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Microbial and Host Factors That Affect Bacterial Invasion of the Gingiva

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S. Ji[1] and Y. Choi[2]

Abstract

Periodontitis is a chronic inflammation of the periodontium caused by the loss of homeostasis between subgingival biofilms and susceptible hosts. Bacterial invasion into the gingival tissue and persistent infection are major events that lead to chronic inflammation. The intratissue bacterial communities are as complex as the subgingival biofilms and can also form biofilm-like structures, which will serve as a reservoir for local and systemic infections. The epithelium forms physical, chemical, and immunological barriers against invading microbes. Nevertheless, many bacterial species can invade the gingival epithelium through transcellular and paracellular pathways. In addition, both genetic and environmental factors of the hosts can affect epithelial barrier functions and thus bacterial invasion of the gingiva. In this review, current evidence for the bacterial invasion of the gingival tissue in periodontitis has been summarized, and the microbial and host factors that determine bacterial invasion of the gingiva have been reviewed.

Keywords: periodontitis, bacterial virulence, epithelia, risk factor(s), genetic predisposition to disease, metagenomics

Introduction

Periodontitis is a chronic inflammation of the periodontium caused by the dysbiosis of subgingival biofilms in susceptible hosts (Sanz et al. 2017). Accumulation of plaque in the subgingival space leads to changes in the structure and composition of the bacterial community, such as the following: reduced proportions of health-associated species, increased Gram-negative proteolytic absolute anaerobes, and increased bacterial diversity (Sanz et al. 2017). Among the increased species, *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola*, and *Aggregatibacter actinomycetemcomitans* have long been considered major pathogens. However, in vitro challenge of neutrophils and oral keratinocytes, the 2 major host cell types that interact with subgingival bacteria, with various oral species showed that periodontal pathogens induce lower levels of inflammatory or tissue-destructive mediators from host cells than nonpathogens (Appendix references 1–2). We recognized the tissue-invading ability of the periodontal pathogens and proposed that bacterial invasion of the gingival tissues and persistent infection are the major events that lead to chronic inflammation (Ji et al. 2015). In this review, we have reevaluated our model incorporating recent advances in the field. Evidence for the bacterial invasion of the gingival tissue in periodontitis has been updated, and microbial and host factors that determine bacterial invasion of the gingiva have been reviewed.

Bacterial Invasion of Gingival Tissue in Periodontitis

During the past 5 y, several groups have examined bacterial infection in periodontitis-affected gingival tissues using diverse methods, including Gram staining, in situ hybridization using digoxigenin-labeled probes, in situ hybridization using fluorescence-labeled peptic nucleic acid probes, and immunohistochemistry using antibodies specific to each bacterial species or virulence factors (Table). Regardless of the methods used, *P. gingivalis, A. actinomycetemcomitans, T. forsythia, T. denticola*, and *Fusobacterium nucleatum* were detected intracellularly in the junctional/sulcular epithelium and extracellularly in the disrupted epithelium and the area of inflammatory infiltration of the lamina propria (Mendes et al. 2016; Listyarifah et al. 2017; Baek et al. 2018; Engstrom et al. 2018; Rajakaruna et al. 2018). Two of 3 studies that compared the presence of bacteria in periodontitis-affected tissues with that in control tissues reported increased bacterial infection in the periodontitis tissues, but 1 study reported comparable levels of *P. gingivalis* and *A. actinomycetemcomitans* in the periodontitis tissues compared with the healthy tissues (Choi et al. 2014; Listyarifah et al. 2017; Engstrom et al. 2018). The discrepancy in results may be attributed to many factors, including differences in the targeted species, the sensitivity of the detection method,

[1]Department of Periodontology, Institute of Oral Health Science, Ajou University School of Medicine, Suwon, Republic of Korea
[2]Department of Immunology and Molecular Microbiology, BK21 CLS, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Republic of Korea

A supplemental appendix to this article is available online.

Corresponding Author:
Y. Choi, Department of Immunology and Molecular Microbiology, BK21 CLS, School of Dentistry and Dental Research Institute, Seoul National University, 101 Daehak-ro, Jongno-gu, Seoul, 110-744, Republic of Korea.
Email: youngnim@snu.ac.kr

Table. Studies That Investigated Bacterial Invasion in Periodontitis.

Author	Methods and Target Bacteria	Study Samples	Major Findings
Choi et al. 2014	ISH using a universal probe	Healthy sites ($n = 10$) and periodontal lesions ($n = 10$) from patients with chronic periodontitis Korean	Sulcular/junctional epithelium: 90% of healthy individuals and 100% of patients with periodontitis Oral epithelium: 20% of healthy individuals and 70% of patients with periodontitis Entire CT from sulcular to oral epithelium: 20% of healthy individuals and 50% of patients with periodontitis Higher levels in lesions than in healthy sites
Moutsopoulos et al. 2015	Gram staining	Periodontal lesions of LAD-1 patients ($n = 3$)	No bacteria within gingival tissues but strong widespread staining for LPS
Mendes et al. 2016	PNA-FISH using Pg-specific probe, Aa-specific probe	Periodontitis ($n = 5$) Portugal	Epithelial cells (intracellular): only Pg detected CT (near to neutrophils): both Pg and Aa detected
Listyarifah et al. 2017	IHC using anti-Td CTLP Ab	Gingivitis ($n = 5$) Periodontitis ($n = 10$) 9 Finish and 6 Indonesian	None in gingivitis 100% in periodontitis Localized to sulcular epithelium (intracellular) and granulation tissue (extracellular)
Rajakaruna et al. 2018	IHC using anti-Pg mAb, anti-Tf mAb	Chronic or aggressive periodontitis ($n = 82$) Japanese	Extracellular: Pg in 67% and Tf in 85% Intracellular: Pg in 28% and Tf in 52% Localized to epithelium (both extracellular and intracellular) and CT (inflammatory and endothelial cells)
Engstrom et al. 2018	IHC using anti-Pg mAb	Healthy individuals ($n = 15$) Periodontitis ($n = 15$) Swedish	60% of healthy tissues 73% of periodontitis tissues Comparable levels between healthy and periodontitis groups Localized to epithelium and CT (leukocytes, fibroblast-like cells, endothelial cells)
Baek et al. 2018	ISH using Fn-specific probe, Pg-specific probe	Periodontitis ($n = 10$) Korean	Both Fn and Pg detected in 100%. Most abundant at the area of inflammatory infiltration but observed also at the likely-to-be past battlefield.

Aa, *Aggregatibacter actinomycetemcomitans*; Ab, antibody; CT, connective tissue; CTLP, chymotrypsin-like proteinase; Fn, *Fusobacterium nucleatum*; IHC, immunohistochemistry; ISH, in situ hybridization; LAD-1, leukocyte adhesion deficiency-1; LPS, lipopolysaccharide; mAb, XXX; Pg, *Porphyromonas gingivalis*; PNA-FISH, peptide nucleic acid fluorescence in situ hybridization; Td, Treponema denticola; Tf, *Tannerella forsythia*.

differences in nationality/ethnicity (Appendix reference 3), and small sample sizes. The sample size in most studies was quite small except the study by Rajakaruna et al. (2018), but the variations in the aforementioned factors hamper to combine or compare the results of different studies. Bacterial invasions of gingival tissue by *P. gingivalis* and *T. denticola* have been also shown in the murine models of periodontitis by in situ hybridization using digoxigenin-labeled or fluorescence-labeled probes (Appendix references 4–7). Within the limitations of currently available approaches, it seems clear that bacterial invasion of gingival tissue is a common phenomenon.

Characterization of the intratissue bacterial communities obtained from periodontal lesions by high-throughput sequencing revealed several important findings (Baek et al. 2018). First, the intratissue communities were as complex as the plaque communities. Second, *F. nucleatum*, particularly subspecies *animalis*, and *P. gingivalis* were highly enriched in the tissue, composing 15% to 40% of the total bacteria. Furthermore, biofilm-like structures containing extracellular polymeric substances were observed in the tissue. The presence of complex bacterial communities in the form of biofilm-like structures within the gingival tissues is not surprising. Because bacteria exist as biofilms composed of hundreds of species in the subgingival space, the tissue-invading bacteria are more likely to be in the form of multispecies aggregates rather than a planktonic single cell. The enrichment of *F. nucleatum animalis* and *P. gingivalis* suggests better adaptation of these species to the intratissue environment, including resistance to clearance by the host immune system. The role of the complex community in inflammation needs to be further studied. Bacteria embedded in the extracellular polymeric substances resist phagocytic killing (Appendix reference 8). The intratissue biofilm-like structures may account for the chronic nature of periodontitis.

Bacterial invasion of gingival tissues in periodontitis also provides an important link for the association of periodontitis and systemic diseases. *P. gingivalis* was detected in and isolated from atherosclerotic plaques (Rafferty et al. 2011). *P. gingivalis* was also identified in the brains of patients with Alzheimer disease, the levels of which correlated with tau pathology (Dominy et al. 2019). Two groups presented *P. gingivalis*–infected endothelial cells by immunohistochemistry (Engstrom et al. 2018; Rajakaruna et al. 2018). The intratissue bacterial communities will serve as a reservoir for infection, and bacteria will migrate to remote sites by entering the circulatory system directly through endothelial cells or via the lymphatic systems.

Microbial Factors That Govern Bacterial Invasion of the Gingiva

Bacterial invasion of the gingival tissues may be initiated by either transcellular or paracellular invasion of the junctional and sulcular/pocket epithelium.

Factors Involved in Transcellular Invasion

Oral bacteria can invade gingival epithelial cells depending on the species and strain. Periodontitis-associated bacteria, including *P. gingivalis, T. forsythia, T. denticola, F. nucleatum*, and *Prevotella intermedia*, had greater invasive abilities than *Streptococcus sanguinis* and *Veillonella atypica* (Ji et al. 2010). In addition, as shown with *F. nucleatum*, bacteria with invasive abilities can transport noninvasive bacteria into epithelial cells via coaggregation (Edwards et al. 2006). This finding suggests that accumulation of periodontitis-associated bacteria will lead to increased transcellular invasion of the epithelium. Interstrain variations in the invasive capacity were also found among diverse strains of *P. intermedia* and clinical isolates of *P. gingivalis* (Baek et al. 2015; Appendix reference 9). Interestingly, the invasion index of *P. gingivalis* had strong positive correlations with the clinical parameters of subjects who harbored the isolates (Baek et al. 2015), but this observation should be accepted with caution considering the small case number.

Bacterial invasion into epithelial cells involves the interaction between various molecules of bacteria and host cells. Binding of the major structural protein of fimbriae (FimA) of *P. gingivalis* to β1 integrins expressed on the surface of oral epithelial cells induces the phosphorylation of the focal adhesion protein paxillin, indicating initiation of signal transduction through integrin and integrin-mediated cytoskeletal rearrangements (Yilmaz et al. 2002). In addition to FimA, the involvement of gingipain proteases (Park and Lamont 1998), the HAD family serine phosphatase SerB (Tribble et al. 2006), and the components of the Clp proteolytic complex ClpC and ClpXP (Capestany et al. 2008) in the internalization process of *P. gingivalis* have been shown. *T. forsythia* invasion into epithelial cells involves binding of the surface protein BspA to host cells (Inagaki et al. 2006). The host cell receptor for BspA is not known. The binding of *T. forsythia* NanH sialidases to sialyl Lewis sugars also mediates epithelial cell attachment and invasion by *T. forsythia* (Frey et al. 2018). Invasion of *T. denticola* into epithelial cells depends on lipid raft–mediated processes, and involvement of the chymotrypsin-like protease dentilisin has been shown (Inagaki et al. 2016). FadA, a unique adhesin of oral *Fusobacteria*, mediates *F. nucleatum* binding and invasion of epithelial cells (Xu et al. 2007). In endothelial cells, vascular endothelial-cadherin was identified as the endothelial receptor for FadA (Fardini et al. 2011); however, its receptor on gingival epithelial cells has not been discovered. Receptor-mediated endocytosis of *P. intermedia* involving type C fimbriae has been shown (Appendix reference 9). Interestingly, pretreatment of eukaryotic cells with the *P. intermedia* surface protein AdpF increased the adhesion and invasion of *P. intermedia*, suggesting a triggering effect of AdpF that seemed to be internalized by endocytosis (Sengupta et al. 2014).

Because the junctional and sulcular epithelia are composed of multiple cell layers, intracellular survival and spreading to neighboring cells are critical for internalized bacteria to reach the lamina propria. To date, intraepithelial cell survival of *P. gingivalis, A. actinomycetemcomitans, T. denticola*, and *F. nucleatum* has been reported (Meyer et al. 1996; Yilmaz et al. 2006; Gursoy et al. 2008; Shin and Choi 2012). However, detailed molecular mechanisms for intracellular survival and spread to neighboring cells have been reported only for *P. gingivalis*. In primary gingival epithelial cells, *P. gingivalis* was presumed to survive in the cytosol after infection (Lamont et al. 1995). However, a recent study using advanced technologies revealed that more than half of the intracellular bacteria are rapidly trafficked into autophagosomes. Whereas these autophagic replicative niches can shield *P. gingivalis* from targeting for lysosomal degradation, the cytosolic bacteria are targeted to lysosomes by selective ubiquitin adaptor proteins, NDP52 and p62 (Lee et al. 2018). Furthermore, intracellular *P. gingivalis* can spread to neighboring cells through actin-based membrane protrusions (Yilmaz et al. 2006). According to another study using immortalized gingival epithelial cells, *P. gingivalis* is first internalized with early endosomes, and approximately half of the intracellular bacteria are then sorted to lytic compartments via autophagosomes and late endosomes. Most of the remaining intracellular bacteria are sorted to recycling endosomes to exit from infected cells to neighboring cells (Takeuchi et al. 2011). Whether the intercellular translocation of *P. gingivalis* in primary gingival epithelial cells also involves the recycling endosome pathway needs to be clarified. The Clp system protein ClpB of *P. gingivalis* has a role in intracellular survival (Capestany et al. 2008).

Factors Involved in Paracellular Invasion

Bacteria can also invade the gingival tissues by penetrating the intercellular space in the junctional and sulcular/pocket epithelium. Epithelial cells are tightly joined to each other by intercellular junctions, including tight junctions (TJs), adherens junctions (AJs), and desmosomes, providing a physical barrier to invading microbes (Takahashi et al. 2019). The junctional epithelium with large intercellular spaces and decreased levels of E-cadherin AJs has been considered a relatively weak barrier to invading bacteria (Lee et al. 2016). A negative correlation between the levels of the TJ protein ZO-1 and bacterial invasion of the gingival tissue has been shown in the murine model of periodontitis and periodontitis patients, supporting the role of the paracellular route in bacterial invasion (Choi et al. 2014; Appendix reference 4). The mechanisms for paracellular bacterial invasion can be classified into 2 categories: direct destruction of intercellular junctions and modulation of junctional protein expression.

The periodontitis-associated microbial communities are characterized by the ability to use abundant proteins as nutrients; thus, many periodontal pathogens produce potent proteases (Sanz et al. 2017). *P. gingivalis* gingipains can directly degrade the AJ protein E-cadherin and the TJ proteins occludin and junctional adhesion molecule 1, resulting in increased paracellular permeability (Takeuchi et al. 2019; Appendix reference 11). The chymotrypsin-like protease dentilisin of

T. denticola also degrades the TJ proteins ZO-1 and claudin 1 (Kikuchi et al. 2018). These results suggest that bacteria-derived proteases can cleave junctional proteins and facilitate the paracellular invasion of bacteria.

Another way to loosen intercellular junctions is to modulate the expression of junctional proteins. Incubation of the human gingival epithelial monolayer with *P. gingivalis*–lipopolysaccharide (LPS) decreased the E-cadherin gene and protein levels, which increased the penetration of LPS across the monolayer (Abe-Yutori et al. 2017). In addition, butyrate, a metabolite of anaerobic bacteria, severely impaired the gingival epithelial barrier by triggering pyroptosis and downregulation of diverse junctional proteins (Liu et al. 2019). In contrast, 10-hydroxy-cis-12-octadecenoic acid, a metabolite produced by several *Lactobacillus* strains, suppressed the *P. gingivalis*–induced degradation of E-cadherin in vitro and in a mouse periodontitis model by facilitating posttranslational modifications of E-cadherin (Yamada et al. 2018). These results suggest that not only bacterial components but also metabolites play a role in the regulation of epithelial barriers. Recently, the *P. gingivalis* LPS-induced impairment of the epithelial barrier was shown to be mediated by targeting grainyhead-like 2 (GRHL2), an epithelial-specific transcription factor. Importantly, Grhl2-knockout mice presented decreased expression of claudins, ZO-1, and β-catenin, which allowed increased translocation of oral bacteria to the blood (Chen et al. 2019).

In addition to opening intercellular junctions, the motility and chemotaxis of bacteria will help them pass through intercellular spaces. Indeed, *T. denticola* that lacked flagella (*flgE*-mutant) completely lost the ability to penetrate tight-junctioned keratinocyte cell layers, and mutants of chemotaxis receptors (CheA, DmcA, and DmcB) also presented substantially reduced penetrating abilities (Lux et al. 2001).

Functional Characteristics of Periodontitis-Associated Microbial Community and Factors Involved in Invasion

The functional characteristics of the periodontitis-associated microbial community revealed by community-wide transcriptome analysis of the subgingival microbiome reflect the microbial factors involved in invasion. Iron acquisition, LPS synthesis, and flagellar synthesis were reported as major activities that define periodontitis compared to healthy gingiva (Duran-Pinedo et al. 2014). As mentioned earlier, the LPS and flagella-dependent motility of bacteria are important in paracellular invasion. Overexpression of the ClpB protease in *P. gingivalis*, NanH sialidases in *T. forsythia*, and homologs to internalins, surface proteins used by *Listeria monocytogenes* to invade mammalian cells, in *T. forsythia* and *T. denticola* were also observed. All of these suggest increased transcellular invasion of bacteria in periodontitis. Lipid A biosynthesis, ciliary and flagellar motility, and chemotaxis genes were also part of the signature activities at the initial stages of the progression sites (Yost et al. 2015). During periodontitis progression, virulence factors involved in chemotaxis and lipid A biosynthesis were overexpressed in the whole community, and members of the orange complex also upregulated the genes involved in cell adhesion, pilus assembly, and proteolysis. Yost et al. (2015) also reported upregulation of putative virulence factors by species that have not been associated with periodontitis. Among them, *Pseudomonas fluorescens* upregulated all genes associated with flagellar synthesis and chemotaxis-related genes at the initial and progression stages of periodontitis. Collectively, the functional characteristics of the subgingival biofilms at progression sites also reflect bacterial invasion.

Host Factors That Govern Bacterial Invasion of Gingiva

The epithelia provide the first line of defense against invading microbes. Therefore, host factors that affect the physical, chemical, and immunologic barrier functions of the gingival epithelium govern bacterial invasion of the gingiva. The physical barrier function of the gingival epithelium depends on the stratified structure and aforementioned intercellular junctions, including TJs, AJs, and desmosomes (Takahashi et al. 2019). The chemical barrier function is provided by diverse antimicrobial peptides and proteins secreted by epithelial cells, leukocytes, and salivary glands (Appendix reference 12). The immunologic barrier function is provided by neutrophils that migrate through the junctional epithelium to the gingival sulcus and dendritic cells and T cells that are located within the epithelium (Appendix reference 13). Both genetic and environmental factors can affect the epithelial barrier functions as described below.

Genetic Risk Factors That Affect Epithelial Barrier Functions

Congenital diseases that accompany severe periodontitis were described in our previous review (Ji et al. 2015). Most of them are associated with defects in the number or function of neutrophils, including congenital neutropenia, type I leukocyte adhesion deficiency, defective polymorphonuclear leukocyte formyl peptide receptor, Chédiak-Higashi syndrome, and Papillon-Lefèvre syndrome. In addition, the lack of the antimicrobial peptide LL-37 in neutrophils and saliva is associated with the early periodontal destruction observed in patients with Kostmann syndrome (severe congenital neutropenia) who maintain normal blood neutrophil counts by granulocyte colony-stimulating factor therapy.

During the past decade, a number of genome-wide association studies (GWAS) of periodontitis have been published. Some of the periodontitis risk genes identified from the GWAS suggest a potential link with bacterial invasion.

Offenbacher et al. (2016) reported that 80 genes at 37 loci were significantly associated with 6 periodontal complex traits (PCTs) that were defined by combining clinical criteria, the levels of 8 periodontal pathogens, and gingival crevicular fluid interleukin (IL)–1β. Many of the identified genes were associated with the immune response and epithelial barrier function.

For example, candidate genes associated with PCT1, which was defined by high positive loading with all pathogens and reflects the most common form of periodontitis, included *IFI16* and *AIM2*. Both IFI16 and AIM2 are cytoplasmic sensors of foreign DNA and are involved in the clearance of intracellular bacteria (Ulland et al. 2015; Appendix reference 14), suggesting a potential role in the transcellular bacterial invasion of the gingiva. PCT5, defined as a *P. gingivalis*–dominant trait, had significant associations with *PKP2, PNN*, and *KIEC3*, which encode the component proteins of desmosomes and AJs, suggesting an association with paracellular invasion (Offenbacher et al. 2016).

SIGLEC5 and *DEFA1A3* loci were reported to be associated with both aggressive and chronic periodontitis at the genome-wide significance level (Munz et al. 2019). SIGLEC-5 encoded by *SIGLEC5* is a cell surface lectin expressed on phagocytes that binds sialic acids. SIGLEC-5 transduces an inhibitory signal through an immunoreceptor tyrosine-based inhibitory motif and inhibits the activation of cells. Several human pathogens use inhibitory SIGLEGs to dampen leukocyte activation, and group B *Streptococcus* (GBS) binds SIGLEC-5 and escapes phagocytic killing. The function of SIGLEC-5 is balanced by SIGLEC-14, which is encoded by the adjacent gene *SIGLEC14.* SIGLEC-14 has an almost identical ligand-binding domain but signals via an activating motif. There is a *SIGLEC14*-null polymorphism, and homozygous *SIGLEC14*-null neutrophils showed increased susceptibility to GBS immune evasion (Ali et al. 2014). If periodontal pathogens can bind SIGLEC-5 and transduce an inhibitory signal, they would escape phagocytic killing, particularly in people with the *SIGLEC14*-null polymorphism. The associated genetic variants at the *DEFA1A3* locus were reported to affect the expression of *DEFA4* by expression quantitative trait locus (eQTL) analysis. The *DEFA4* gene encodes human neutrophil defensin 4 (HNP4), which presents greater potency against Gram-negative bacteria than HNP1 to HNP3 (Ericksen et al. 2005). Recently, reduced levels of *DEFA4* expression in periodontitis patients have been reported, corroborating its potential association with periodontitis (Jourdain et al. 2018). The genetic variants at the *SIGLEC5* and *DEFA1A3* loci may affect the function of neutrophils as the immunologic barrier at the gingival sulcus.

Environmental Risk Factors That Affect Epithelial Barrier Functions

Environmental risk factors for periodontitis include smoking, poorly controlled diabetes, obesity, osteoporosis, low dietary calcium and vitamin D, and stress (Genco and Borgnakke 2013). Among them, smoking, hyperglycemia, and obesity commonly induce heightened inflammatory responses, providing a link to inflammation-mediated periodontal destruction. Osteoporosis and low dietary calcium and vitamin D can accelerate alveolar bone destruction. Other potential mechanisms for each risk factor were reviewed by Genco and Borgnakke (2013), but effects on epithelial barriers were not addressed. Notably, except for osteoporosis and calcium, the other risk factors are associated with epithelial barrier dysfunction.

Cigarette smoke–induced airway epithelial barrier dysfunction has been extensively studied and recently reviewed (Aghapour et al. 2018). Cigarette smoke induces disassociation of occludin and ZO-1, reduced expression of TJ-associated proteins, and endogenous protease calpain-mediated TJ protein degradation, resulting in disrupted TJ integrity (Appendix reference 15). Cigarette smoke also induces the disruption of AJs by decreasing E-cadherin expression (Appendix reference 16). Cigarette smoke is expected to have a similar effect on the physical barrier function of the gingival epithelium.

Hyperglycemia-induced barrier dysfunction and increased permeability have been reported in the airway and intestinal epithelia. In airway epithelial cells, hyperglycemia-induced downregulation of connexin 43 mediated the decreased expression of ZO-1 and occludin, suggesting an association of epithelial barrier dysfunction with an increased risk for respiratory infection in hyperglycemia (Yu et al. 2016). The intestinal epithelial cells exposed to hyperglycemia reprogrammed the transcriptome, including ZO-1 and E-cadherin, leading to increased intestinal permeability. Importantly, inhibition of glucose metabolism or glucose transport to the intestinal epithelium restored the barrier function and protected hosts from enteric infection (Thaiss et al. 2018).

In obese patients, abnormal esophageal barrier function has been reported (Blevins et al. 2018). TJ impairments in the jejunal epithelium, evidenced by reduced expression of occludin and tricellulin and increased intestinal permeability, were also observed in patients with severe obesity (Genser et al. 2018). Increased intestinal permeability allows translocation of microbial contents to the intestinal tissue and systemic circulation, leading to inflammation. Thaiss et al. (2018) showed that hyperglycemia underlies the intestinal barrier dysfunction observed in multiple mouse models of genetic and acquired obesity. Similarly, a lipid challenge exacerbated intestinal barrier dysfunction, and the increased permeability after lipid challenge was linked to diabetic status in a human study (Genser et al. 2018), confirming the role of hyperglycemia in obesity-associated epithelial barrier dysfunction.

Vitamin D has a protective effect on epithelial barriers in diverse mucosal tissues. Vitamin D deficiency is associated with various inflammatory diseases in the lung, inflammatory bowel diseases, and increased rates of infection (Sun 2010). Vitamin D receptor (VDR)–deficient mice presented increased alveolar permeability and severe lung injuries accompanied by decreased expression of occludin and ZO-1 (Shi et al. 2016). Epithelial VDR signaling protects the integrity of the intestinal barrier by several mechanisms, including upregulation of junctional protein expression, stabilization of TJ structures, upregulation of cathelicidin (the precursor of LL-37) and β-defensins, and inhibition of inflammation-induced epithelial cell apoptosis (Sun 2010). Menzel et al. (2019) reported that treatment of oral epithelial cells with $1,25(OH)_2D_3$, the active form of vitamin D, upregulated LL-37 expression but downregulated inflammatory cytokine IL-1α. Interestingly, treatment of oral epithelial cells with $1,25(OH)_2D_3$ also reduced invasion by *P. gingivalis*, the mechanisms for which await further elucidation.

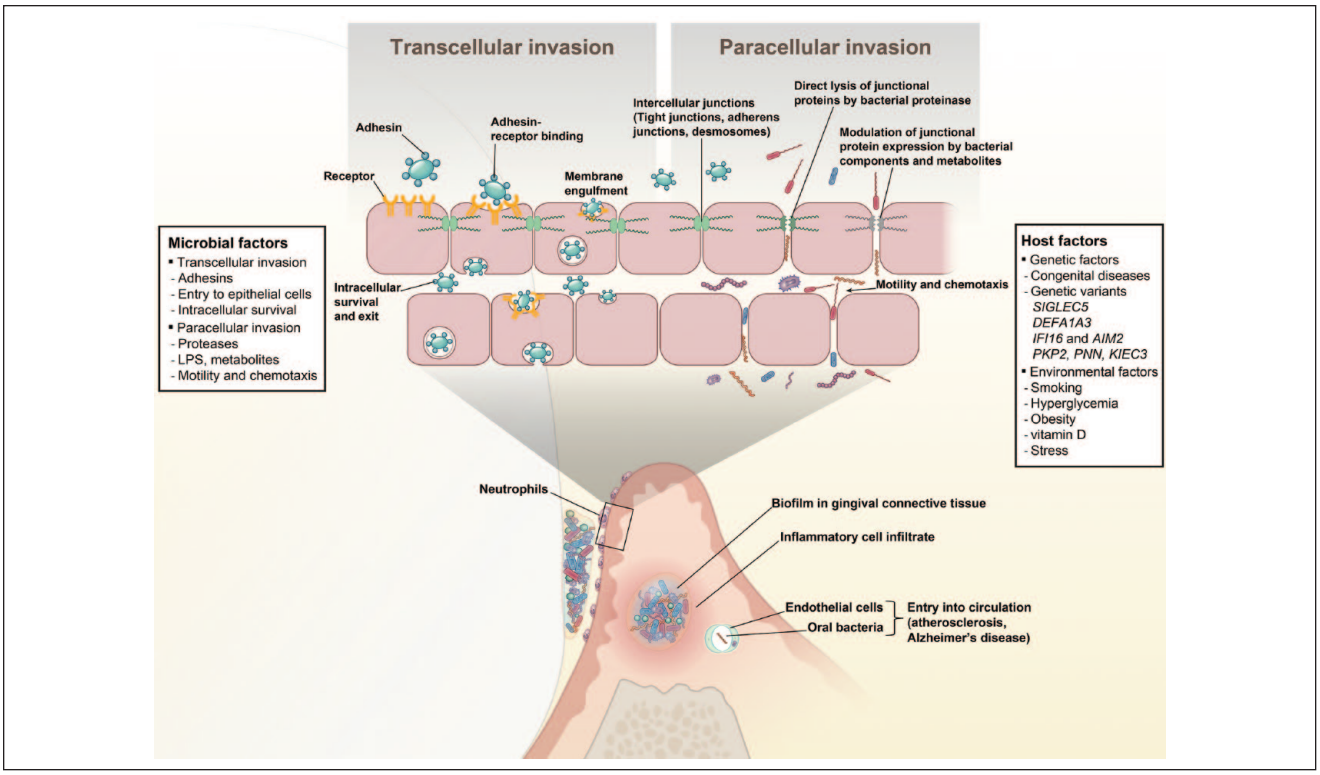

Figure. Bacterial invasion pathways and factors that affect bacterial invasion. Multiple oral bacterial species can invade the gingival epithelium through transcellular and paracellular pathways. In transcellular invasion, entry into epithelial cells is initiated by the binding of bacterial adhesins to a specific receptor on host cells. The internalized bacteria can survive in the epithelial cells and spread to the adjacent cells. Epithelial cells are tightly joined to each other by intercellular junctions, including tight junctions, adherens junctions, and desmosomes. Paracellular bacterial invasion can occur by directly destroying the intercellular junctions and/or modulating junctional protein expression. Both genetic factors, such as congenital disease and genetic variants, and environmental factors, such as smoking, hyperglycemia, obesity, vitamin D, and host stress, can affect epithelial barrier functions. The bacteria that reach the lamina propria can form biofilm-like structures and migrate to remote sites by entering the circulatory system directly through endothelial cells or via lymphatics. The intratissue bacterial community will serve as a reservoir for the source of local and systemic (atherosclerosis and Alzheimer disease) infections.

Stress in humans is associated with diverse gastrointestinal diseases, including gastroesophageal reflux disease, peptic ulcer disease, irritable bowel syndrome, and inflammatory bowel disease (Appendix reference 17). Reber et al. (2011) showed that adrenal hormone–mediated local immune suppression and impaired intestinal barrier functions are key events in a murine model of psychosocial stress-induced colitis. The stress-related increase in intestinal permeability was found to be associated with a region-specific decrease in the levels of glucocorticoid receptor and tight junction proteins in the colon (Zheng et al. 2013).

Concluding Remarks

The microbial and host factors that govern bacterial invasion of the gingiva are summarized in the Figure. In this article, the interactions between microbial and host factors are not reviewed. The bacterial communities and hosts affect each other through continuous communication. For example, smoking enriches periodontal pathogens in clinically healthy subjects (Appendix reference 18), and increased periodontal pathogens can contribute to epithelial barrier destruction (Takahashi et al. 2019). Vitamin D deficiency is known to cause changes in the gut microbiota (Appendix reference 19) and may have a similar effect on oral microbiota. The effects of environmental factors on epithelial barrier functions studied in other anatomical sites need to be confirmed in the oral epithelium. Within these limitations, the reviewed knowledge on the determinants of bacterial invasion would be useful for the development of vaccines or new therapeutics.

Vaccines that induce salivary IgA against microbial factors involved in bacterial invasion, such as adhesins and proteases, may successfully prevent periodontitis, which has been shown in a mouse model (Appendix reference 20). Considering the increasing role of periodontitis in diverse systemic diseases, the impact of successful vaccines would not be limited to periodontitis.

Author Contributions

S. Ji, contributed to design, drafted and critically revised the manuscript; Y. Choi, contributed to conception and design, drafted and critically revised the manuscript. Both authors gave final approval and agree to be accountable for all aspects of the work.

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ORCID iD

Y. Choi https://orcid.org/ 0000-0002-6496-5560

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