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Herpesvirus-bacteria synergistic interaction in periodontitis

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1 | INTRODUCTION

Periodontitis is a polymicrobial infectious disease characterized by loss of the periodontal ligament and alveolar bone. Most periodontitis starts in the third decade of life but can debut at any age.¹ Severe periodontitis is estimated to affect 8.9% of US adults² and 11% of the global population.³ Traditional (mechanical) periodontal therapy and follow-up supportive care can reduce gingival inflammation and restore partly damaged gingival tissue, but may not arrest ongoing loss of periodontal attachment.^{4,5} Differences in periodontitis progressivity and response to treatment are related to the composition of the periodontal microbiome and to levels of protective and destructive immune reactions to the infectious agents. Major pathogenic determinants of severe periodontitis are active herpesviruses, specific bacterial species, and proinflammatory cytokines.⁶

Different hypotheses on the etiology of periodontitis have been proposed during the past century.^{7,8} Prior to 1930, periodontitis was thought to be caused by four distinct microscopic groups of micro-organisms: amebae, spirochetes, fusiforms, and streptococci. In the 1940s, periodontal disease became associated with dental plaque, with little consideration for the microbial composition of the dental plaque, and periodontitis was regarded as a microbiologically non-specific disease with plaque removal as the major treatment goal. In the 1970s, with advancements in anaerobic culture techniques and bacterial taxonomy, several facultative and obligate anaerobes became associated with periodontitis. These findings led to the specific plaque hypothesis, which emphasized the role of individual bacterial species in the development of periodontitis. Based on disease association, effect of elimination, host response, virulence factors, and animal studies,⁹ oral bacteria were divided into major pathogens and low pathogenic commensals, and a consensus list of periodontopathic species was produced.¹⁰ Because periodontitis involves a polymicrobial infection in a susceptible host, in the 1990s Marsh¹¹ applied ecological principles to understand the bacteria-host association in periodontal health and disease. The ecology hypothesis recognizes that the stability of the oral microbial community depends on complex interactions among bacteria, the host, and environmental factors, and disease occurs as a

result of upsetting those relationships. The components that make up the gingival ecosystem, with differing weight, are commensal bacteria of low virulence, specific pathogenic species, environmental factors (including nutrients, redox potential, pH, and temperature), and host immune responses. Recently, Hajishengallis and Lamont¹² assigned distinct pathogenic roles to different bacterial groups and proposed a polymicrobial synergy and dysbiosis model for periodontal disease.¹² Keystone pathogens are bacteria of low abundance that exert a disproportionately high influence in the microbial community (eg, *Porphyromonas gingivalis*). Accessory pathogens are commensal bacteria that facilitate the colonization and growth of the pathogens. Pathobionts are commensal bacteria that can cause disease under conditions of dysbiosis. As a keystone pathogen, *P. gingivalis* modulates both the commensal bacteria and the host immune system, causing a dysbiotic state, which may lead to increased bacterial load, inflammation, and bone loss. Also, as described in this review, active herpesviruses may provide a particularly powerful inducement of dysbiosis with overgrowth of keystone pathogens and development of periodontitis.

During the last 20 years, periodontitis has been linked statistically to more than 50 seemingly unrelated systemic diseases and conditions, such as adverse pregnancy outcome,¹³ rheumatic fever,¹⁴ cardiovascular disease and stroke,¹⁵ dementia,¹⁶ and cancer.^{17,18} Herpesviruses, however, can infect any organ system of the body and, arguably, periodontal herpesviruses comprise the most likely linkage between periodontitis and various systemic diseases.¹⁸ Periodontitis is not a fatal disease per se, but activated periodontal herpesviruses which enter the systemic circulation may pose life-threatening disease risks, such as cytomegalovirus and herpes simplex virus inducing atherosclerosis,¹⁹ and Epstein-Barr virus causing cancer.¹⁷ Periodontal bacteria can also give rise to serious extraoral diseases, but on a relatively small scale.^{20,21}

Traditional treatment of periodontitis sought to prevent loss of teeth mainly for reasons of esthetics and general wellness, but was largely unaffordable for low-income individuals.²² However, the potential contribution of periodontitis to several systemic diseases provides a compelling argument and even a professional obligation to expand periodontal treatment to individuals of all income groups. By necessity, population-wide periodontal healthcare has to be based on inexpensive treatment with a long-lasting impact. A focus solely on bacterial biofilms tends to oversimplify the pathogenesis and management of periodontitis, and a template for a low-cost, high-performance periodontal treatment that includes herpesviruses was described recently.⁴ Periodontal herpesvirus infections in immunocompetent individuals are conceptually readily prevented and cured. Effective control of gingivitis restricts herpesviruses imbedded in inflammatory cells from entering the periodontium, and a number of basic periodontal therapies combined with systemic antiviral chemotherapy can resolve established periodontal herpesvirus infections.⁴ Maintaining a healthy periodontium depends on proper self-care, but current methods of oral hygiene require considerable patient commitment, and are often inadequately implemented.²³ Oral rinsing twice-weekly with 0.25% sodium hypochlorite (dilute household bleach) can markedly improve the subgingival microbiota and the periodontal status, even in unscaled deep pockets,²⁴ and constitutes a valuable adjunct to traditional patient self-care.^{1,23}

As clearly exemplified in localized (“aggressive”) juvenile periodontitis, herpesvirus-related periodontitis, like other herpesvirus diseases, debuts with rapid tissue breakdown followed by disease stability that may last for several years or even a lifetime, possibly interrupted by relatively minor relapses.²³ Cell-mediated immunity limits activation and local spread of herpesviruses more effectively during reactivation events than at the primary infection, and morbidity is typically less severe at disease recurrences. Because the initial outbreak of aggressive periodontitis tends to be the most severe, predisease detection of periodontal herpesviruses followed by preemptive anti-herpesvirus therapy may help avert sizable tissue destruction. Proactive intervention based on molecular identification of pathogenic agents instead of reactive treatment relying on the simple idea of measuring changes in clinical variables may significantly improve periodontal healthcare.

Herpesvirus-bacteria coinfection is associated with particularly severe diseases in humans and experimental animals,²⁵ and synergistic collaboration between active herpesviruses and bacterial pathogens may be responsible for severe periodontitis.⁶ In a 2-way pathogenetic interaction, periodontal herpesviruses may promote upgrowth of periodontopathic bacteria, and bacterial virulence factors may reactivate latent herpesviruses. The current review analyzes the morbidity of periodontopathic bacteria (*A. actinomycetemcomitans* and *P. gingivalis*) and herpesviruses (cytomegalovirus and Epstein-Barr virus), and the coinfection of these agents in the development of periodontitis. Putative virulence factors of the infectious agents are described in detail to illustrate the wide range of potential synergistic interactions. Dissecting the molecular relationships among periodontal herpesviruses and bacteria may uncover novel pathogenic determinants that can be exploited for more efficient periodontal disease prevention and treatment.

2 | BACTERIAL PERIODONTAL PATHOGENS

Aggregatibacter actinomycetemcomitans and *P. gingivalis* are key pathogens of periodontitis. The association of *A. actinomycetemcomitans*²⁶ and *P. gingivalis*^{27,28} with severe periodontitis was established in Buffalo, New York, in the late 1970s, and virulence factors and the immune response of the same bacteria were published soon thereafter in 1984.^{29,30} Studies on the pathogenicity of *A. actinomycetemcomitans* and *P. gingivalis* have continued unabated until the current time.^{31,32} *Aggregatibacter actinomycetemcomitans* is a toxin-producing species of bacteria, and *P. gingivalis* is a strongly proteolytic species. *Aggregatibacter actinomycetemcomitans* periodontitis typically exhibits minimal gingival inflammation, while *P. gingivalis* periodontitis is frequently associated with bleeding gingivitis.²⁹ Studies on the molecular biology of *A. actinomycetemcomitans* and *P. gingivalis* may further clarify the pathogenic potential and role in periodontitis of these bacteria.

2.1 | *Aggregatibacter actinomycetemcomitans*

The gram-negative facultative *A. actinomycetemcomitans* is a member of the *Pasteurellaceae* family, and 16S ribosomal RNA and whole genome sequence analyses have demonstrated a close relationship with *Aggregatibacter aphrophilus*, *Haemophilus paraphrophilus*, *Haemophilus segnis*, and *Haemophilus influenzae*.^{33,34} These species

colonize mucosa and exhibit numerous similarities in physiology, tissue tropism, host range, and virulence mechanisms. Table 1 lists the virulence factors of *A. actinomycetemcomitans* as they may relate to periodontitis.

Bacterial evolutionary divergence and heterogeneity within species occur as a result of gain or loss of genes and genomic rearrangement,^{35–42} or acquisition of genomic islands (large DNA blocks that encode multiple virulence or fitness factors) by horizontal gene transfer.^{43–47} Considerable genetic/phenotypic variability exists within the *A.*

actinomycetemcomitans species.^{34,48,49} Seven *A. actinomycetemcomitans* serotypes (a–g) have been identified based on the immunodominant lipopolysaccharide O-antigen, with serotypes a–c accounting for >80% of all clinical isolates.^{34,50,51} Whole-genome sequencing of 31 *A. actinomycetemcomitans* strains of serotypes a–f and two *A. aphrophilus* strains identified 3220 unique genes, including 1550 core genes (present in all genomes) and 1670 variable genes (missing in at least 1 genome), and 387 genomic islands, most without known function.³⁴ Variable genes make up 14%–23% of individual *A. actinomycetemcomitans* genomes. Five clades of *A. actinomycetemcomitans* were distinguished by phylogenetic analysis of the core genes: Clade b (serotype b strains); Clade c (serotype c strains); Clade e/f (serotype e and f strains); Clade a/d (serotype a and d strains); and Clade e' (serotype e strains). Strains of distinct clades may differ by as much as 20% in their genomic content. Strains of Clade e' lack the genomic island that carries genes encoding the cytotoxic distending toxin. The available data suggest a pattern of evolutionary divergence of *A. actinomycetemcomitans* through gain or loss of genes and large-scale genomic reversion, leading to variability in virulence within the *A. actinomycetemcomitans* species.

Aggregatibacter actinomycetemcomitans serotype b predominates in severe periodontitis and serotype c is associated with a healthy periodontium in the USA and Finland,⁵² which may point to strain difference in virulence potential,^{53,54} but could also reflect different dominance of genotypes in the study populations or distinct host responses to *A. actinomycetemcomitans* infections among subjects of different ethnicity.^{50,55,56} With this caveat in mind, the best-documented example of a virulent *A. actinomycetemcomitans* genotype is the serotype b JP2 clone, which is prevalent in individuals of African descent^{57–60} and is closely associated with localized juvenile periodontitis.⁶¹ The JP2 clone exhibits a 530 base pair deletion in the promoter region of the *Itx* operon and elaborates high leukotoxic activity.⁶² While the high leukotoxin activity presumably contributes to virulence, it cannot be excluded that other differences in the gene content of the JP2 strain account for its particularly high pathogenicity.⁶³

Aggregatibacter actinomycetemcomitans is not a highly transmissible bacterium. Genetic fingerprinting of *A. actinomycetemcomitans* strains has found both vertical transmission (parents to children with a transmission rate of 32%) and horizontal transmission (between adult cohabitants with a transmission rate of 36%).^{64–66} *Aggregatibacter actinomycetemcomitans* appears to colonize buccal epithelium before translocating to the tooth surface and the gingival crevice.^{67,68} *Aggregatibacter actinomycetemcomitans* elaborates three autotransporter proteins, Aae, EmaA, and ApiA, which may play roles in initial oral attachment. Autotransporter proteins function in the colonization of numerous bacterial species and possess three domains, each with a distinct function.^{69–71} The N-

terminal and C-terminal domains guide the proteins through the inner and outer bacterial membranes, allowing the central effector domain to surface on the bacterial cell and interact with host tissues. Aae mediates the binding of *A. actinomycetemcomitans* to buccal epithelial cells of humans, Old World monkeys, and several other animals, but not to the buccal epithelial cells of New World monkeys.⁷² EmaA is a homotrimeric protein that forms an antenna-like structure on the surface of *A. actinomycetemcomitans*.^{73,74} As shown by transposon mutagenesis,⁷⁵ the EmaA protein may mediate binding of *A. actinomycetemcomitans* to collagen. The sequence of the *emaA* gene is almost identical to that of *apiBC* identified in a subset of *A. actinomycetemcomitans* strains, but the translation products of *emaA* and *apiBC* appear to be different.^{75,76} The gene *emaA* is translated into one longer protein, while *apiBC* is translated into two individual proteins (ApiB and ApiC). Presumably ApiB/ApiC serve functions different to that of EmaA.^{73,77} The ApiB protein has been related to invasion of *A. actinomycetemcomitans* into epithelial cells, as deletion of *apiB* resulted in *A. actinomycetemcomitans* mutants exhibiting a 4-fold diminished capability of invading KB cells.⁷⁶ The ApiA is a 32-kDa heat-stable trimer protein which shares some homology with adhesins to epithelial cells of several bacterial species and evokes a strong serum immune response in patients with aggressive periodontitis.⁷⁶ ApiA mediates bacterial adhesion and invasion of KB cells, as deletion mutants of *apiA* have shown a 60% decrease in adhesion and invasion efficiency,⁷⁸ although not in every study.⁷⁶ *Aggregatibacter actinomycetemcomitans*, in coculture with *Streptococcus gordonii*, has demonstrated increased expression of ApiA,⁷⁹ perhaps as a result of the hydrogen peroxide production by *S. gordonii*. ApiA also binds factor H of the alternative complement pathway to inhibit C3b, thereby impeding complement-mediated killing of the bacterial cells.⁷⁹

Fresh oral isolates of *A. actinomycetemcomitans* are invariably fimbriated.^{80–83} The genes encoding the fimbria biogenesis of *A. actinomycetemcomitans* reside in a 12-kilobase 14-gene *flp-tad* operon.^{82,84–90} The operon is known as a widespread colonization island, as evidenced by its homology with that of a variety of bacterial species and its acquisition by horizontal gene transfer.⁹¹ Fimbriated *A. actinomycetemcomitans* strains exhibit a stronger tendency to form aggregates and to adhere to saliva-coated hydroxyapatite than isogenic nonfimbriated variants.^{80–83} In a rat model, fimbriated

A. actinomycetemcomitans caused persistent oral infections and alveolar bone loss, but isogenic nonfimbriated *A. actinomycetemcomitans* failed to colonize or to induce bone loss.⁹² The conversion from a fimbriated to a nonfimbriated variant occurs in vitro after repeated subculture due to the occurrence of random mutations at the promoter of the *flp-tad* operon.⁹³ The nonfimbriated *A. actinomycetemcomitans* variant has a higher growth rate than the parental fimbriated strains, and outcompetes the wild-type bacteria in vitro.⁹⁴ There is no evidence for spontaneous reversion of the nonfimbriated phenotype of *A. actinomycetemcomitans*, or the occurrence of nonfimbriated *A. actinomycetemcomitans* in vivo.

A number of bacterial species produce an exopolysaccharide polymer of beta(1,6)-linked N-acetyl-D-glucosamine residues, which mediates cell-to-cell aggregation.^{95–97} In *A. actinomycetemcomitans*, N-acetyl-D-glucosamine is encoded by the genetic locus *pgaABCD*, an operon of 4 genes.⁹⁶ *Aggregatibacter actinomycetemcomitans* N-acetyl-D-

glucosamine mediates aggregation between bacteria but may play an insignificant role in initial surface attachment and biofilm formation.⁹⁶ *Aggregatibacter actinomycetemcomitans* also produces dispersin B enzyme, which degrades *N*-acetyl-D-glucosamine to facilitate dissemination of *A. actinomycetemcomitans* cells from mature biofilm.^{96,98,99} Dispersin B can degrade *N*-acetyl-D-glucosamine-like structures in a variety of bacterial species and may serve as an antibiofilm agent.^{100,101}

Aggregatibacter actinomycetemcomitans generates a 116-kDa leukotoxin of the repeat-in-toxin family of bacterial cytotoxins.^{102–105} The genetic locus of *A. actinomycetemcomitans* leukotoxin is in a single operon of 4 genes, namely, *ltxC*, *ltxA*, *ltxB*, and *ltxD*. The leukotoxin protein is encoded by *ltxA*, and the other 3 genes encode proteins involved in the transportation (*ltxB* and *ltxD*) and post-translational modification (*ltxC*) of the toxin. *LtxA* was thought initially to be cell-associated and not secreted,^{106,107} but was later convincingly shown to be secreted abundantly into the culture medium.¹⁰⁸ As stated above, *A. actinomycetemcomitans* strains can be distinguished into JP2 and non-JP2, based on their leukotoxic activity. The former is characterized by a shorter promoter which drives the higher expression level of the leukotoxin. Another type of high leukotoxic activity is related to an insertion sequence in the promoter region of the *ltx* operon.¹⁰⁹ The mechanism of how distinct promoters lead to different expression levels of leukotoxin is not completely understood.^{110,111} The expression of leukotoxin depends also on growth conditions, nutrients, and autoinducers.^{112–116} *Aggregatibacter actinomycetemcomitans* leukotoxin binds lymphocyte-function associated antigen 1 to a CD11/CD18 dimer and kills the neutrophil cell via apoptosis.^{117–119} The involvement of lymphocyte-function associated antigen 1 explains the specificity of the *A. actinomycetemcomitans* leukotoxin for neutrophils and lymphocytes. *Aggregatibacter actinomycetemcomitans* leukotoxin can activate macrophages to secrete interleukin-1 β ^{120–122} and also exhibit hemolytic activity.¹²³ The hemolytic activity of the highly leukotoxic JP2 strains may provide hemoglobin for iron acquisition, thereby compensating for a nullmutation in *hgpA* (the gene encoding hemoglobin-binding protein).¹²⁴

Cytolethal distending toxin produced by *A. actinomycetemcomitans* is a trimeric holotoxin that belongs to the AB toxin super-family found in a diverse group of gram-negative pathogens.^{125–133} While cytolethal distending toxins from a number of bacterial species share their namesake's active toxin-binding protein structure, they differ in affinity to hostcell-surface receptors and in mechanisms of cell entry and toxicity. *Aggregatibacter actinomycetemcomitans* cytolethal distending toxin is encoded by the operon *cdtABC*. CdtB is the toxin, while CdtA and CdtC, after forming a complex with CdtB at a 1:1:1 ratio, facilitate the binding and entry of the toxin into the cells. The binding of *A. actinomycetemcomitans* cytolethal distending toxin is thought to involve N-linked fucose-containing glycoproteins, gangliosides, and membrane rafts.^{134–137} After binding, the CdtB-CdtC dimer enters the cells via endocytosis, leaving CdtA on the cell surface. The CdtB toxin traffics to the Golgi apparatus, endoplasmic reticulum, and finally the nucleus. CdtB exhibits DNase and lipid phosphatase activities, leading to DNA damage, and induces apoptosis and subsequently cell death in a variety of cell types.^{126,132,133,138–141} CdtB may also elevate the expression level of receptor activator of nuclear factor kappa-B ligand with potential for osteoclastogenesis and bone loss.^{142,143} Expression of the cytolethal distending

toxin varies among *A. actinomycetemcomitans* clonal types, with some strains devoid of the *cdtABC* operon.^{34,144} A possible correlation between cytolethal distending toxin expression and virulence of *A. actinomycetemcomitans*, or an association between *cdtABC*-negative strains and periodontal status, remains to be established.

Lipopolysaccharide forms the outer leaflet of the outer membrane of gram-negative bacteria and is composed of a highly variable O-antigen, a semiconserved core oligosaccharide, and a highly conserved lipid A.¹⁴⁵ The lipid A of *Escherichia coli*, a well-characterized prototype of the lipid A structure, consists of a phosphorylated disaccharide backbone with 4 primary acyl chains added at the C2, C3, C2', and C3' positions.¹⁴⁶ Two additional acyl chains are linked to 2 of the primary acyl chains at the C2' and C3' positions.¹⁴⁶ *Escherichia coli* lipid A, as a pathogen-associated molecular patterns molecule, is recognized by the host via toll-like receptor-4, and a proinflammatory immune is subsequently elicited. The structure of lipid A of *A. actinomycetemcomitans* is similar to that of *E. coli*, except that a C14 acyl chain, instead of a C12 acyl chain, is present on the C2'-linked primary fatty acid chain. Perhaps not surprisingly, the lipid A of *A. actinomycetemcomitans* is an agonist of toll-like receptor-4, and a strong inflammatory response elicited in murine macrophages can induce bone resorption through the induction of membrane interleukin-1.^{147–150} *Aggregatibacter actinomycetemcomitans* lipopolysaccharide also induces apoptosis of human and murine macrophages,^{151,152} interacts with toll-like receptor to initiate the mitogen-activated protein kinases c-Jun N-terminal kinase, extracellular signal-regulated kinase, and p38, and activates nuclear factor kappa-B which induces a proinflammatory response and alveolar bone loss in animal models.^{153,154} *Aggregatibacter actinomycetemcomitans* lipopolysaccharide may also function as an adhesin,^{155–157} as *A. actinomycetemcomitans* mutants lacking the O-antigen, but with an intact core and lipid A structure, exhibit reduced attachment and biofilm formation on glass surfaces.¹⁵⁷

Other less well-characterized virulence factors have been identified in *A. actinomycetemcomitans*. Nalbant et al¹⁵⁸ showed that a GroEL-like molecule of *A. actinomycetemcomitans* induced apoptosis of T cells independently of leukotoxin and the cytolethal distending toxin, as well as bone resorption in murine calvaria.¹⁵⁹ Teng and Hu¹⁶⁰ identified a cytotoxin-associated gene E protein (a homolog of *Helicobacter pylori* cytotoxin-associated gene E) that was able to induce apoptosis of human epithelial cells.

Bacterial interaction may be of importance in *A. actinomycetemcomitans* colonization. Mandell et al^{161,162} found that the levels of *Eikenella corrodens* were elevated in disease-active juvenile periodontitis lesions that also harbored *A. actinomycetemcomitans*, suggesting a synergistic relationship between these 2 species. Periasamy et al¹⁶³ showed *Fusobacterium nucleatum* strain ATCC 10953 to exhibit synergism with *A. actinomycetemcomitans* strain JP2 (serotype b), probably because of a consumption of lactic acid released by *F. nucleatum*. Rupani et al¹⁶⁴ identified *A. actinomycetemcomitans* serotype b O-lipopolysaccharide as one of the receptors that binds *F. nucleatum* (strain PK1594). *Aggregatibacter actinomycetemcomitans* produces catalase to neutralize hydrogen peroxide released by organisms such as *S. gordonii*,¹⁶⁵ and utilizes lactic acid produced by *S. gordonii*, to reduce the accumulation of hydrogen peroxide synthesized by the bacteria.

^{166,167} In vivo, however, the virulence and viability of *A. actinomycetemcomitans* is enhanced by *S. gordonii*, attesting to a complex relationship between these 2 species.^{168,169}

2.2 | Porphyromonas gingivalis

Porphyromonas gingivalis is a gram-negative obligate anaerobe that is strongly associated with severe types of chronic periodontitis. The species has undergone a series of taxonomic changes over the past half-century and was previously known as *Bacteroides melaninogenicus*, *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, or black-pigmented *Bacteroides*. The phylogeny and the taxonomy of *P. gingivalis* was finally settled by 16S ribosomal RNA sequence analysis.¹⁷⁰ The *Cytophaga-Flavobacter-Bacteroides* phylum includes the *Bacteroides* subgroup, which is divided into major clusters of *Prevotella*, *Bacteroides*, and *Porphyromonas*. *Porphyromonas gingivalis* belongs to the *Porphyromonas* cluster, and is closely related to *Porphyromonas endodontalis*, *Porphyromonas circumdentaria*, *Porphyromonas salivosa*, and *Bacteroides macacae* (later renamed *Porphyromonas macacae*). Table 2 summarizes the major virulence factors of *P. gingivalis*.

Chen et al¹⁷¹ performed comparative genomic analysis of 19 *P. gingivalis* strains and found extensive differences in genetic content. A total of 37,667 proteins were identified and clustered based on sequence homology. Approximately 1,000 proteins (designated core proteins) were shared by all strains. About half of the total proteins in each genome were noncore proteins, and the majority of the strain-specific proteins (ie, found in one genome only) were hypothetical proteins. However, there were also nonhypothetical strain-specific proteins, which may account for biologic differences among strains. The genetic diversity of the *P. gingivalis* species has also been revealed by heteroduplex and sequence analyses of the ribosomal intergenic spacer region and sequence analysis of the fimbria gene, *fimA*.^{172–176} The phylogeny of *P. gingivalis* revealed by different approaches are in general agreement, including a particularly close relationship between the strains W50 and W83, and between the strains ATCC 33277 and ATCC 381.

Porphyromonas gingivalis expresses 2 types of fimbriae, FimA (long fimbriae) and Mfa1 (minor or short fimbriae), which mediate adherence to biotic or abiotic surfaces.^{177–180} However, the names may not adequately denote the relative amounts or the lengths of the 2 types of fimbriae.¹⁸¹ The *fimA*-encoded FimA protein possesses several domains that interact with salivary proteins and epithelial cells through integrin receptors, fibrinogen, fibronectin, lactoferrin, erythrocytes, and glyceraldehyde-3-phosphate dehydrogenase of oral streptococci.^{178,182,183} Downstream of *fimA* are genes that encode FimC, FimD, and FimE, which are accessory proteins of the mature FimA fimbriae. The genetic determinant for Mfa1 fimbriae has been identified as a cluster of 5 genes (*mfa1*, *mfa2*, *mfa3*, *mfa4*, and *mfa5*).¹⁸¹ The Mfa1 fimbriae attach to SspA/B proteins of oral streptococci.^{184,185} Both FimA and Mfa1 fimbriae exhibit the capacity to exploit host immune responses.^{186–192}

Distinct *P. gingivalis* strains may exist in periodontal health and in disease. As an example, genetically discrete fimbriae of *P. gingivalis*, encoded by *fimA*, occur at different rates in periodontal health and disease. *Porphyromonas gingivalis* strains with types II, IV, or Ib FimA tend to predominate in periodontitis, while strains of *P. gingivalis* with types I and III FimA are more prevalent in nonperiodontitis sites.¹⁷⁶ Heteroduplex analysis of subgingival

plaque samples has confirmed the association between strains with type II FimA and periodontitis.¹⁹³ *Porphyromonas gingivalis* strains with types II, IV, or Ib FimA also exhibit greater virulence in a rodent subcutaneous infection model.¹⁷⁶ Recombinant type II FimA-conjugated beads exhibit greater adhesiveness and invasiveness with human epithelial cells than other types of FimA proteins.¹⁷⁶ Moreover, most clinical isolates of *P. gingivalis* are encapsulated, and 6 capsular serotypes have been identified.¹⁹⁴ The *P. gingivalis* capsule confers resistance to phagocytosis and reduces the efficacy of the host response to support in vivo survival of the species.¹⁹⁵ Encapsulated *P. gingivalis* strains were more virulent than nonencapsulated strains in a mouse abscess model.^{195,196} These observations corroborate the notion of differences in virulence among genetically distinct strains of *P. gingivalis*.

Porphyromonas gingivalis, being an asaccharolytic organism, utilizes peptides and proteins as nutritional sources, and expresses a wide array of proteolytic enzymes to acquire host tissue proteins for nutritional purposes. The best characterized proteolytic enzymes of *P. gingivalis* are gingipains, namely RgpA, RgpB, and Kgp. These 3 enzymes account for 85% of the extracellular proteolytic activity of *P. gingivalis*.¹⁹⁷ RgpA and RgpB (arginine-specific) and Kgp (lysine-specific) cleave proteins at the C-terminus. RgpA and RgpB are released after proteolytic removal of the N-terminal pro-fragment and the C-terminal domain. They share structural and sequential similarity in the N-terminal catalytic caspase-like domains, but RgpA contains additional hemagglutinin domains that are not present in the shorter RgpB. The hemagglutinin domains of RgpA are released to form a noncovalent complex with the enzyme. The Kgp enzyme has a catalytic domain that is distinct from that in RgpA and RgpB, while the hemagglutinin domains are similar to those in RgpA. After proteolytic processing of Kgp, the released catalytic and hemagglutinin domains form a multidomain complex. Gingipains can also be linked in a multidomain complex with lipopolysaccharide and other nonenzymatic components. Gingipains can degrade extracellular matrix proteins, fibronectin, laminin, collagens, fibrinogen, immunoglobulins, complement proteins, leukocyte surface receptors, and antimicrobial peptides, can activate matrix metalloproteinases and the kallikrein/kinin cascade, can bind heme and hemoglobin and release heme and iron, can expose cryptitopes to promote bacterial adhesion, can induce post-translational processing of fimbrillin and outer membrane proteins, can interact with epithelial cells, and can inactivate plasma proteinase inhibitors.^{177,178} *Porphyromonas gingivalis* mutants devoid of gingipains invariably demonstrate decreased morbidity in animal and in vitro models. Gingipains have proven to be one of the most critical virulence factors of *P. gingivalis*.

Porphyromonas gingivalis produces additional hemagglutinins, HagA, B, and C.^{177,178} These proteins mediate binding and lysis of red blood cells to release iron from hemoglobin and heme. The hemagglutinins also mediate adherence to endothelial cells and other host tissues and induce platelet aggregation.^{177,178} Intracellular *P. gingivalis* produces the serine phosphatase, SerB,^{198–200} which dephosphorylates serine 536 in the Rel homology domain of the protein P65 subunit of nuclear factor kappa-B, thereby suppressing interleukin-8 production²⁰¹ to help in paralyzing the host defense.²⁰⁰

The lipopolysaccharide of *P. gingivalis* has a distinct lipid A structure and biologic activity which differs from that of *E. coli* lipid A.^{145,202,203} Four major variants of *P. gingivalis* lipid

A exist. Lipid A varies in the number of residues phosphorylated in the disaccharide backbone (from 0 to 2) and primary acyl chains (3 or 4), with only 1 secondary acyl chain added to the acyl chain at the C3 position. The penta-acylated lipid A, either monophosphorylated or diphosphorylated, is a weak toll-like receptor-4 agonist. The tetra-acylated monophosphorylated lipid A is a toll-like receptor-4 antagonist, and the tetra-acylated nonphosphorylated lipid A is inert to toll-like receptor-4. The growth environment regulates the synthesis of different lipid A species.^{204–206} At low hemin concentration, the tetra-acylated dominant nonphosphorylated (inert) and minor components of penta-acylated lipid A (weak agonist) may elicit, at best, a relatively weak inflammatory response. At high hemin concentration, tetra-acylated monophosphorylated lipid A (antagonist) is produced, which dampens the host immune response and thereby enhances the survival of *P. gingivalis*.

The subgingival colonization of *P. gingivalis* requires the presence of early colonizers, such as streptococci. The formation of polymicrobial biofilms involving these species occurs via surface adhesins and activation of specific genes of both species.^{178,207–211} At the individual level, transmission of *P. gingivalis* between adults has been reported by Asikainen et al.⁶⁶ Vertical transmission of *P. gingivalis* occurs rarely, probably because relatively few children are able to sustain an oral *P. gingivalis* infection.^{64–66}

Porphyromonas gingivalis is a potent modulator of the host immune response. Manipulation of the complement system appears to be a key feature in the pathogenesis of *P. gingivalis*. The proteolytic enzymes produced by *P. gingivalis* degrade complement components to prevent opsonization-mediated phagocytosis and formation of the complement membrane attack complex.^{12,189,191,212} However, *P. gingivalis* gingipains also convert C5 to C5a, activating C5aR and C5aR toll-like receptor-2 crosstalk to manipulate the host response, leading to the survival of the bacteria while eliciting proinflammatory mediators to acquire nutrition for growth.^{12,141,189,191,212} Gingipains can also suppress antimicrobial peptides and phagocytosis by neutrophils and macrophages. The Mfa1 fimbriae mediate entry into and survival of *P. gingivalis* in dendritic cells via a specific nonintegrin intercellular adhesion-grabbing molecule.²¹³ *Porphyromonas gingivalis* in association with macrophages induces toll-like receptor-2-mediated transactivation of CR3 through an inside-out pathway, and the activated CR3 interacts with *P. gingivalis* major fimbriae to down-regulate interleukin-12, allowing *P. gingivalis* to enter and persist in macrophages.^{189,212} Overall, *P. gingivalis* plays an essential role in subgingival microbial dysbiosis because of its participation in biofilm formation and modulation of host defenses, and the organism is recognized as a prototypic keystone pathogen of periodontitis.

3 | HERPESVIRUS PERIODONTAL PATHOGENS

Herpesviruses are ubiquitous in humans and their infections cause significant morbidity and mortality in immune-compromised individuals, such as patients with AIDS. A common theme in the pathogenesis of herpesvirus diseases is the ability of the viruses to modulate host immune responses. Table 3 summarizes key herpesvirus pathogenic factors and their targets.

The mature herpesvirus virion contains 4 structurally distinct layers: a nuclear core; an icosahedral capsid; an amorphous tegument; and a lipid bilayer envelope (Figure 1). Herpesvirus genomes are double-stranded DNA ranging from 130 to 220 kilobases, which encode ~ 100–200 polypeptides and noncoding RNAs. The icosahedral capsid provides a framework for packaging all virion structural components and shapes the viral 3-dimensional structure.²¹⁴ The envelope is derived from the cellular membrane and integrates a large number of viral glycoproteins that mediate cellular attachment and entry for a new cycle of infection. The amorphous layer of tegument between the nucleocapsid and the envelope packs a large number of cellular and viral proteins that, when released into the cytoplasm of infected cells, collectively establish a cellular environment conducive for viral infection. Notable functions of tegument proteins include immune modulation, transcription regulation, and signal transduction.^{215–217}

One hallmark of all herpesviruses is their ability to establish lifelong latent infections in the human host. Based on biologic properties and genomic similarities, herpesviruses are classified into alpha-, beta-, and gamma-herpesviridae. Alpha-herpesviridae include herpes simplex virus types 1 and 2 and varicella zoster virus. After primary infection, usually of epithelial cells, alpha-herpesviruses can establish latent infection in peripheral trigeminal ganglia.²¹⁸ Beta-herpesviridae include human cytomegalovirus and human herpesvirus 6 and –7. The cellular tropism of beta-herpesviruses is broad in vivo, although cytomegalovirus is preferentially found in monocytes/macrophages and endothelial cells.²¹⁹ The lymphotropic gamma-herpesviridae include Epstein-Barr virus and human herpesvirus 8 (also known as Kaposi sarcoma-associated herpesvirus) that establish latent infections in lymphoid cells. Epstein-Barr virus human herpesvirus 8 are the causative agents of human malignancies. Epstein-Barr virus infection is implicated in a broad range of malignancies, including lymphomas (Burkitt, Hodgkin, and diffuse large B cell lymphoma), nasopharyngeal carcinoma, gastric cancer, and T/natural killer cell proliferative diseases.^{220,221} Human herpesvirus 8 is the leading oncogenic agent in immune-compromised individuals, such as patients with AIDS and organ transplantation recipients. Human herpesvirus 8 infection is also causatively associated with 2 types of B cell lymphoma: primary effusion lymphoma; and multicentric Castleman's disease.^{222–225} Although Kaposi sarcoma is primarily found in the skin and internal organs, the cancer is derived from endothelial cells that express multiple lymphatic markers.^{226,227}

3.1 | Cytomegalovirus

Cytomegalovirus infection causes no apparent illness in healthy individuals but can be life-threatening in immune-compromised individuals, such as patients with AIDS, organ transplantation recipients, and newborn infants. Cytomegalovirus infection is also the leading infectious cause of deafness, learning disabilities, and intellectual disability in children.²²⁸ Because cytomegalovirus can infect a broad spectrum of cell types in vivo, the virus may be recovered throughout the human body.²²⁹ Cytomegalovirus lytic infection is found more frequently in salivary glands than in other organs/tissues.²³⁰ The salivary glands may be an immune-privileged site for cytomegalovirus replication, as salivary gland CD4⁺ T cells secrete high levels of the cellular immune-suppressive interleukin-10, which can dampen the anti-herpesvirus host response and facilitate cytomegalovirus replication and

shedding.²³¹ Two features of mucosal shedding of cytomegalovirus are relevant to the oral cavity. First, cytomegalovirus can be secreted into saliva for months following a primary acute infection. Studies have found that nearly 80% of toddlers attending daycare centers in the USA shed cytomegalovirus in saliva for up to 2 years.^{232,233} Second, cytomegalovirus secretion and shedding in saliva can be unapparent or intermittent clinically, which is particularly pronounced in patients with HIV and in organ transplantation recipients, who excrete cytomegalovirus into saliva at levels of ~ 30% and ~ 45%, respectively.^{234,235} These observations suggest that the herpesvirus-host interaction, similar to other polymicrobial interactions, can shape the course and outcome of infectious events in the oral cavity.

The cytomegalovirus genome encodes a large array of polypeptides that modulate the innate and adaptive immune responses. Antigen presentation by major histocompatibility complex-I and -II surface molecules is crucial for adaptive immunity against cytomegalovirus and, consequently, cytomegalovirus proteins seek to inhibit nearly every step of the antigen presentation.²³⁶ These inhibitory activities tap into peptide generation (UL83),^{237,238} peptide processing by means of luminal endoplasmic reticulum aminopeptidase (US4-1),²³⁹ antigenic peptide translocation by the transporter associated with antigen processing complex (US6),^{240,241} peptide-major histocompatibility complex formation (UL82),²⁴² and major histocompatibility complex-I and -II presentation at the cell surface (US3, US11, US2, and US10).²⁴³⁻²⁵¹ These lytic gene products are either expressed at immediately early or early phases of infection, or are imported into the infected cells as virion structural tegument proteins. The key to containing bacterial pathogens in the oral cavity is the recruitment and activation of neutrophils at the site of infection.^{190,252} Notably, the cytomegalovirus genome contains four G protein-coupled receptors that are functional homologues of the human interleukin-8 receptor (also known as chemokine [C-X-C motif] receptor 2).^{253,254} The viral G protein-coupled receptors bind to interleukin-8 and may function as decoy receptors to delay neutrophil recruitment.^{255,256} During latent infection, cytomegalovirus infection suppresses the major histocompatibility complex-II molecule in monocytes, probably through the action of the cytomegalovirus homologue of interleukin-10,^{257,258} as this immune suppression is independent of US2 and US10. Additionally, cytomegalovirus can derail the development or differentiation of dendritic cells from their progenitor monocytes, thereby repressing the function of one of the main classes of professional antigen-presenting cells.^{257,259,260} These immune-suppressive activities of cytomegalovirus can potentially foster exacerbation of bacterial and other microbial oral infections, leading to synergy in microbial propagation and pathogenesis.

3.2 | Epstein-Barr virus

The Epstein-Barr virus is one of the most successfully adapted human pathogens, and is known to infect more than 90% of the human adult population. Although Epstein-Barr virus infection is typically asymptomatic because of a robust immune response, the virus causes infectious mononucleosis during adolescence as a result of hyperactivation of T cells,^{261,262} various diseases in immune-deficient individuals (such as patients infected with HIV or organ transplantation recipients), and several malignancies of lymphoid and epithelial cell origin.^{221,263} The ability of the Epstein-Barr virus to produce a diverse spectrum of diseases probably stems from its distinct gene expression programs during latency. Numerous latent

phase gene products have been implicated in transformation or oncogenicity of the Epstein-Barr virus, and Epstein-Barr virus infection can readily transform human B lymphoid cells into clonal lymphoblastoid cell lines.^{264,265} Depending on the type of latency program, latent gene transcripts may include 6 Epstein-Barr virus nuclear antigens, 3 latent membrane proteins, and Epstein-Barr virus encoded small RNAs.^{266,267} The latency products modulate cellular signaling pathways to promote uncontrolled cell proliferation and/or immortalization by deterring cell death.^{268,269} Although Epstein-Barr virus-associated malignancies are driven primarily by latent gene products, recent studies have suggested that lytic replication of the Epstein-Barr virus is also an integral part of Epstein-Barr virus-induced diseases.²⁷⁰ How the Epstein-Barr virus lytic program contributes to the array of human diseases merits further study.

Latency of Epstein-Barr virus converts to a lytic cycle by activating the master transcription factor, ZEBRA (also known as BZLF1, Zta, EB1, or Z).²⁷¹ BZLF1 acts on its own promoter to amplify its expression and induces a cascade of ordered expression lytic genes that ultimately culminates in the production of infectious virions and lysis of the host cell.^{272,273} A second transactivator, RTA (also known as BRLF1 or R), also induces the lytic cycle of the Epstein-Barr virus by activating ZEBRA expression in a limited number of Epstein-Barr virus cell lines.^{274,275} In cells supporting Epstein-Barr virus lytic replication, more than 100 viral gene products are expressed, which in turn choreograph extensive cellular events to impact cell growth and proliferation.²⁷⁶ As a result of a tight regulation of latency, reactivating BZLF1 often results in abortive replication, in which a subset of lytic genes is expressed without producing infectious virions. These early gene products are responsible for modulating the cellular environment, such as immune evasion to facilitate productive infection.^{277,278} For example, Epstein-Barr virus-encoded homologues of the Bcl-2 protein demonstrate pro- and anti-apoptotic activity, which may extend the survivability of Epstein-Barr virus-infected cells during lytic replication.^{279,280} The Epstein-Barr virus also encodes a viral G protein-coupled receptor that functions to downregulate major histocompatibility complex class-I molecules on the cell face, thereby dampening herpesvirus antigen presentation.²⁸¹ Moreover, an Epstein-Barr virus-produced interleukin-10 homologue can potentially suppress the cellular inflammatory response and promote an anti-inflammatory environment that can nurture productive Epstein-Barr virus infection and facilitate dissemination within the host.^{282,283} The activity of these immediate early gene products or the structural components brought into the infected cells may have a profound effect on the host as well as other pathogens that are spatially and temporally relevant to the Epstein-Barr virus infection.

4 | SYNERGISTIC HERPESVIRUS-BACTERIA INTER ACTIONS IN PERIODONTITIS

Studies in mice provided the first indication of a synergistic pathogenic interaction between herpesviruses and periodontal microorganisms. Mice inoculated intraperitoneally with murine cytomegalovirus together with *Pseudomonas aeruginosa* and either *Staphylococcus aureus* or *Candida albicans* exhibited mortality rates of 80%–100%, whereas immunization against murine cytomegalovirus abrogated the mortality for all combinations of the

infectious agents studied.²⁸⁴ Stern et al²⁸⁵ found a markedly higher mortality rate and a significantly lower systemic interferon-gamma level in mice coinfecting with murine cytomegalovirus and *P. gingivalis* than in monoinfected mice, suggesting that the *P. gingivalis* infection diminished the level of antiviral interferon-gamma, causing increased morbidity of the coinfecting cytomegalovirus.

Pathogenic synergism is deduced if the odds ratio for disease is significantly higher for a coinfection of infectious agents than for the sum of the odds ratios of each individual agent. Synergistic pathogenic interactions are expected to occur with particularly high frequency in rapidly progressive periodontitis, such as localized juvenile periodontitis. Classic localized juvenile periodontitis disease begins at puberty and affects the proximal surfaces of permanent incisors and first molars.^{286,287} Three studies on localized juvenile periodontitis have examined the pathogenic interaction between herpesviruses and periodontopathic bacteria.

A study in Los Angeles included 11 patients (aged 10–23 years) with localized juvenile periodontitis, who demonstrated periodontal breakdown at incisors and first molars, but minimal dental plaque and gingivitis.²⁸⁸ Each patient with localized juvenile periodontitis contributed a pooled sample from 3 lesions of the incisor teeth and first molars (5–11 mm probing depths), and from gingivitis/healthy sites at the canines (2–3 mm probing depths). In 11 patients with localized juvenile periodontitis and 11 control individuals, subgingival cytomegalovirus was detected in 8 patients and 2 individuals, Epstein-Barr virus type 1 in 7 and 2, Epstein-Barr virus type 2 in 1 and 0, herpes simplex virus in 6 and 1, and viral coinfection in 8 and 2, respectively ($P = 0.03$). Six patients with localized juvenile periodontitis showed active cytomegalovirus infection and lacked crestal alveolar lamina dura in angular bony defects, a radiographic feature consistent with progressive periodontitis.²⁸⁹ Cytomegalovirus activation was detected in all 5 cytomegalovirus-positive patients aged 10–14 years (early localized juvenile periodontitis), but only in 1 of the 3 patients who were older than 14 years, and latent cytomegalovirus was found in periodontal samples from 2 control individuals. In another study, cytomegalovirus activation was identified in deep periodontal pockets in 2 of 3 patients aged 14–17 years with localized juvenile periodontitis, and in 2 of 6 patients with adult periodontitis.²⁹⁰ In addition, herpesvirus-like virions have been detected in localized juvenile periodontitis lesions, signifying an active viral infection.^{291,292} *Aggregatibacter actinomycetemcomitans*, a major pathogen of localized juvenile periodontitis,²⁸⁷ was found to be present at higher levels in localized juvenile periodontitis lesions with an active, as opposed to with a latent, cytomegalovirus infection.²⁸⁸ From these observations it is possible that the development of localized juvenile periodontitis involves cytomegalovirus-active infection causing upgrowth of *A. actinomycetemcomitans*.

Michalowicz et al,²⁹³ in a study in Jamaica, assessed the presence of subgingival cytomegalovirus, Epstein-Barr virus type 1, *A. actinomycetemcomitans*, and *P. gingivalis* in Afro-Caribbean adolescents, aged 14–18 years, with localized juvenile periodontitis ($n = 15$), incidental periodontal attachment loss ($n = 20$), or a normal periodontium ($n = 65$). The patients with localized juvenile periodontitis showed the characteristic incisor-first molar tissue destruction with occasional involvement of other teeth, but several patients also had

plaque accumulation and gingivitis. Of the patients with localized juvenile periodontitis, 73% harbored cytomegalovirus, 33% Epstein-Barr virus, 67% *A. actinomycetemcomitans*, and 87% *P. gingivalis*. Significant associations were found between localized juvenile periodontitis and cytomegalovirus (odds ratio = 10.0), *A. actinomycetemcomitans* (odds ratio = 8.0), and *P. gingivalis* (odds ratio = 12.7), but not between localized juvenile periodontitis and Epstein-Barr virus (odds ratio = 2.4). Epstein-Barr virus (odds ratio = 4.0) and *P. gingivalis* (odds ratio = 4.6) were significantly linked to incidental periodontal attachment loss. The odds ratio for having localized juvenile periodontitis was substantially higher when both cytomegalovirus and *P. gingivalis* were present (odds ratio = 51.4) than when cytomegalovirus (odds ratio = 4.6) and *P. gingivalis* (odds ratio = 7.8) occurred alone. The odds of detecting *A. actinomycetemcomitans* in localized juvenile periodontitis increased 31.8-fold if cytomegalovirus was present and by 9.3-fold if Epstein-Barr virus was present. Cytomegalovirus-*P. gingivalis* coinfection was associated with an average periodontal breakdown of 3.7 teeth, whereas cytomegalovirus sole infection only affected 0.9 teeth, and *P. gingivalis* sole infection only 1.4 teeth. Thus, localized juvenile periodontitis in Jamaican adolescents was strongly associated with cytomegalovirus and *P. gingivalis*, and the markedly higher odds ratio of the cytomegalovirus-*P. gingivalis* coinfection than that of the sum of the individual pathogens suggests a periodontopathogenic synergy between the infectious agents. Therefore, cytomegalovirus and *P. gingivalis* seemed to influence both the onset and the extent of localized juvenile periodontitis.

A recent study of localized juvenile periodontitis in Sudan supported periodontopathogenic relationships between cytomegalovirus, Epstein-Barr virus type 1, *A. actinomycetemcomitans*, and *P. gingivalis*.²⁹⁴ Seventeen patients with localized juvenile periodontitis (mean age, 15.5 ± 1.6 years) and 17 periodontally healthy control subjects of a similar age were studied. A total of 70.6% of patients with localized juvenile periodontitis and 11.8% of control subjects harbored subgingival cytomegalovirus ($P = 0.0001$), and 64.7% of patients with localized juvenile periodontitis and 47.1% of control subjects harbored subgingival Epstein-Barr virus ($P = 0.3$). A total of 70.6% of patients with localized juvenile periodontitis and 5.9% of control subjects harbored subgingival *A. actinomycetemcomitans* ($P = 0.0001$), and 82.4% of patients with localized juvenile periodontitis and 41.2% of control subjects harbored subgingival *P. gingivalis* ($P = 0.013$). There was no association between *Tannerella forsythia* and *Treponema denticola* and localized juvenile periodontitis. The odds ratio for having localized juvenile periodontitis was 39.1 for cytomegalovirus-*A. actinomycetemcomitans* coinfection, 49.0 for Epstein-Barr virus-*A. actinomycetemcomitans* coinfection, 29.3 for cytomegalovirus-*P. gingivalis* coinfection, and 10.7 for Epstein-Barr-*P. gingivalis* coinfection.

To explain the clinical characteristics of localized juvenile periodontitis, Ting et al²⁸⁸ hypothesized that a primary cytomegalovirus infection at the time of root formation of permanent incisors and first molars at 3–5 years of age resulted in an abnormal periodontium. Viruses infecting odontogenic cells of developing hamster teeth can disrupt normal cell differentiation,²⁹⁵ and an active cytomegalovirus infection can change the morphology of developing human teeth.^{296,297} Cementum hypoplasias have been identified in localized juvenile periodontitis-affected teeth on the intracrestal part of the root, excluding the periodontal pocket environment or treatment as causes of the cemental defects.²⁹⁸

During puberty, hormonal changes may trigger reactivation of periodontal cytomegalovirus or Epstein-Barr virus, followed by an upgrowth of *A. actinomycetemcomitans* or *P. gingivalis* and rapid periodontal destruction around teeth with a damaged periodontium. The periodontal breakdown appears to proceed along the path of the cementum hypoplasias.²⁹⁹ Cementum abnormalities have also been described in generalized juvenile periodontitis²⁹⁹ and prepubertal periodontitis.³⁰⁰ A single Hopi American-Indian adolescent demonstrated generalized juvenile periodontitis and yielded (as the only study person) both cytomegalovirus and Epstein-Barr virus, presumably in an active stage.³⁰¹ Similarly to localized juvenile periodontitis, the pathogenesis of generalized juvenile periodontitis and prepubertal periodontitis may involve an active cytomegalovirus infection interfering with root formation, followed at a later time by reactivation of periodontal herpesviruses and upgrowth of periodontopathic bacteria.

Pathogenic interactions between infectious agents may also occur in adults with “chronic” periodontitis, although with lower odds ratios, probably because of a predominance of nonprogressive sites in adult periodontitis.²³ A study in Los Angeles of 68 adults with moderate to severe periodontitis and 72 adults with gingivitis or slight periodontitis associated cytomegalovirus and Epstein-Barr virus type 1 to *P. gingivalis* with significant odds ratios between 2.06 and 4.39.³⁰² Herpesvirus-bacteria coinfection in gingivitis occurred with a frequency that was to be expected from a random distribution of the infectious agents.³⁰² Coinfections with cytomegalovirus or Epstein-Barr virus and *P. gingivalis* or *A. actinomycetemcomitans* have also been observed in periodontitis studies from Japan,^{303–305} India,^{306–310} Turkey,³¹¹ Iran,³¹² China,^{313,314} Colombia,³¹⁵ Russia,³¹⁶ Greece,³¹⁷ Romania,^{318,319} and Italy.³²⁰ Initial data also incriminate herpesvirus-bacteria coinfection in the pathogenesis of pericoronitis at mandibular third molars,³¹⁸ peri-implantitis,^{304,321} and symptomatic periapical pathosis.^{322,323} Moreover, adult types of periodontitis demonstrate a tendency to site-specificity, although this is not as predictable and distinct as in localized juvenile periodontitis,³²⁴ which may also involve herpesvirus reactivation, major bacterial pathogens, and host defenses capable of preventing the spread of the herpesvirus infection.³²⁵ Individuals with genetically or acquired compromised cellular immunity experience a more frequent and longer lasting herpesvirus reactivation, which may overwhelm the host defense and cause the herpesvirus infection and periodontitis to expand beyond the initially affected teeth.

In summary, herpesviruses and bacterial pathogens can separately contribute to periodontal disease but, in a reciprocal interrelationship, may synergistically increase the disease severity. The coinfection of herpesviruses and periodontopathic bacteria appears to be a key pathogenic determinant of severe periodontitis and of site-specificity of the disease. Virus-bacteria coinfections can also exacerbate the morbidity of systemic diseases, and findings from systemic diseases may provide clues to the pathogenic mechanisms of severe periodontitis.

5 | SYNERGISTIC VIRUS-BACTERIA INTERACTIONS IN SYSTEMIC DISEASES

The concept of synergistic morbidity of a coinfection by viruses and bacteria is a research topic of increasing importance in medicine. Viruses may exploit bacterial factors to invade host cells, and viral infections may enhance the morbidity of bacterial infections. The following summarizes evidence gathered from polymicrobial interactions of the intestines and the lung, and outlines possible mechanisms by which two or more transkingdom microbes may interact with each other in systemic diseases.

Synergism between viruses and bacteria may be direct or indirect and can be classified accordingly. The direct mode of synergism may entail physical interaction during infection. Viruses explore environmental cohabitants in the gastrointestinal tract to enhance infectivity. For example, intestinal bacteria can aggregate multiple virion particles of the poliovirus to enhance the efficiency of viral entry into gastrointestinal epithelial cells.³²⁶ A hallmark of poliovirus is a high genomic mutation rate, and a poliovirus infection of intestinal epithelial cells gives rise to numerous genetic recombinations and a variety of quasispecies.³²⁷ A quasispecies viral population helps the viral pathogen to outwit the host immune response and prolong the viral infection. Intestinal bacteria also seem to stabilize the poliovirus in the feces of infected mice, pointing to an additional direct mode of interaction between bacterial and viral pathogens.³²⁸ Noroviruses are nonenveloped plus-strand RNA viruses, which demonstrate tropism for mouse and human primary B cells, and are a leading cause of epidemic and sporadic gastroenteritis. Norovirus binds to the histo-blood group antigens expressed by intestinal bacteria (such as *Enterobacter cloacae*) and hitchhikes with bacteria to breach the intestinal epithelium to reach B cells.^{329,330} Purified histo-blood group antigen can augment norovirus attachment and entrance into B cells and viral infectivity. The virion structure and content of herpesviruses are significantly more complex than those of poliovirus and norovirus, and thus bacteria may enhance herpesvirus infectivity in a greater number of mechanisms.

Coinfection between the influenza virus and bacteria has been recognized since the beginning of the 19th century.^{331,332} Up to 70% of the fatalities in the Spanish influenza pandemic in 1918 and subsequent influenza pandemics resulted from coinfection with *Streptococcus pneumoniae*, the most common bacterial cause of pneumonia.^{333,334} Mechanisms of the influenza virus-bacteria interaction have been related to either tissue physical damage or immune response alteration in the respiratory tract.³³⁵ Influenza virus-induced damage of mice respiratory epithelial cells increases the number of pneumococcal cells in the respiratory tract and in the lung.^{336,337} The underlying mechanism appears to be upregulation of the receptor for platelet-activating factor, a putative entry molecule to which pneumococci can adhere via phosphocholine on the bacterial cell surface.^{338–340} Infection with influenza virus also stimulates mucus production, fibrin secretion, edema, and influx of inflammatory cells, which cumulatively promotes propagation of bacteria and hampers bacterial clearance.^{341,342} Moreover, influenza virus and pneumococcus both express neuraminidase during infection. Influenza virus relies on neuraminidase to cleave sialic acid for dissemination³⁴³ and pneumococcus uses neuraminidase to catabolize sialic acid for

entry into the respiratory epithelium.^{344,345} Influenza virus genes of the sialic acid metabolism provide sialated intermediaries for pneumococcal propagation in a synergistic utilization of sialic acid.³⁴⁶ In addition, the influenza virus-induced mucinous environment provides a culture medium for pneumococcal proliferation in an otherwise suffocating nasopharynx environment. Studies in mice have confirmed that an influenza virus infection can enhance pneumococcal colonization of the murine nasopharynx, a nutrient-deprived organ.³⁴⁶

The lung immune response to influenza virus-*S. pneumoniae* and influenza virus-*S. aureus* coinfections remains an important research topic.³³⁵ Both innate and adaptive immune responses have been implicated in the pathogenesis of secondary bacterial infection after initial influenza infection. The innate immune defense is crucial for controlling pathogen propagation immediately after infection.^{347,348} Viruses are highly susceptible to interferon-mediated antiviral activities, and bacteria are usually cleared by antibody-mediated opsonization and neutrophil-mediated phagocytosis. In innate immunity, pathogen-associated molecular patterns of viruses and bacteria engage various toll-like receptors to elaborate an array of overlapping and distinct interferons and inflammatory cytokines.^{349,350} Lipopolysaccharide of gram-negative bacteria attaches to toll-like receptor-4,³⁵¹ while bacterial RNA can bind to multiple toll-like receptors (toll-like receptors 3, 7, or 8) on the plasma membrane or endosomal vesicles.^{352–354} Also, the 2 cytosolic RNA sensors, namely, retinoic acid-inducible gene I and melanoma differentiation-associated gene 5, can recognize double-stranded RNA in the cytosol to activate the mitochondrial antiviral-signaling protein-dependent immune pathway.^{355–358} The enhanced severity of an influenza virus-bacteria coinfection is most likely the result of immune stimulation by two distinct pathogens, each inducing high levels of inflammatory cytokines and a cytokine storm which exacerbates the tissue destruction and the functional impairment of the lung.^{359,360} Certain interferons and cytokines can also produce contrasting effects on influenza virus and bacterial pathogens. For example, *S. pneumoniae* can exploit the antiviral type I interferon to promote colonization in mice,^{361,362} and mice deficient in type I interferon receptor are relatively resistant to *S. pneumoniae* superinfection.³⁶² Influenza virus may inhibit production of tumor necrosis factor-alpha and increase induction of interferon-gamma, which may impair bacterial clearance and enhance the risk of a secondary bacterial infection.

Herpesvirus genomes encode a large array of cytokines (eg, v-macrophage-inflammatory protein, v-interleukin-6, and v-inter-leukin-10) and their receptors (such as interleukin-8 receptor).^{363–365} These signaling molecules may derail host defenses and exacerbate herpesvirus infections, but the immune effect may also extend to “bystander” infecting bacteria. One intriguing example is neutrophils, which are attracted by interleukin-8 and are critical for preventing epithelial invasion by oral bacteria. Epstein-Barr virus encodes a G protein-coupled receptor that is a functional homologue of the human interleukin-8 receptor.²⁵³ Herpesvirus-8 G protein-coupled receptor bound to interleukin-8 activates inflammatory immune signaling by the AKT-mTOR pathway and fuels inflammation and angiogenesis.^{366,367} Herpesvirus G protein-coupled receptor also stimulates cytokine production, such as interleukin-8; nevertheless, herpesvirus G protein-coupled receptor is expected to diminish neutrophil recruitment.³⁶⁸ Interleukin-10 is a potent anti-inflammatory cytokine produced in response to microbial infection^{369,370} and, interestingly, cytomegalovirus expresses a

homologue of human interleukin-10, while Epstein-Barr virus upregulates the production of cellular interleukin-10 during infection.³⁷¹ Moreover, interleukin-10 in an influenza viral infection may synergistically facilitate *S. pneumoniae* superinfection.^{362,372} The role of interleukin-10 in periodontal herpesvirus-bacteria coinfections awaits further investigation.

6 | MOLECULAR SYNERGISTIC INTERACTIONS BETWEEN HERPESVIRUSES AND PERIODONTOPATHIC BACTERIA

The oral cavity represents a unique environment with numerous opportunities for interactions between herpesviruses and bacteria. The oral epithelium supports a productive infection of human herpesviruses, and saliva can exhibit a high herpesvirus load and serve as a major vehicle for herpesvirus transmission among individuals. The oral cavity is also host to more than 700 bacterial taxa. Clinical evidence implicates herpesvirus-bacteria synergism in the etiopathogenesis of severe periodontitis, but research into the molecular interactions (mechanisms) between herpesviruses and periodontopathic bacteria is still in its infancy. Periodontal herpesvirus-bacteria coinfection remains a virgin field of research.

Table 4 lists plausible synergistic mechanisms between periodontal herpesviruses and *A. actinomycetemcomitans* and *P. gingivalis*, ranked by plausibility, strength of evidence of virus-bacteria synergism in other organ systems, and virulence mechanisms of herpesviruses, *A. actinomycetemcomitans*, and *P. gingivalis*.

Physical binding between viruses and bacteria, with or without prior modifications, may yield a pathway for viruses to reach subgingival sites. This hypothesis has a precedent from virus-bacteria interactions in the gastrointestinal tract, and even though evidence is lacking for the oral microbiome, the importance of herpesvirus-bacteria physical binding should not be dismissed outright. Also, bacterial lipoteichoic acid and the lipopolysaccharide can upregulate the expression of heparin sulfate, which may mediate, for example, the entry of herpesvirus-8 into the periodontal ligament and gingival fibroblasts.³⁷³ The exposure of cryptic viral receptors following bacterial enzymatic modification of cell-surface structures constitutes another possibility for herpesvirus colonization. Herpesvirus glycoproteins expressed on infected eukaryotic cell membranes may serve as receptors for bacteria, and herpesvirus damage of epithelial cells may expose bacterial attachment sites on the basement membrane. In addition, the tissue destroyed by a herpesvirus lytic infection generates nutrients for bacteria through direct (tissue degradation) or indirect (inflammation) mechanisms. Furthermore, *P. gingivalis* and other periodontal bacteria produce short-chain fatty acid metabolic end products, such as butyric acid, which can activate latent Epstein-Barr virus, herpesvirus-8, and HIV infections via modifications of histone proteins.^{374–377} The downstream effects of converting a latent herpesvirus infection to active herpesvirus infection and its clinical impact is an exciting new area for periodontal investigation.

Modulation of innate and adaptive immunity and the cytokine/ chemokine network is a common theme of both bacterial and herpesvirus pathogenicity. Table 4 lists several plausible mechanisms of herpesvirus-bacteria synergism via innate and adaptive immunity. *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* can interfere with the function of neutrophils and macrophages and manipulate the complement system to maintain a level of

inflammation favorable for bacterial growth. The ability of *P. gingivalis*, Epstein-Barr virus, and herpesvirus-8 to suppress the production or function of interleukin-8 may prevent bacterial clearance from periodontal sites. Interference of herpesvirus with antigen presentation on macrophages may hamper the generation of protective immunity against bacterial infections. On the other hand, bacterial infection may lead to activation of latent herpesvirus infections, elevated levels of herpesviruses in periodontal sites and saliva, and an increased risk of viral transmission within and between subjects. In summary, herpesviruses and bacterial pathogens may support each other's pathogenic potential and give rise to diseases which, to be controlled, may require adequate anti-herpesvirus host responses or treatment.

7 | CONCLUDING REMARKS

Important progress has been made in our understanding of the infectious agents of periodontal disease, and in particular of the herpesvirus-bacteria-host immune interactions in severe periodontitis. Periodontal herpesvirus-bacteria coinfections may share pathogenic features with similar coinfections in systemic diseases, and that notion may help to clarify the pathogenesis of periodontitis. Increased insights into periodontal herpesvirus-bacteria interactions may also suggest new means for the management of destructive periodontal disease.

The current state of investigation into herpesvirus-bacteria synergism in periodontitis is comparable with the research on periodontopathic bacteria in the 1970s, which changed our concept of the pathogenesis of periodontitis and caused a conceptual shift in the treatment of the disease. The pathogenetic synergy between herpesviruses, *A. actinomycetemcomitans*, and *P. gingivalis* is supported by clinical studies and is highly plausible mechanistically. Herpesviruses, *A. actinomycetemcomitans*, and *P. gingivalis* possess virulence mechanisms that are overlapping as well as complementary. This article provides an extensive review of the pathogenic potential of herpesviruses and periodontopathic bacteria, and presents a list of mechanisms for herpesvirus-bacteria synergism ranked by biologic plausibility and strength of evidence. The ranking provides an approach for mechanistic studies. The low-ranking mechanisms may initially be examined by high-throughput low-cost experiments, such as in vitro screening for phenotypes. Mechanisms with moderate plausibility may be studied in in vitro systems before embarking on more time- and resource-consuming experiments. Mechanisms with both high plausibility and strength of evidence may prompt studies in animal models. The herpesvirus and bacterial manipulation of innate and adaptive immunity represents a convincing mode of synergism and is fitting for mechanistic studies. However, there are other novel, but less well-characterized, mechanisms of herpesvirus-bacteria synergy that remain to be explored. For example, several studies have identified herpesvirus-encoded microRNAs in inflammatory gingival and pulpal tissues.^{378,379} These microRNAs may serve as master regulators of host genes/pathways that may influence the clinical course of periodontal infections.

In conclusion, recognition of the herpesvirus-bacteria interaction in periodontitis has unraveled many of the intricacies of the disease and opened new means of preventing and treating destructive periodontal disease. Herpesviruses and bacteria play important roles in

the onset and progression of periodontitis, and antimicrobial treatment that targets both herpesviruses and bacterial pathogens may reduce the risk of developing severe periodontitis and possibly certain systemic diseases. Also, a detailed molecular understanding of the periodontal herpesvirus-bacteria coinfection may identify critical inflammatory pathways that are suited for pharmacologic intervention.

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FIGURE 1.

Structures of herpesvirus virion. The mature virion contains 4 major structures: a nuclear core of double-stranded DNA; an icosahedral capsid; an amorphous tegument; and a host-derived lipid bilayer envelope with surface glycoproteins

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TABLE 1

Major virulence determinants of *Aggregatibacter actinomycetemcomitans*^a

Virulence determinants	Function
Flp-1 pili	Adherence to biotic and abiotic surfaces; autoaggregation
ApiA (Omp100)	Adherence and invasion of epithelial cells; protects bacteria from complement-mediated serum bactericidal activity
ApiB	Invasion of epithelial cells
EmaA	Adherence to collagen
Aae	Adherence to epithelial cells
Polyglandular autoimmune syndrome polysaccharide	Autoaggregation
Dispersin B	Dissemination of bacteria to start a new cycle of transmission and colonization
PilA, PilB, PilC, PilD	DNA uptake and natural competence
Lipopolysaccharide	Adherence to abiotic surfaces; activation of toll-like receptors and induction of proinflammatory responses; activation of osteoclasts and induction of alveolar bone loss
Phosphorylcholine	Adherence and invasion of endothelial cells
Leukotoxin	Killing of neutrophils and lymphocytes; induction of proinflammatory responses; hemolysis
Cytolethal distending toxin	Inhibition or induction of apoptosis of a variety of cells including epithelial cells, fibroblasts, T cells, and periodontal ligament cells; induction of proinflammatory responses; osteoclastogenesis and alveolar bone loss

^aReferences: 72,75,91,98,100,108,153,157,180.

TABLE 2

Major virulence determinants of *Porphyromonas gingivalis*^a

Virulence determinants	Function
FimA	Adherence to biotic and abiotic surfaces; binding to salivary proteins, epithelial cells, fibrinogen, fibronectin, lactoferrin, erythrocytes, oral streptococci; binding of chemokine (C-X-C motif) receptor 4 to suppress toll-like receptor 2/1-mediated proinflammatory and antimicrobial responses; binding of complement receptor 3 to inhibit interleukin-12 production to promote intracellular survival of bacteria, without affecting tumor necrosis factor- α -mediated destructive inflammation
MfaI pili	Adherence to oral streptococci; binding to dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin of dendritic cells to initiate the entry and the survival of the bacteria
Hemagglutinins HagA, B, and C	Hemolysis for iron acquisition as a nutrient; adherence to epithelial cells; induction of platelet aggregation
Hemoglobin binding protein	Binding of hemoglobin for nutrient acquisition
Gingipains (RgpA, RgpB, Kgp)	Degradation of host tissue and antibodies; activation of the complement system, or inactivation of the complement system by degradation of its components; generating C5a to initiate C5aR toll-like receptor-2 crosstalk to promote bacterial survival in neutrophils and macrophages; degradation of toll-like coreceptors to suppress immune response
Capsule	Antiphagocytosis
Lipid A of lipopolysaccharide	Weak agonist or antagonist of toll-like receptor-4 to induce or inhibit inflammatory response
Phosphoserine phosphatase SerB	Suppress interleukin-8 production to induce host defense paralysis

^aReferences: 145, 178, 189, 191.

TABLE 3

Pathogenic mechanisms of herpesvirus infections^a

Immune response	Herpesviruses	Viral factor/cellular targets	Effect
Innate immunity (cytosolic sensing of nucleic acids)	HSV-1, HSV-2	UL37 (RIG-I, cGAS, TRAF6); ICP0 (IFI16, STING, p50); US3 (IRF3, p65); ICP27 and ICP24 (TBK-1); VPI6 (CBP)	Downregulation (except TRAF6)
	HCMV	pUL83 (IFI16, cGAS, IRF3, p50-p65); UL31 (cGAS); pUL82 (STING); US9 (MAVS, STING, IRF3); IE86 (p50-p65)	Downregulation
Adaptive immunity (antigen presentation)	KSHV, EBV	RTA (IRF3, IRF7, p65); ORF36 (IRF3); dUTPase vGAT/ORF75 (RIG-I); ORF63 (RIG-I); ORF52 (cGAS); LANA (cGAS); vIRF1 (STING, IRF3)	Downregulation
	HSV-1, HSV-2	ICP47: inhibition of TAP-mediated antigen presentation US3: MHC-I and CD1d downregulation from cell surface	Downregulation of antigen presentation
	HCMV	US2, US10, and US11: MHC-I retention or degradation US6: inhibition of TAP-mediated antigen presentation US3 and UL82: MHC-I retention and MHC-II mislocalization UL83 and US4-1:	Downregulation of antigen presentation
	KSHV, EBV	K3 and K5 (KSHV): MHC-I degradation	Downregulation of antigen presentation
Cytokine/chemokine (production and signaling)	HSV-1, HSV-2	Vhs: mRNA degradation to avoid cytokine production	Immune suppression
	HCMV	UL11: induction of IL-10 via CD45 vIL-10: mimicry of cellular IL-10 US27, US28, UL33, and UL78: hijacking of IL-8-mediated chemotaxis	Immune suppression
	KSHV, EBV	vGPCR: hijacking of IL-8-mediated chemotaxis vIL-6: stimulation of B cell proliferation/viral dissemination microRNA: IL-6 and IL-10 induction	Immune suppression (IL-8 and IL10) and activation (IL-6)

Abbreviations: CBP, CREB-binding protein; CD1b, cluster of differentiation 1b; cGAS, cyclic GMP-AMP synthase; dUTPase, deoxyuridine triphosphatase; EBV, Epstein-Barr virus; GPCR, G protein-coupled receptor; HCMV, human cytomegalovirus; HSV-1 and HSV-2, herpes simplex virus-1 and -2; ICP, infected cell protein; IFI16, gamma-interferon-inducible protein Ifi-16; IL, interleukin; IE86, immediate early 86 kDa protein; IRF, interferon regulatory factor; KSHV, Kaposi sarcoma-associated human herpesvirus; LANA, latency-associated nuclear antigen; MAVS, mitochondrial antiviral signaling; MHC, major histocompatibility complex; ORF, open reading frame; RIG-I, retinoic acid-inducible gene I; RTA, replication and transcription activator; STING, stimulator of interferon genes; TAP, transporter associated with antigen processing; TBK1, TANK-binding kinase 1; TRAF6, TNF receptor associated factor 6; UL, unique long; US, unique short; vGAT, viral glutamine amidotransferase; vGPCR, viral G protein-coupled receptor; Vhs, virion host shutoff; vIFR, viral interferon regulatory factor; vIL, viral interleukin; VP, virion protein.

^aReferences: 237–258.

Mechanisms of the synergistic relationships between viruses and bacteria in periodontitis

TABLE 4

Steps	Mechanisms of synergism	Bacterial and herpesvirus factors	Plausibility and strength of evidence
Initial colonization and infection	Exposure of cryptic receptors of the host cells via degradation for bacterial or viral adherence	<i>Porphyromonas gingivalis</i> proteolytic enzymes	Moderate
	Physical interaction between bacteria and viruses to increase access of the microorganisms to the host tissue	Not available	Low
	Activation of cell surface receptors for bacterial or viral adherence	Not available	Low
	Exposure of new tissues for bacterial adherence	Herpesvirus lytic infection	Moderate
Bacterial growth and viral replication	Tissue destruction associated with viral lytic infection, leading to an increase in available nutrients for bacteria	Herpesvirus lytic infection	High
	Activation of latent viral infection via bacterial infection	Various mechanisms of bacterial infection	High
Modulation of host innate immunity	Killing of neutrophils and macrophages	<i>Aggregatibacter actinomycetemcomitans</i> leukotoxin and cytolethal distending toxin	High
	Suppression of production or functions of cytokines, including interleukin-8 and -10, leading to immune suppression and host defense paralysis	<i>Porphyromonas gingivalis</i> SerB; herpesvirus Vhs, UL11, vIL-10, US27, US28, UL33 and UL78, vGPCR, vIL-6, microRNA	High
	Suppression of pathogen-associated molecular pattern recognition/activation by the innate immunity to promote bacterial and viral infection	Atypical <i>P. gingivalis</i> lipopolysaccharide as a toll-like receptor-4 antagonist; herpesvirus UL37, ICP0, US3, ICP27, ICP24, VP16, pUL83, UL31, pUL82, US9, IE86, RTA, ORF36, dUTPase, vGAT/ORF75, ORF63, ORF52, LANA, vIRF1	High
Modulation of host adaptive immunity	Interfering with antigen presentation leading to dampened adaptive host immune response	<i>Porphyromonas gingivalis</i> Mfa1 and gingipains to promote intracellular survival of bacteria in macrophages and dendritic cells; herpesvirus ICP47, US3, US2, US10 and US11, US6, US3, UL82, UL83, US4-1, K3, K5, and BILF1/vGPCR to interfere with antigen presentation	Low
Tissue destruction	Killing of epithelial cells, fibroblasts, endothelial cells	<i>Aggregatibacter actinomycetemcomitans</i> leukotoxin and cytolethal distending toxin; herpetic lytic infection	High
Transmission	Shedding of viruses in the saliva for transmission	Activation of latent herpesvirus infection by bacterial infection	High

Abbreviations: BILF1, G-protein coupled receptor BILF1; ICP, infected cell protein; IE86, immediate early 86 kDa protein; LANA, latency-associated nuclear antigen; ORF, open reading frame; SerB, phosphoserine phosphatase; RTA, replication and transcription activator; UL, unique long; US, unique short; v, viral; Vhs, virion host shutoff; VP, virion protein.