

Evasive Mechanisms of Oral Microflora

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Abstract

Oral microflora is an inherent and essential component in maintaining the oral health status of an individual. It is a necessary evil that can turn pathogenic if the oral microenvironment undergoes imbalance in homeostatic mechanisms. Thus, the oral microbiome is an intricate ecosystem where the host defense mechanisms keep in check the exuberant display of its otherwise foreign colonizers. This article presents with an overview of mechanisms that enable these microorganisms to evade the defense mechanisms in play in oral cavity.

Introduction

Biofilms are organized complex niches with a high degree of organization within which biological organisms form structured, coordinated and functional communities embedded in a self-created extracellular matrix. Oral microflora is a complex habitat containing a plethora of microorganisms such as *mutans streptococci*, *S. salivarius*, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. Bacteriocin and genotyping of most of these organisms have exhibited both horizontal and vertical transmissibility routes. The development of a climax community takes place by both allogenic and autogenic microbial succession. In allogenic microbial succession, non-microbial factors are responsible for community development where as, in autogenous succession, microbial factors play a central role [1]. Saliva is an immunological fluid rich in antimicrobial components such as agglutinin and immunoglobulins. Hence, it is quite an interesting fact that despite a variety of host defense mechanisms, the resident microflora tends to persist in complex biofilm communities with relative ease. This paper explores the evasive mechanisms developed by these microorganisms that help in their persistence within a biofilm.

Recognition of Oral Microbes

Microbes can be recognized by identification of conserved structures such as 'pathogen-associated molecular patterns'. These structures include lipopolysaccharides, peptidoglycans and DNA. Recognition of these structures is dependent on genome-encoded host receptors that allow detection of non-self entities that can activate the host defense mechanisms. This innate recognition property can be avoided by steric-shielding or modifications in the pathogen-associated molecular patterns [2].

The production of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukins-1, -8 and -12 (IL-1, -8 and -12) is an important host immune response. These cytokines aid in enhancing the bactericidal phagocytic activity, recruit the innate cellular response and dendritic cell maturation [2]. Salivary defense mechanisms exhibit antimicrobial properties in a myriad of ways [3,4]:

- Salivary immunoglobulins like sIgA, sIgG and sIgM act by inhibition of bacterial adhesion/aggregation and neutralization of viral particles.
- Lactoferrin exhibits bacteriostatic activity in lieu of its iron-binding property.
- Lysosomal enzymes lyse bacterial cells.
- Agglutinin inhibits bacterial proliferation due to its agglutination property.

e. Myeloperoxidase enzyme has bactericidal property in presence of thiocyanate/halide- H_2O_2 mechanism.

f. Complement pathway

g. Leukocytic activation

4. Mechanisms of Evasion

Oral bacteria use salivary molecules as decoy molecular receptors, hence, masking their foreign origin and assuming host-like immunological features. Immune evasion mechanisms such as variability in carbohydrates and protein antigens have been observed in different *S. mitis* genotypes. Resident microflora and oral mucosal tissues exist in a harmonious state due to constant molecular cross-talk that suppresses the inflammatory mechanisms [3].

The constant exfoliation of epithelial cells limits the microbial surface colonization. Besides this, generation of cytokines like IL-1 β , IL-6, TNF- α , GM-CSF, TGF- β and IL-8 provides the microbes with an immunological advantage. For instance, Mycobacteria inhibit T-cell activation by up regulation of IL-10 and TGF- β (transforming growth factor- β) which possess immunosuppressive properties [2]. Secretory immunoglobulin A (sIgA) forms the major antibody salivary constituent. IgA2 subclass predominates in the salivary secretion. sIgA acts by blocking microbial adherence to oral epithelium. It also opsonizes the bacteria for phagocytosis, activates alternative pathway of complement system and neutralizes few viruses [4] (Figure 1).

Periodontal diseases are manifestations of pathogenic microbial activity related to *P. gingivalis* and *T. denticola*. *Porphyromonas gingivalis* is a hemin-dependent bacterium. It acquires hemin from gingival crevicular fluid with the aid of secretory protease/haemagglutinins such as gingipain, haemagglutinins B and C. Local hemin concentration rises in the gingival crevicular fluid during periodontitis, providing a competitive advantage to these bacteria. *P. gingivalis* is capable of shifting its lipopolysaccharide structure from penta-acylated lipid A to tetra-acylated lipid, depending upon hemin concentration. This is

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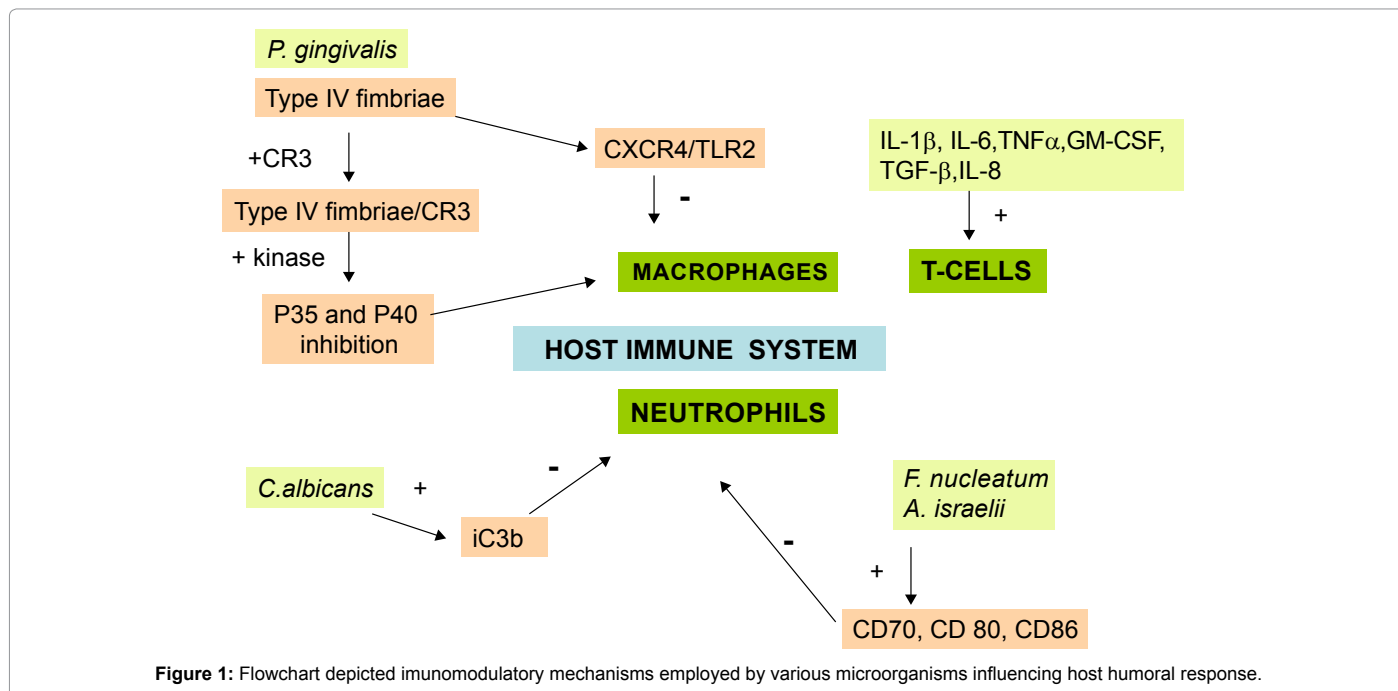


Figure 1: Flowchart depicted immunomodulatory mechanisms employed by various microorganisms influencing host humoral response.

possibly the evasive mechanism as a tetra-acylated lipopolysaccharide structure has been found to arrest the local cytokine network. Besides this, type IV fimbriae also acts as virulent contributors of periodontal disease [5]. Additionally, its fimbriae have CR3 binding property. This binding induces extracellular signal-regulated kinase 1/2 signaling which selectively inhibits the p35 and p40 IL-12 subunits. The fimbrial proteins co-ligate with the CXC-chemokine receptor 4 (CXCR4) and TLR2 contained within lipid rafts inhibiting the cross-talk and hence, phagocytic activity of the macrophages [6].

Veillonella dispar can transfer Tn916, a conjugative transposon to *Streptococcus* species in oral biofilms thus, rendering the organism tetracycline resistance. Symbiotic relationship among bacterial species such as *P. gingivalis* and *F. nucleatum* has been found to provide increased survival advantage in a biofilm community. Similarly, presence of some species can counter-inhibit growth of other species. For example, *S. salivarius* has been shown to inhibit quorum sensing and biofilm formation among mutans streptococci species. Quorum sensing controls growth of microbial community by signaling bacteria to migrate from one biofilm to another. It is mediated by molecules, such as competence stimulating peptide and autoinducer-2, which are involved in both intra- and interspecies communities among bacterial species in a biofilm. Competence stimulating peptide is involved in multiple activities such as biofilm formation, acid tolerance, antimicrobial resistance and horizontal transfer [5].

Candidal biofilms are resistant to antibiotic activity. Though the exact mechanism is unclear, the presence of biofilm matrix has been thought to act as diffusion barrier to any agent [7]. An increased prevalence of oral candidiasis in HIV affected individuals can be related to the decline in immune status and an elevation in salivary mucins. These factors provide an increase ability of mucosal adherence of *C. albicans* [8].

C. albicans has been found to activate the alternate complement pathway where it binds to both the iC3b and C3d fragments. *C. albicans* can evade host defensive mechanisms by binding iC3b which blocks neutrophilic CR3 recognition of iC3b. This inhibits the neutrophilic

Summary of strategies for bacterial evasion from host defensive Mechanisms
Prevention of opsonization
Toxin secretion
Modification of pattern molecule presentation or interference with intracellular signaling or cell trafficking
Escape from phagosomal activity
Antigen masking
Molecular mimicry
Enzymatic degradation (IgA1 protease production)
Immune suppression or immune indifference
Antigenic variation due to clonal turnover

Table 1: Strategies for bacterial evasion from host defensive Mechanisms.

phagocytosis of iC3b-coated *C. albicans*. Thus, helping in persistence of *C. albicans* in oral biofilm [9] (Table 1).

The candida antigen CR3-RR (complement receptor-3 related protein) is a 'mimicry' protein as it can bind against the α-subunit of mammalian neutrophilic CR3 receptor (CD11b/CD18) aiding in virulence and host evasion strategies [10].

Fusobacterium nucleatum and *Actinomyces israelii* enhance major histocompatibility complex II expression on oral epithelial cells while at the same time decrease surface costimulatory molecules such as CD70, CD80 and CD86. Thus, initiating a state of dormancy of the oral epithelial cells thus, maintaining the quiescent state of immune effector cells. *P. gingivalis* has been shown to promote this dormant state by IL-8 inhibition. It also inhibits phagocytic killing by via initiation of the anaphylatoxin, C5a-receptor (C5aR)-TLR2 crosstalk, CXC-chemokine receptor-4-TLR2 crosstalk and suppression of IL12 induction via the complement receptor 3-TLR2 or C5aR-TLR2 crosstalk [11,12].

Similarly, *Treponema denticola* evades the TLR9-mediated antimicrobial response by inhibiting endosomal degradation [12]. "*Bacterium actinomycetemcomitans*" was first described as a coccobacillus by Klinger (1912). It was reclassified as '*Actinobacillus actinomycetemcomitans*' by Topley and Wilson (1929) [13]. *A. actinomycetemcomitans* constitutes a major periodontopathogenic

Microorganism	Virulence factor	Role
<i>S. mutans and mitis</i>	Alterations in carbohydrate and protein antigenic moieties	Evasion of protective host inflammatory response.
<i>Porphyromonas gingivalis</i>	a. Secretory haemagglutinins b) Change in lipopolysaccharide structure c) Type IV fimbriae	a) Rise in local hemin concentration. b) Evasion of cytoprotective cytokines. c) Inhibits macrophage phagocytosis.
<i>Veillonella dispar</i>	Transposon transfer	Tetracycline resistance.
<i>S. salivarius</i>	Enhancement of competence stimulating peptide and C3d fragments	Migration within biofilm.
<i>C. albicans</i>	a) Binding to both the iC3b and c3d fragments b) CR3-RR proteins	a) Blocks neutrophilic phagocytosis. b) Binds to α -subunit of mammalian neutrophilic CR3 receptor.
<i>Fusobacterium nucleatum</i> <i>Actinomyces israeli</i>	Enhance major histocompatibility protein	Maintenance of immune quiescence.
<i>T. denticola</i>	Inhibition of TLR-9 mediated antimicrobial response	Inhibition of endosomal degradation.
<i>Actinobacillus actinomycetemcomitans</i>	a) Leukotoxin b) Fc binding protein c) 65 kDa protein d) Lipopolysaccharide e) Capsular polysaccharide antigen	a) Selective leukocytic toxicity. b) Osteoclast activation. c) Osteoclast activation and osteoblast apoptosis.

Table 2: Virulence factors of different oral pathogens.

organism responsible for periodontal diseases and in particular, localized aggressive periodontitis [14]. It is a Gram-negative, nonmotile, nonspore forming, facultative coccobacillus and is slightly curved rod with rounded ends [15]. *A. actinomycetemcomitans* possesses six serotypes (a-f) based on O-polysaccharide component of lipopolysaccharides. Virulence factors of *A. actinomycetemcomitans* can be broadly classified into three categories: a) Modulation of inflammation; b) Tissue destruction and c) tissue repair inhibition [15].

a) Immunomodulation: Leukotoxin, a member of RTX family binds to the β 2-integrin molecule present on leukocyte surface. This toxin is responsible for selective cytotoxicity. Fc-binding protein, an outer membrane protein also contributes to its immunosuppressive behaviour. A 65-kDa macromolecule protein binds to the IL-10 receptor found on macrophage cell surface, thus, modulating macrophage/monocytes activity [15].

b) Tissue destruction: Lipopolysaccharide component of *A. actinomycetemcomitans* has been found to stimulate Osteoclastic activity, thus, enhancing bone resorption. This activity is enhanced by another bone degrading protein, chaperonin 60. A serotype-specific capsular polysaccharide antigen stimulates osteoclasts formation while at the same time, inducing osteoblastic apoptosis [15].

c) Tissue repair inhibition: *A. actinomycetemcomitans* is characterized by rapid proliferation rate and quick exit from cells to infiltrate other cells. This property has been attributed to its close interaction with host microtubular assembly [15] (Table 2).

Conclusion

Oral microflora has evolved various intricate mechanisms for evading the oral defense strategies in order to maintain survival. Understanding of these evasive mechanisms are essential in development of pharmacological approaches such as gene therapy, immunomodulators etc. The increase in prevalence of hospital acquired infections and immuosuppressive states require a deeper understanding of these evasive mechanisms at physiological levels. This paper overviews these mechanisms in an attempt to enlighten and update a researcher in this area of interest.

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