other eosinopoietic factors. Eosinophils also show a brisk chemotactic response to several materials liberated during the immune responses, e.g. ECF [from mast cells], C5a and certain chemokines. ECF and C5a display synergism in their chemotactic effects on eosinophils.

Eosinophils phagocytose poorly but degranulate promptly in the presence of chemotactic factors and when membrane-bound then endocytosis following triggering of their surface membrane, in contrast to the neutrophil. Eosinophils have Fc receptors for both IgG and IgE isotypes, although the later [FcRII] are of lower affinity than the IgE receptors on mast cells [FcRI] and, like neutrophils, they also possess C3b receptors. They are able to form phagolysosomes following membrane triggering but this phenomenon is much less marked than in the neutrophils, and eosinophils display only limited proteolytic activity. A prominent role of neutrophils is the intracellular digestion of microbes [e.g. bacteria] which are readily phagocytosed. Eosinophils are more effective in the extracellular digestion of infectious agents that are too large to be engulfed. [27]

EOSINOPHIL PRODUCTS

Eosinophils display an oxidative burst with generation of H_2O_2 and probably, superoxide but it is uncertain whether they produce the other more lytic oxygen radicals found in the neutrophils. Eosinophil peroxidase [EPO] is different from myeloperoxidase [MPO] but may be able to work in concert with hydrogen peroxide and iodide or chloride ions to lyse some microorganisms, e.g. Trichinella. However the major source of lytic activity in the eosinophil is the basic or cationic proteins contained within characteristic granules which are freely exocytosed during the degranulation response and are directly toxic to parasites e.g. Schistosomes, as well as to host cells.

The characteristic granules have a crystalloid core consisting largely of a major basic protein and a peripheral matrix containing other basic proteins, e.g. eosinophil cationic protein, and eosinophil derived neutrotoxin, as well as eosinophil peroxidase. Separate smaller granules contain aryl sulphatase and acid phosphatase. Eosinophil granules do not contain lysozyme. The exact location of other enzymes released by the cell, e.g. histaminases, β-glucuronidase and phospholipase D is unclear. The protein which forms Charcot-Leyden crystals in various tissues and body fluids subjected to eosinophil degranulation is a lysophospholipase which resides in the plasma membrane of eosinophils [and basophils]. Eosinophils also metabolize arachidonic acid to produce large amounts of platelet activating factor, leukotrienes, e.g. LTB_4 and LTC_4 and PG E_2. The cationic proteins and arachidonic acid metabolites derived from eosinophils contribute, together with mast cell products, to the acute and chronic phases of allergic inflammation. However, several of the other eosinophil products have an inhibitory effect on mast cell mediators, and so may be anti-inflammatory. [27]

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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