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J DENT RES 2006 85: 392
DOI: 10.1177/154405910608500502

CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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J Dent Res 85(5):392-403, 2006

ABSTRACT

Emerging data on the consequences of the interactions between invasive oral bacteria and host cells have provided new insights into the pathogenesis of periodontal disease. Indeed, modulation of the mucosal epithelial barrier by pathogenic bacteria appears to be a critical step in the initiation and progression of periodontal disease. Periodontopathogens such as *Porphyromonas gingivalis* have developed different strategies to perturb the structural and functional integrity of the gingival epithelium. *P. gingivalis* adheres to, invades, and replicates within human epithelial cells. Adhesion of *P. gingivalis* to host cells is multimodal and involves the interaction of bacterial cell-surface adhesins with receptors expressed on the surfaces of epithelial cells. Internalization of *P. gingivalis* within host cells is rapid and requires both bacterial contact-dependent components and host-induced signaling pathways. *P. gingivalis* also subverts host responses to bacterial challenges by inactivating immune cells and molecules and by activating host processes leading to tissue destruction. The adaptive ability of these pathogens that allows them to survive within host cells and degrade periodontal tissue constituents may contribute to the initiation and progression of periodontitis. In this paper, we review current knowledge on the molecular cross-talk between *P. gingivalis* and gingival epithelial cells in the development of periodontitis.

KEY WORDS: periodontitis, epithelial cell, *Porphyromonas gingivalis*, adhesion, invasion.

Received June 16, 2005; Accepted November 8, 2005

(I) INTRODUCTION

Periodontal disease is a complex multifactorial disorder involving Gram-negative anaerobic bacteria and host cell interactions, the combined effects of which lead to the destruction of tooth-supporting tissue. More specifically, periodontitis results from chronic inflammation of the gingiva and occurs by its spread into the deeper structures of the periodontium, leading to progressive destruction of periodontal tissues, including the alveolar bone (Williams, 1990). Approximately 15% of the population is affected by severe forms of the disease, which, if untreated, may result in tooth loss and systemic complications (American Academy of Periodontology, 1996). In addition, periodontitis has been associated with cardiovascular disease and pre-term delivery of low-birthweight infants (Teng *et al.*, 2002). The progression of periodontitis is episodic, with active and inactive phases of tissue destruction, which reflects the opposing actions of bacterial challenges and host immune responses. The intimate interactions between periodontopathogens and host cells have become the subject of intensive investigations.

Porphyromonas gingivalis is a Gram-negative black-pigmented strict anaerobic bacterium that has been implicated as a major etiologic agent in the development and progression of periodontitis, more particularly, the chronic form (Lamont and Jenkinson, 1998; Holt *et al.*, 1999). *P. gingivalis* produces a broad array of potential virulence factors involved in tissue colonization and destruction as well as in host defense perturbation (Holt *et al.*, 1999). *P. gingivalis* is in close contact with the epithelium in periodontal pockets *in vivo* (Noiri *et al.*, 1997) and can invade various cell lines, including epithelial cells (Sandros *et al.*, 1994; Lamont *et al.*, 1995; Belton *et al.*, 1999; Rudney *et al.*, 2001), endothelial cells (Deshpande *et al.*, 1998; Dorn *et al.*, 2000), and fibroblasts (Amornchat *et al.*, 2003). The gingival epithelium is a stratified squamous epithelium that is an interface between the external environment, which is exposed to bacterial challenges, and the underlying periodontal tissue. The basal layer of the gingival epithelium is separated from and attached to the connective tissue by the basement lamina. The gingival epithelium can be divided into oral, sulcular, and junctional epithelia, based on their architecture. The sulcular epithelium, which extends from the oral epithelium to the gingival sulcus facing the teeth, and the junctional epithelium, which mediates the attachment of teeth to gingiva, are not keratinized, in contrast to the oral gingival epithelium. The sulcular and the coronal margins of the junctional epithelium are in close contact with bacteria in the gingival sulcus and appear to be crucial sites with regard to the development of periodontal diseases. During periodontitis, loss of connective tissue attachment and bone resorption associated with the formation of periodontal pockets is related to the pathologic conversion of the junctional and the sulcular epithelium to a pocket epithelium. Invasion of mammalian epithelial cells is an important strategy developed by pathogenic bacteria to evade the host immune system and cause tissue damage. Gingival epithelial cells are the primary physical barrier to infections by periodontopathogens *in vivo*. While the epithelium was previously thought to be passive, Dale

(2002) proposed a new perspective, assigning an active role to the epithelium in the host response to bacterial infections. The epithelium reacts to bacterial challenges by signaling host responses and integrating innate and acquired immune responses. This review focuses on the current understanding of host epithelial cell-*P. gingivalis* interactions in the pathogenesis of periodontitis.

(II) ADHESION TO EPITHELIAL CELLS

There is a strong correlation *in vivo* between the number of bacteria attached to the periodontal epithelium and the severity of the inflammation (Vaahtoniemi *et al.*, 1993). The capacity of *P. gingivalis* to attach to a variety of squamous human epithelial cell lines *in vitro* has been reported by several investigators. *P. gingivalis* adheres to human primary gingival epithelial cells (Isogai *et al.*, 1988; Lamont *et al.*, 1992; Weinberg *et al.*, 1997; Yilmaz *et al.*, 2002) as well as to epithelial cell lines, such as KB cells (epidermoid carcinoma) (Duncan *et al.*, 1993; Sandros *et al.*, 1993; Huard-Njoroge *et al.*, 1997; Delcourt *et al.*, 1998), HEp-2 cells (laryngeal origin) (Nakagawa *et al.*, 2002a), HeLa cells (cervical carcinoma), and Ca9-22 cells (gingival carcinoma) (Watanabe *et al.*, 1992; Hamada *et al.*, 1994).

Adhesion of *P. gingivalis* to host cells is multimodal (Lamont and Jenkinson, 1998) and involves a variety of cell-surface and extracellular components, including fimbriae, proteases, hemagglutinins, and lipopolysaccharides (LPS) (Cutler *et al.*, 1995). Among the large array of virulence factors produced by *P. gingivalis*, the major fimbriae (FimA), as well as cysteine proteinases (gingipains), contribute to the attachment to and invasion of oral epithelial cells *via* different receptors (Weinberg *et al.*, 1997; T Chen *et al.*, 2001). Adhesion and subsequent invasion of epithelial cells by *P. gingivalis* are likely critical in the pathogenesis of periodontitis, especially during the initial stages of infection.

Roles of FimA in Adhesion to Epithelial Cells

P. gingivalis major fimbriae FimA is considered a critical determinant for the colonization of the oral cavity by this micro-organism. The major fimbriae FimA is composed of a subunit protein (fimbrillin) with a molecular mass ranging from 41 to 45 kDa, depending on the strain (Lee *et al.*, 1991). The gene coding for fimbrillin (*fimA*) is present in a single copy in the chromosome and is monocistronic (Dickinson *et al.*, 1988; Hamada *et al.*, 1994). Amino acid sequence analysis has revealed no significant homology with fimbrial proteins from other bacteria, indicating that *P. gingivalis* produces a unique class of fimbriae (Dickinson *et al.*, 1988). The *fimA* gene is present in all fimbriated strains of *P. gingivalis* so far examined and is absent in afimbriated strains (Holt *et al.*, 1999). Several groups of investigators have provided clear evidence to support the key role of *P. gingivalis* major fimbriae FimA in adhesion to and invasion of many types of mammalian cells, including epithelial cells (Isogai *et al.*, 1988; Njoroge *et al.*, 1997; Sojar *et al.*, 1999). FimA-deficient mutants of *P. gingivalis* have been constructed, and all have an attenuated capacity to adhere to and invade epithelial cells (Njoroge *et al.*, 1997; Weinberg *et al.*, 1997; Umemoto and Hamada, 2003). Invasive strains of *P. gingivalis* carrying a FimA mutation are non-invasive in a tissue culture invasion model and have a significantly reduced ability to cause disease in mice following oral inoculation (Malek *et al.*, 1994). In addition, synthetic peptides analogous to the fimbrillin sequence (Lee *et al.*, 1991) and antibodies directed against fimbriae significantly inhibit the capacity of *P. gingivalis* to adhere to and invade epithelial cells (Isogai *et al.*, 1988; Njoroge *et al.*, 1997; Dorn *et al.*, 2000; Sojar *et al.*, 2002). The allelic variations in *fimA* observed among strains of *P. gingivalis* result in fimbrial diversity in terms of the sizes and N-terminus amino acid sequences of the proteins (Dickinson *et al.*, 1988). The terminal region corresponding to amino acid residues 49 to 90 of the fimbrillin protein has been identified as the potential epithelial cell-binding domain of *P. gingivalis* fimbriae (Sojar *et al.*, 1999). *fimA* genes encoding fimbrillin (FimA) can be grouped into six variants (types I to V and Ib) on the basis of their nucleotide sequences (Hamada *et al.*, 1994; Nakagawa *et al.*, 2002b).

Functional differences in *P. gingivalis* FimA variants with regard to the adhesion to and invasion of human epithelial cells have been the focus of recent investigations. A type II FimA strain (HW24D1) was found to adhere to and invade significantly more epithelial cells than strains with the other known *fimA* genotypes (*fimA* types I, III, IV, and V) (Nakagawa *et al.*, 2002a; Amano *et al.*, 2004). Interestingly, recombinant type II FimA (rFimA) protein adheres to and is internalized by human epithelial HEp-2 cells more efficiently than other rFimA types and accumulates around the nucleus (Nakagawa *et al.*, 2002a). The adhesion and internalization of *P. gingivalis* with type II FimA are inhibited by anti-FimA type II antibodies (Nakagawa *et al.*, 2002a). In contrast, Dorn *et al.* (2000) did not observe any correlation between invasiveness and specific FimA type in *P. gingivalis*.

Electron microscopic analyses revealed that, while epithelial-cell-adhering strains of *P. gingivalis* have abundant peritrichous fimbriae on their surfaces, poorly adhering strains such as W50 and W83 possess very few fimbriae and are sparsely covered with short fimbriae-like structures, referred to as minor fimbriae (Watanabe *et al.*, 1992). Like some of the naturally occurring non-adhering *P. gingivalis* strains, FimA-deficient mutants are devoid of classic fimbriae and produce short fimbriae-like structures that do not react with anti-FimA antibodies (Hamada *et al.*, 1994; Hamada *et al.*, 1996; Arai *et al.*, 2000). Little research has been done on the role of *P. gingivalis* minor fimbriae in adhesion to epithelial cells. Recently, Umemoto and Hamada (2003) demonstrated the importance of the *mfa1* gene, which codes for the minor 67-kDa fimbriae (Hamada *et al.*, 1996), in binding to and invasion of gingival epithelial cells by *P. gingivalis*. Using the homologous recombination technique, they constructed *fimA* (MPG1), *mfa1* (MPG67), and double-knock-out (MPG4167) mutants of strain ATCC 33277. Consistent with previous reports, these authors showed that FimA-deficient mutant MPG1 has a reduced ability to bind to epithelial cells. They also observed that inactivation of the *mfa1* gene results in an increased binding ability of mutant MPG67 compared with the wild-type strain, suggesting that *mfa1* gene mutation may cause changes in cell-surface properties. The mutant MPG67 was shown to exhibit numerous long fimbriae in its surface, and to adhere to human epithelial cells by forming larger clumps by auto-aggregation than did the wild-type strain. In contrast, the capacity of the double-knock-out mutant MPG4167 to adhere to gingival epithelial cells is completely abolished. In addition, all three mutants have a decreased ability to invade gingival epithelial cells, suggesting that both minor Mfa1 (67 kDa) and major FimA (41 kDa) proteins contribute to the ability of *P. gingivalis* to invade epithelial cells.

Integrins as Epithelial Cell Cognate Receptors for FimA

Integrins are a super-family of heterodimeric transmembrane molecules made up of diverse non-covalently-bound α- and β-chains. The nature of the β-chains defines the family of integrins, and both α- and β-chains contribute to the binding of ligands. α2β1, α3β1, α6β1, and α6β4 integrin subunits are expressed by gingival epithelial cells (Hormia *et al.*, 1992; Del Castillo *et al.*, 1996; Thorup *et al.*, 1997). Integrins are involved in cell-extracellular matrix and cell-cell interactions and function as host cell receptors for microbial adhesins. For instance, α5β1 integrin can act as a receptor for the integrin-binding proteins of *Yersinia* spp., *Shigella flexneri, Bordetella pertussis,* and *Pseudomonas aeruginosa* (Watarai *et al.*, 1996; Roger *et al.*, 1999; Ishibashi *et al.*, 2001). More attention is also being paid to the involvement of the target adhesin-receptors expressed on human epithelial cells in the attachment of *P. gingivalis* fimbriae.

Weinberg *et al.* (1997) first identified a 48-kDa surface protein on gingival epithelial cells that binds fimbriated *P. gingivalis* but not afimbriated strains. These authors suggested that the 48-kDa protein may function as a cognate fimbriae receptor and hypothesized that the interaction between fimbriae and this protein may be the first step in a signaling process that mediates the uptake of the bacteria into the host cells. Yilmaz *et al.* (2002) recently reported that there is a physical association between *P. gingivalis* rFimA protein and the β1 integrin and α5β1 integrin heterodimers expressed on gingival epithelial cells. Moreover, the adhesion of type II rFimA-coupled microspheres to HEp-2 cells and the adhesion of *P. gingivalis* cells (type I FimA) to gingival epithelial cells are significantly reduced by anti-α5β1 integrin and anti-β1 integrin antibodies, respectively. The fact that binding inhibition is not completely abolished suggests that there are additional receptors for fimbriae. Nevertheless, antibodies against αVβ3 integrin as well as RDG (arginine-aspartic acid-glycine) peptide have a negligible effect on fimbriae adhesion to epithelial cells (Nakagawa *et al.*, 2002b). A recent study pointed to the participation of host neuraminic acid and glucuronic acid in *P. gingivalis* adherence to KB oral cells (Agnani *et al.*, 2003). The addition of either carbohydrate in a soluble form caused a significant decrease in *P. gingivalis* adhesion to KB cells. However, these authors did not identify cadherins, cellular adhesion molecules (CAM), or β1, β3, and αV integrins as potential receptors that mediate *P. gingivalis* binding to epithelial cells. Carbohydrate chains on epithelial cell membrane glycolipids have been reported to act as receptors for *P. gingivalis* (Hellström *et al.*, 2004). This study also identified a β1 integrin, independent of the RGD binding motif of integrin, as a cognate receptor that mediates *P. gingivalis* fimbriae attachment to epithelial cells.

Fimbriae have been implicated in *P. gingivalis* internalization by gingival epithelial cells (Weinberg *et al.*, 1997). Numerous studies have revealed that ligand binding to integrins initiates a signal transduction cascade that coordinates and regulates a variety of cellular responses that induce the uptake of bacteria by host cells (Rankin *et al.*, 1992; Rosenshine *et al.*, 1992) (see "Internalization" section). Antibodies directed against β1 integrin 2 inhibit the invasion of gingival epithelial cells by *P. gingivalis* by up to 94% (Yilmaz *et al.*, 2002). However, the internalization into gingival epithelial cells of a fimbriae-deficient mutant was not completely blocked by the anti-β1 integrin antibodies. The authors suggested that fimbriae-integrin interactions initiate one pathway that leads to *P. gingivalis* internalization, and that there may be other fimbriae-independent pathways that promote the uptake of bacteria.

Weinberg *et al.* (1997) reported that fimbriae bind to more than one epithelial cell receptor. Two major components with molecular masses of 50 kDa and 40 kDa bind with high affinity to *P. gingivalis* fimbriae (Sojar *et al.*, 2002). The 50-kDa protein corresponding to cytokeratin was identified as an epithelial cell ligand for native fimbriae.

Roles of Gingipains in Adhesion to Epithelial Cells

P. gingivalis is an asaccharolytic bacterium that produces and releases a large array of proteolytic enzymes that play essential roles in the growth of this bacterial species. Among these enzymes, trypsin-like proteinases, called gingipains, have been purified and characterized, and their functions and pathological roles in periodontitis have been extensively investigated over the past decade (Potempa *et al.*, 1995; Genco *et al.*, 1999; Nakayama, 2003). Gingipains are responsible for most of the extracellular and cell-bound proteolytic activities produced by *P. gingivalis*. Three different genes code for arginine-X (Arg-gingipain A and B [*rgpA* and *rgpB*]- and lysine-X (Lys-gingipain [*kgp*])-specific cysteine proteinases, which occur in multiple forms due to proteolytic processing of the initial polypeptides (Potempa *et al.*, 1995; Potempa and Travis, 1996).

Gingipains contribute to the virulence potential of *P. gingivalis* in a multifactorial way, especially by influencing the binding of the bacterium to host tissues. These proteinases may play a role in binding to host cells, either by binding to a cognate receptor or by exposing cryptitope receptors. *P. gingivalis* strains with high levels of trypsin-like protease activity (Arg-gingipain activity) adhere better to human epithelial cells than do strains with lower levels of such activity (Grenier, 1992). The mature forms of Arg-gingipain A and Lys-gingipain possess a catalytic domain and three or four hemagglutinin/adhesin (HA) domains (HA1 to HA4) linked by strong non-covalent bonds (Potempa *et al.*, 1995; DeCarlo and Harber, 1997). The HA domains of Arg-gingipain A and Lys-gingipain share a high degree of homology (over 97%) and have been implicated in the adherence of *P. gingivalis* to gingival epithelial cells (T Chen *et al.*, 2001; Chen and Duncan, 2004). Chen and Duncan (2004) provided additional evidence for the involvement of gingipain adhesin domains in the binding of *P. gingivalis* to epithelial cells. They showed that antibodies against the recombinant adhesin domain of Arg-gingipain block the attachment of native gingipain adhesins to epithelial cells (HEp-2) and inhibit the adherence of *P. gingivalis* to epithelial monolayers. Furthermore, Scragg *et al.* (2002) have suggested that the adhesin domain is involved in the nuclear targeting of *P. gingivalis* W50 proteinases in epithelial cells. More recently, Rautemaa *et al.* (2004) reported that the *P. gingivalis* thiol proteinase localizes near the perinuclear region in the cytoplasm of periodontal epithelial cells.

The catalytic domains of gingipains, and, more specifically, Arg-gingipains A and B, can modulate *P. gingivalis* binding to epithelial cells (T Chen *et al.*, 2001). Chen *et al.* (T Chen *et al.*, 2001) proposed that while the attachment of *P. gingivalis* to epithelial cells is mediated by Kgp and RgpA gingipain HA domains from Kgp and RgpA, detachment of bacterial cells is mediated by RgpA and RgpB catalytic activities. Gingipain

catalytic activities may thus enhance the binding of *P. gingivalis* to host cells by a mechanism, previously described by Gibbons *et al.* (Gibbons, 1989; Gibbons *et al.*, 1990), in which hidden segments of cell adhesion molecules, referred to as 'cryptitopes', are exposed following enzymatic degradation of host matrix proteins.

Gingipains have been shown to play important physiological roles, more particularly in controlling the expression of virulence factors and the stability and/or processing of extracellular and cell-surface proteins (Kadowaki *et al.*, 1998). Both subunits of the two types of fimbriae are regulated by proteolytic processing involving Rgp and Kgp. Rgp processes the precursor form of fimbrillin to the mature form FimA and is involved in fimbrial formation (Onoe *et al.*, 1995; Xie *et al.*, 2000). This is supported by the fact that a double *rgpA/rgpB*-deficient mutant possesses very few fimbriae on its cell surface (Nakayama *et al.*, 1996; Weinberg *et al.*, 1997).

Other Components Involved in Adhesion to Epithelial Cells

The binding of *P. gingivalis* to epithelial cells is a multimodal process involving several bacterial cell-surface structures that may act in concert to allow for binding to host cells. Chandad and Mouton (1995) and Du *et al.* (1997) provided evidence that HA-Ag2—which possesses antigenic, structural, and functional similarities with *P. gingivalis* fimbriae—may be a bacterial ligand involved in the binding of *P. gingivalis* to epithelial cells. Glycosyltransferase, which is coded for by the *gtfA* gene, also has a role in the binding of *P. gingivalis* to epithelial cells (HEp-2) (Narimatsu *et al.*, 2004). A *gtfA*-deficient mutant without mature fimbriae had a reduced ability to auto-aggregate and attach to epithelial cells as well as several extracellular matrix proteins, including type I collagen, laminin, and fibronectin. However, the expression of FimA protein and mRNA in the mutant was not altered. From these observations, the authors suggested that the *P. gingivalis* GtfA is required for a sugar transfer reaction in fimbriae formation, and that GtfA plays an essential role in auto-aggregation and binding to epithelial cells. Consistent with these findings, Duncan *et al.* (1996) previously showed that the overexpression of an open reading frame (ORF) for a putative glycosyltransferase can enhance binding to the cells.

Capsular polysaccharides protect bacterial cells from the host immune system. However, the presence of a capsule may also interfere with the initial step of bacterial binding to epithelial cells. Recently, Dierickx *et al.* (2003) demonstrated that unencapsulated *P. gingivalis* strains adhere significantly more than do their encapsulated variants to epithelial cells from the periodontal pockets of patients with periodontitis. This observation was also reported for various human pathogens, including *Klebsiella pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae* (St Geme and Falkow, 1992; Virji *et al.*, 1995; Sahly *et al.*, 2000). The capsule of *P. gingivalis*, unlike its fimbriae, which make the cell surface hydrophobic (Watanabe *et al.*, 1992), lowers the hydrophobicity of the bacterial surface (van Winkelhoff *et al.*, 1993). This suggests that bacterial surface hydrophobicity contributes to their capacity to adhere to epithelial cells. Encapsulated strains of *P. gingivalis* that are virulent in a mouse model can be classified into six serogroups (K-antigen types; K1 to K6) based on their capsular antigens (Laine and van Winkelhoff, 1998). Correlations between FimA type and capsular antigen type have been established by Amano *et al.* (1999): K1 for type IV FimA; K2, K3, and K5 for type II FimA; K4 for type II FimA; and K6 for type Ib FimA. Interestingly, *P. gingivalis* strains belonging to the K4 serogroup and those with type II FimA both adhere significantly better to epithelial cells than do the other K-antigen and FimA types (Nakagawa *et al.*, 2002a; Yilmaz *et al.*, 2002; Dierickx *et al.*, 2003).

(III) INTERNALIZATION BY EPITHELIAL CELLS

P. gingivalis can be internalized by primary cultures of gingival epithelial cells (Lamont *et al.*, 1992, 1995), oral epithelial cell lines (Duncan *et al.*, 1993; Sandros *et al.*, 1993), and multilayered pocket epithelial cells (Sandros *et al.*, 1994). *P. gingivalis* has also been observed in gingival epithelial cells *in vivo* (Noiri *et al.*, 1997; Rudney *et al.*, 2001). The binding of *P. gingivalis* to epithelial cells induces the formation of membrane invaginations that surround and engulf the bacteria (Lamont *et al.*, 1992; Lamont and Jenkinson, 2000; Houalet-Jeanne *et al.*, 2001). The invasive process occurs within 20 minutes, with large numbers of bacteria localized in the perinuclear region (Belton *et al.*, 1999; Houalet-Jeanne *et al.*, 2001; Park *et al.*, 2004). Once inside the cells, *P. gingivalis* remains viable and is capable of multiplying (primary and KB cells) and surviving for prolonged periods (Papapanou *et al.*, 1994; Lamont *et al.*, 1995; Madianos *et al.*, 1996; Houalet-Jeanne *et al.*, 2001; Yilmaz *et al.*, 2003). Recent studies have shed considerable light on the mechanisms involved in *P. gingivalis*-epithelial cell interactions. *P. gingivalis* has developed strategies to ensure survival in host cells and elicit host responses that result in tissue destruction. Invaded epithelial cells are thought to provide a protective environment for the micro-organisms. The mechanisms *P. gingivalis* uses to internalize into host cells are similar to those described for invasive enteric pathogens (Lamont and Jenkinson, 1998). Many recent studies have focused on *P. gingivalis*' interactions with and invasion of epithelial cells. We used a three-dimensional (3-D) engineered human oral mucosa model, in which epithelial cells interact with fibroblasts in the lamina propria, to demonstrate, for the first time, that *P. gingivalis* can migrate through the basement membrane and reach the underlying connective tissue (Fig. 1B), which is consistent with previous *in vivo* observations of *P. gingivalis* in periodontal connective tissue (Saglie *et al.*, 1988; Andrian *et al.*, 2004). Ultrastructural analyses showed that the infiltrating bacteria penetrate beneath the superficial cell layer and are internalized within multilayered gingival epithelium, as well as at the junction between the stratified epithelium and the lamina propria (Fig. 1A). No visible histological changes were observed in that junction in the 3-D model when a gingipain-null mutant was used, thus providing additional evidence for a critical role of gingipains in tissue destruction.

Bacterium-Host Cell Interactions Contributing to Internalization

P. gingivalis can induce its internalization into normally non-phagocytic gingival epithelial cells by exploiting host cell signaling pathways (Lamont *et al.*, 1995; Watanabe *et al.*, 2001; Yilmaz *et al.*, 2002, 2003). The induction of self-uptake by non-professional phagocytic cells is a property of several major pathogens, including species of the genus *Salmonella, Shigella, Listeria,* and *Yersinia,* and is considered an important virulence determinant (Rosenshine *et al.*, 1992). Recently, there have been in-depth investigations aimed at identifying the signaling pathways used by *P. gingivalis* to enter epithelial cells (Izutsu *et al.*, 1996; Watanabe *et al.*, 2001; Yilmaz *et al.*, 2002, 2003). *P. gingivalis* stimulates signaling pathways that involve mitogen-activated protein kinase (MAPK) activation, protein

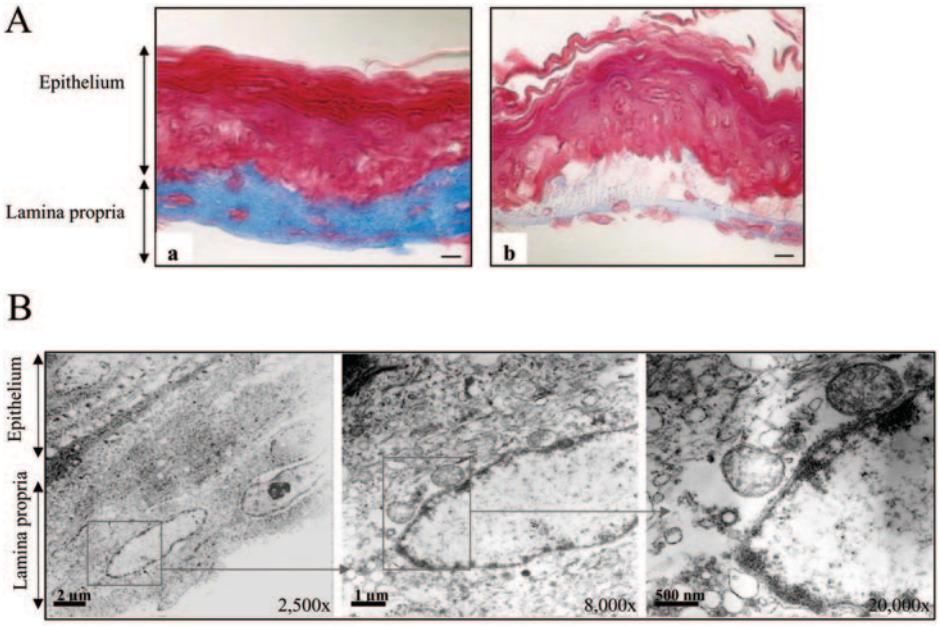

Figure 1. *P. gingivalis* invasion of a three-dimensional (3-D) engineered human oral mucoasa model. **(A)** Structural modifications to the 3-D engineered human oral mucosa model following a *P. gingivalis* ATCC 33277 infection. (a) Uninfected control model; (b) *P. gingivalis* ATCC 33277-infected model. Scale bars, 50 µm. **(B)** Transmission electron micrograph of *P. gingivalis* in a multilayer of epithelial cells and in the underlying connective tissue of a 3-D engineered human oral mucosa model. These Figs. are from Andrian *et al.* (2004), and are reprinted with permission.

phosphorylation, calcium ion fluxes, and the re-organization of cytoskeletal structural proteins. Signals that activate pathways are detected by sensors in the plasma membrane. Integrin receptor activation by *P. gingivalis* fimbriae initiates one of the pathways leading to *P. gingivalis* internalization (Yilmaz *et al.*, 2002). In response to a stimulation, the receptors are activated and initiate intracellular signaling events leading to various cellular responses.

The invasive process of pathogenic bacteria is frequently associated with signaling activities involving MAPKs. MAPKs are serine-threonine protein kinases that play a central role in transmitting the signals from a diverse group of extracellular stimuli to the nucleus, controlling many cell-signaling responses, including cell proliferation and differentiation, stress responses, apoptosis, and cell cycles (Robinson and Cobb, 1997). The MAPK superfamily includes the stress-activated protein kinase c-Jun N-terminal (JNK), the extracellular signal-regulated kinase (ERK), and the p38 MAP kinase (Robinson and Cobb, 1997). Phosphorylation of MAPKs results, in many cases, in subcellular translocation and subsequent activation of diverse substrate proteins, including transcription factors such as nuclear transcriptional factor (NF-κB), other kinases, and cytoskeletal proteins. *P. gingivalis* specifically activates JNK and down-regulates the extracellular signal-regulated kinase ERK1/2 in human gingival epithelial cells, whereas p38 and NF-κB are not activated (Watanabe *et al.*, 2001). JNK activation is related to bacterial invasion, whereas the inhibition of ERK1/2 activity is likely mediated by internalized bacteria, a phenomenon that possibly prevents the activation of NF-κB. Specific inhibitors of MEK1/2, which is the upstream regulator of ERK1/2 activation, do not affect the invasion rate of bacteria. Exposure of epithelial cells to Rho family guanosine-5′-triphosphatase (GTPase)-specific inhibitor toxin B does not prevent JNK phosphorylation, suggesting that stimulation of JNK may occur at a step subsequent to GTPase activation. These results indicate that internalization of *P. gingivalis* is independent of MEK/ERK1/2 signaling pathways. Other evidence points to the involvement of MAPK responses in the *P. gingivalis* invasion process, since bacteria rendered non-invasive by heat or sodium azide treatments (Lamont *et al.*, 1995; Belton *et al.*, 1999) do not disrupt MAPK responses.

Internalization of *P. gingivalis* is correlated with tyrosine phosphorylation of eukaryotic cells. Genistein, a tyrosine kinase inhibitor, strongly impairs *P. gingivalis* internalization by epithelial cells, suggesting the involvement of tyrosine phosphorylated proteins in signal transduction during invasion (Sandros *et al.*, 1996; Watanabe *et al.*, 2001). A 43-kDa eukaryotic cell protein corresponding to MAPK has been identified as a target for protein tyrosine phosphorylation (Sandros *et al.*, 1996). *P. gingivalis* fimbriae interactions with its cognate receptor β1-integrin on the surfaces of gingival epithelial cells can initiate signal transduction that may lead to bacterial uptake by epithelial cells (Lamont *et al.*, 1995; Belton *et al.*, 1999; Yilmaz *et al.*, 2002). When fimbriae bind to integrin, downstream signaling events—including the tyrosine phosphorylation of a 68-kDa focal adhesion signaling component (paxillin) and the activation of a focal adhesion tyrosine kinase (FAK)—have been observed (Yilmaz *et al.*, 2002). Paxillin and FAK are believed to play an important role in an integrin-mediated signal transduction cascade that regulates adhesion, survival, proliferation, differentiation, and migration (Clark and Brugge, 1995). In mammalian cells, the phosphorylation of paxillin and FAK leads to the activation of other specific signaling molecules, thus promoting the assembly of focal adhesion complexes subsequent to integrin activation (Clark and Brugge, 1995). Immunofluorescence staining has been used to demonstrate that there is a significant recruitment of phosphorylated paxillin and FAK from the cytosol to the cell periphery to form focal adhesion complexes. Following prolonged exposure of epithelial cells to *P. gingivalis*, the focal adhesion complexes dissociate, and the paxillin and FAK are redistributed into the cytoplasm, mainly to the perinuclear area. This is correlated with the localization of bacterial cells in the perinuclear region (Yilmaz *et al.*, 2003).

The formation of integrin-associated focal adhesion cytoskeletal proteins is associated with actin microfilament and microtubule cytoskeletal re-organization (Clark and Brugge, 1995). Increasing numbers of reports have highlighted the involvement of both actin microfilaments and microtubule cytoskeleton re-arrangements during the *P. gingivalis* invasion process, which may promote the invaginations of the membrane that bring the bacteria into the host cells (Lamont *et*

al., 1995). *P. gingivalis* is unable to enter epithelial cells that have been treated with microtubule polymerization inhibitors (nocodazole and colchicine) and a microfilament inhibitor (cytochalasin D) (Lamont *et al.*, 1995). Immunofluoresence analyses have revealed that the invasion of *P. gingivalis via* integrin contact induces the nucleation of actin filaments, which form thin filamentous microspike-like structures and long stable filaments distributed throughout the cell. A significant disassembly and nucleation of the actin and microtubule filamentous network after extended periods of infection have also been noted (Yilmaz *et al.*, 2003). Together, these results suggest that bacterial receptors and phosphotyrosine-dependent intracellular signaling trigger an internalization process involving a re-arrangement of the cytoskeleton.

Increased intracellular calcium concentrations in epithelial cells have been associated with invasion by *P. gingivalis*. Following contact with epithelial cells, *P. gingivalis* causes a transient increase in Ca^{2+} in the cells, resulting from the release of calcium ions from thapsigargin-sensitive intracellular stores (Izutsu *et al.*, 1996). Belton *et al.* (2004) reported that *P. gingivalis* induces oscillations in nuclear and cytoplasmic spaces by activating a Ca^{2+} influx through Ca^{2+} channels in gingival epithelial cells. The fluctuation in cytosolic calcium ions, which are important mediators of eukaryotic signaling, may initiate a cascade of intracellular responses that mediate cytoskeletal remodeling. Yilmaz *et al.* (2002) suggested that the integrin receptor initiates one of the pathways by which cell signal transduction may activate cytoskeletal elements. The molecular signaling events that occur during the invasion of gingival epithelial cells by *P. gingivalis* are illustrated in Fig. 2.

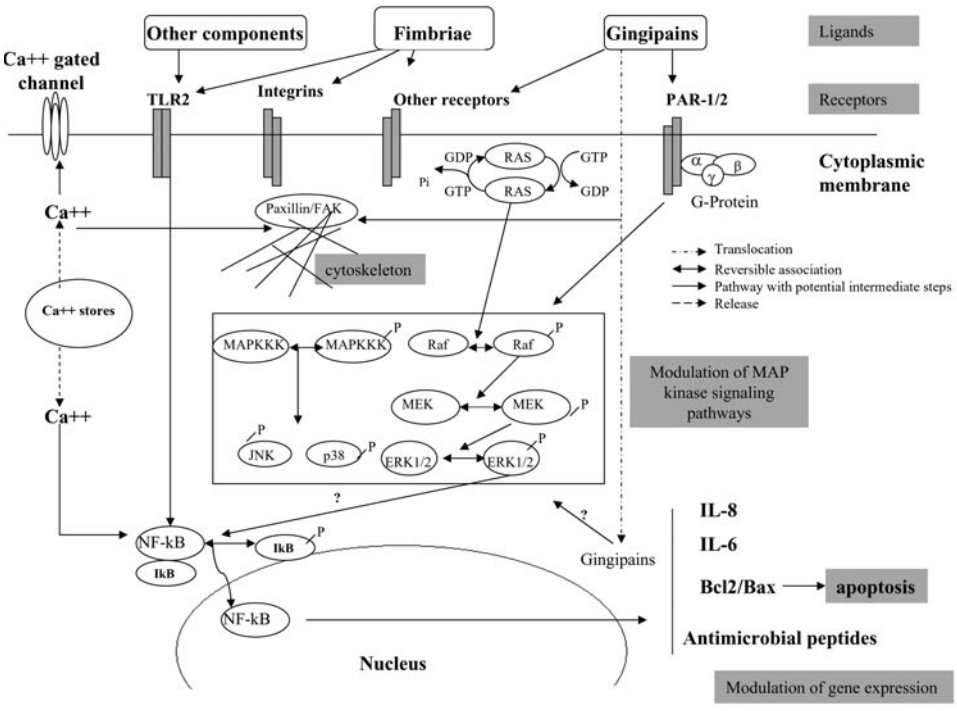

Figure 2. Current model of *P. gingivalis* interactions with gingival epithelial cells. Interactions between *P. gingivalis* fimbriae, gingipains, and other potential adhesins with various epithelial cell-surface receptors (PAR-1/2, TLR2, integrins) lead to the activation of epithelial cell signaling pathways and the modulation of gene expression. The entry of *P. gingivalis* into epithelial cells is associated with the phosphorylation/dephosphorylation of signaling molecules such as MAP kinases, the modulation of calcium influx, and the re-arrangement of the cell cytoskeleton. Interactions of fimbriae with integrins initiate down-stream signaling events, including the formation of focal adhesion molecules such as FAK/paxillin. The intracellular localization of gingipains can interfere with the pathways of the focal adhesion molecules FAK/paxillin and MAP kinase. This model has been adapted from a model proposed by Lamont and Jenkinson (1998). See text for references. Abbreviations: Ca++, calcium; ERK, extracellular signal-regulated kinase; GTP, guanosine triphosphate; IkB, inhibitory factor; ILβ, interleukin; JNK, c-Jun N-terminal; MAPKKK, mitogen-activated protein kinase kinase kinase; MEK, extracellular signal-regulated kinase activator kinase; NF-κB, nuclear transcriptional factor; PAR, protease-activated receptor; RAS, small-GTPase; and TLR, Toll-like receptor.

Differences between KB Cells and Gingival Epithelial Cells

There is evidence that *P. gingivalis* internalization is dependent on a physical association between *P. gingivalis* and cell-surface receptors and the subsequent activation of intracellular signaling pathways. *P. gingivalis* can recognize different host cell types and is capable of targeting specific and distinct eukaryotic signaling pathways to induce uptake into the eukaryotic cell. *P. gingivalis* entry into transformed KB cells is different in several respects from its entry into primary cultures of gingival epithelial cells. Binding to and entry into epithelial cells from primary gingival tissue cultures are more efficient than with transformed cells such as KB cells (Belton *et al.*, 2004), which may be due to the low numbers of *P. gingivalis* receptors on KB cells (Huard-Delcourt *et al.*, 1998). Entry into KB cells involves a receptor-mediated endocytosis pathway and tyrosine phosphorylation of a eukaryotic cell protein corresponding to MAPK ERK1/2 (Sandros *et al.*, 1996), which is down-regulated in gingival epithelial cells (Watanabe *et al.*, 2001). While *P. gingivalis* cells internalized in primary gingival epithelial cells are not surrounded by membrane vacuoles (Lamont *et al.*, 1995; Andrian *et al.*, 2004), bacteria internalized in KB cells are frequently surrounded by endosomal membranes, although free-floating bacteria are also present in the cytoplasm (Sandros *et al.*, 1993; Njoroge *et al.*, 1997; Houalet-Jeanne *et al.*, 2001). The functional significance of the bacterial association with endosomal vacuoles remains to be determined.

Expression of Virulence Factors by *P. gingivalis* during Entry into Epithelial Cells

A crucial part of bacterial pathogenicity involves the expression, in the host cell cytoplasm, of virulence factors that interfere with and alter host processes (Thanassi and Hultgren, 2000). When *P. gingivalis* enters into contact with epithelial cells, it secretes a novel set of proteins that may have intracellular effector

activities (Park and Lamont, 1998). Park and Lamont (1998) identified, in *P. gingivalis*, a contact-dependent protein secretion pathway similar to that required for the translocation of proteins, one that mediates the entry of invasive enteric pathogens into host cells (Zierler and Galan, 1995). However, the *P. gingivalis*-dependent extracellular secretion pathway has not yet been characterized. The attachment of *P. gingivalis* to the epithelial cell surface leads to the secretion of FimA, homologs of a phosphoserine phosphatase, and polysaccharide biosynthetic enzymes (Park and Lamont, 1998; W Chen *et al.*, 2001). In contrast, the secretion of gingipain cysteine proteinases is inhibited following brief contact with primary cultures of gingival epithelial cells, whereas prolonged contact with epithelial cells induces an increased secretion of Lys-gingipain (Park and Lamont, 1998; Agnani *et al.*, 2000). Temporally, up-regulation of the expression of *P. gingivalis* genes, which are essential for maintaining cellular function and viability, is also induced following *P. gingivalis* contact with HEp-2 human epithelial cells (Hosogi and Duncan, 2005). Increased expression of genes involved in oxidative stress—including the superoxide dismutase (*sod*), alkyl hydroxide reductase (*ahpCF*), thioredoxin peroxidase (*tpx*), and thioredoxin reductase (*trxB*) genes, which are involved in the detoxification of reactive oxygen species (ROS) and peroxides—has been reported. Heat-shock genes involved in maintaining protein stabilization and cellular functions—including *groEL, dnaK,* and *htpG*—are also expressed by *P. gingivalis*.

Novel factors have been identified that play a role during *P. gingivalis* entry into gingival epithelial cells. These include a metallo-endopeptidase (PepO), a cation-transporting ATPase, and an ATP-binding cassette (ABC) transporter (Ansai *et al.*, 2003; Park *et al.*, 2004). The independent inactivation of each gene has revealed that most mutants display an indistinct microvillus formation of the actin cytoskeleton and are poorly internalized compared with parent strains (Park *et al.*, 2004). Park *et al.* (2004) have suggested that internalization-defective mutants may not induce the formation of actin stress fibers, suggesting a role for these proteins in the induction of host cytoskeletal responses. Once inside the epithelial cell, *P. gingivalis* releases outer membrane vesicles (Sandros *et al.*, 1994; Houalet-Jeanne *et al.*, 2001). The proteolytic activity of these extracellular structures may be responsible for the degradation of host proteins.

(IV) EPITHELIAL CELL RESPONSES

In addition to providing a physical barrier against invading pathogens, epithelial cells play an important role in innate host immune defenses. Interactions between *P. gingivalis* and epithelial cells lead to the activation of several complex signaling cascades, which ultimately regulate the transcription of target genes that encode effectors and regulators of the immune response. Effectors of the innate immune system, pro-inflammatory cytokines, chemokines, matrix metalloproteinases (MMPs), and antimicrobial peptides are up-regulated and may have a direct impact on disease progression and the inflammation processes.

Cell-surface Modifications and Apoptosis

While gingival epithelial cells containing internalized *P. gingivalis* exhibit morphological changes such as cell rounding and detachment from the substratum (Lamont *et al.*, 1992; Belton *et al.*, 1999), they do not undergo apoptosis and maintain their physiological integrity for extended periods (Nakhjiri *et al.*, 2001). *P. gingivalis* gingipains have been implicated in morphological changes to gingival epithelial cells (Johansson and Kalfas, 1998) through the degradation of cell adhesion molecules, including occludin, E-cadherin, beta-1 integrin (Katz *et al.*, 2000), ICAM-1, vascular cell adhesion molecule-1, and very late antigen-1 (Wang *et al.*, 1999; Tada *et al.*, 2003). We have demonstrated that *P. gingivalis* LPS can potentiate syndecan-1 shedding from the gingival epithelial cell surface by exploiting host shedding signaling pathways. We have also showed that gingipains contribute to the release of syndecan-1 from the gingival epithelial cell surface (Andrian *et al.*, 2006). By shedding the syndecan-1 ectodomain, *P. gingivalis* may modulate the activation of host effectors and disrupt cell-cell interactions mediated by syndecan-1 (Andrian *et al.*, 2005). *P. gingivalis* blocks camphotecin-mediated apoptosis of epithelial cells, up-regulates anti-apoptotic molecule Bcl-2 expression, and down-regulates pro-apoptotic molecule Bax expression (Nakhjiri *et al.*, 2001). Unlike what has been observed with primary gingival epithelial cells, *P. gingivalis* gingipains induce cell detachment and apoptosis in KB oral cells through the cleavage of N-cadherin and β1-integrin (Z Chen *et al.*, 2001). While the signaling pathways mediated by gingipains are not fully understood, β-integrin may be involved in inducing apoptosis (Yilmaz *et al.*, 2002; Sheets *et al.*, 2005). β-integrin is expressed on gingival and junctional epithelial cells (Hormia *et al.*, 1990) and acts as an adhesin receptor for fimbriae (Yilmaz *et al.*, 2002). Integrins are associated with numerous survival pathways, including those leading to apoptosis (Matter and Ruoslahti, 2001).

Membrane-bound mucin 1 (MUC1) is a component of the non-immune host defense system and mediates the attachment of bacteria to host cells (Lillehoj *et al.*, 2001). MUC1 activates anti-apoptotic pathways in rat fibroblasts (Raina *et al.*, 2004). MUC1 is ubiquitously expressed on oral epithelial surfaces and may provide protection against bacterial infections (Offner and Troxler, 2000). An increased expression of MUC1 has been observed in KB oral cells stimulated with *P. gingivalis* whole cells, but not with *P. gingivalis* LPS (Li *et al.*, 2003). Li *et al.* (2003) reported that pro-inflammatory cytokines—including IL-1β, IL-6, TNF-alpha, and IFN-gamma—up-regulate MUC1 expression, leading to an over-expression of MUC1 on the cell surface. These results suggest that MUC1 plays a role in host defenses. However, the mechanism by which MUC1 participates in host defenses and in the maintenance of cell integrity is not known.

Cytokine Production

P. gingivalis induces a strong pro-inflammatory cytokine response in gingival epithelial cells *in vitro*, which is correlated with the adhesive/invasive potential of *P. gingivalis* (Njoroge *et al.*, 1997; Sandros *et al.*, 2000). Kesavalu *et al.* (2002) reported that *P. gingivalis* induces pro-inflammatory cytokine expression in an *in vivo* murine calvarial model. They showed that *P. gingivalis* induces different levels of cytokine expression, the highest being TNF-α, followed by IL-1β and IL-6. Sandros *et al.* (2000) reported that the binding of *P. gingivalis* to the surfaces of epithelial cells results in an increased secretion of IL-1, IL-8, IL-6, and TNF-α. *P. gingivalis* fimbriae and LPS can also up-regulate IL-1β, IL-6, IL-8, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) gene expression and protein synthesis in gingival epithelial cell lines (Njoroge *et al.*, 1997; Sandros *et al.*, 2000). Fimbriae use Toll-like receptor 2 (TLR2), which is predominantly expressed in human gingival epithelial cells, as a co-receptor to induce cell activation and IL-8 expression (Asai *et al.*, 2001). LPS mediates cytokine release in many cell lines, including monocytes

and gingival fibroblasts (Wilson *et al.*, 1996), and acts as an agonist and antagonist of p38 MAPK activation (Darveau *et al.*, 2002). However, LPS-mediated receptor activation on epithelial cells remains to be better-characterized. Kusumoto *et al.* (2004) have reported that *P. gingivalis* component(s) distinct from fimbriae and LPS can induce IL-8 and MCP-1 production through the activation of TLR2 and NF-κB in human gingival epithelial cells. They suggested that polysaccharidic components have a role in this induction.

While pro-inflammatory chemokine IL-8 is up-regulated in oral and gingival epithelial cells following challenge with several periodontopathogens—including *A. actinomycetemcomitans, Fusobacterium nucleatum, Eikenella corrodens,* and *Prevotella intermedia* (Yumoto *et al.*, 1999; Han *et al.*, 2000)—*P. gingivalis* inhibits IL-8 expression and secretion by gingival epithelial cells following an extended period of infection (Madianos *et al.*, 1997; Darveau *et al.*, 1998; Huang *et al.*, 1998, 2001, 2004). This may inhibit neutrophil transepithelial migration and accumulation in infection sites (Madianos *et al.*, 1997), thereby allowing *P. gingivalis* to escape host defense mechanisms and survive for long periods in periodontal tissue. Unlike the IL-1β response, which is strongly correlated with the adhesive and invasive potential of *P. gingivalis*, IL-8 up/down-regulation is independent of the invasive property (Huang *et al.*, 2001). *P. gingivalis* may inhibit IL-8 accumulation at two levels: (i) IL-8 degradation by proteinases, and (ii) IL-8 regulation by unidentified factor(s) (Darveau *et al.*, 1998; Mikolajczyk-Pawlinska *et al.*, 1998; Zhang *et al.*, 1999; Huang *et al.*, 2001). The regulation of IL-8 expression is dependent on the activation of the NF-κB, MAPK p38, and MEK/ERK pathways (Huang *et al.*, 2004). Pre-treatment of *P. gingivalis* with heat or proteases enhances IL-8 mRNA induction, suggesting that proteinaceous components are involved in IL-8 gene regulation. Fimbriae activate NF-κB and up-regulate IL-8 expression *via* TLR2 (Asai *et al.*, 2001). While Kusumoto *et al.* (2004) reported that *P. gingivalis* components induce IL-8 up-regulation and NF-κB activation *via* TLR2, proteinase and heat treatments are ineffective in preventing the induction. In contrast, down-regulation of IL-8 mRNA by viable *P. gingivalis* involves the MEK/ERK, but not the NF-κB or MAPK p38 pathway (Huang *et al.*, 2004). Huang *et al.* (2004) suggested that the up-/down-regulation of IL-8 may involve MEK/ERK pathways that may be regulated by different factors. *P. gingivalis* cysteine proteinases may disrupt multi-signaling pathways, including those leading to the activation of MAPKs and NF-κB, as has been reported for the *Yersinia* YopJ protein (Palmer *et al.*, 1999), suggesting that gingipains may be responsible for the induction of MEK/ERK regulation (Watanabe *et al.*, 2001). *P. gingivalis* gingipains mediate IL-8 up-regulation in gingival epithelial cells and cause proteolysis of focal adhesion molecules such as paxillin and FAK (Hintermann *et al.*, 2002; Chung *et al.*, 2004).

P. gingivalis gingipains may play a pivotal role in the evasion of host defenses by disrupting cytokine signaling networks. Gingipains cleave and degrade most pro-inflammatory cytokines, including IL-1β (Fletcher *et al.*, 1997), IL-6 (Banbula *et al.*, 1999), TNF-α (Calkins *et al.*, 1998), and IL-8 (Mikolajczyk-Pawlinska *et al.*, 1998; Zhang *et al.*, 1999). Interestingly, RgpB activates the protease-activated receptors (PAR) PAR-1 and PAR-2 on the KB cell surface and induces an increase in intracellular calcium levels, resulting in an up-regulation of IL-6 secretion (Lourbakos *et al.*, 2001). Moreover, gingipains can inactivate the effector molecules of the innate and acquired immune systems (Lamont and Jenkinson, 1998; Imamura, 2003) and therefore contribute to the progression of periodontal diseases.

Similarly, *P. gingivalis* down-regulates the expression of intercellular adhesion molecule-1 (ICAM-1) by gingival epithelial cells and degrades secreted ICAM-1 (Huang *et al.*, 2001). Both IL-8 and ICAM-1 are responsible for the accumulation and activation of neutrophils in the epithelium (Sugiyama *et al.*, 2002). *P. gingivalis* also down-regulates the expression of 4 genes related to host innate immunity—including IL-1β, IL-8, macrophage protein-alpha 2, and migration inhibitory factor-related protein-14—in gingival epithelial cells (Huang *et al.*, 2004).

Antimicrobial Peptide Production

Antimicrobial peptides have emerged as potential participants in host defenses at mucosal surfaces. Antimicrobial peptides are small, endogenous, polycationic molecules that constitute a ubiquitous and significant component of innate immunity. Most of these peptides exert their antimicrobial activity by interacting with the bacterial cell membrane, leading to the disorganization of the bilayer and resulting in pore formation (Brogden, 2005). Among the antimicrobial peptides expressed by gingival epithelial cells, calprotectin and β-defensin have been reported to provide protection against *P. gingivalis* infections. Elevated calprotectin levels have been detected in gingival crevicular fluid (GCF) from patients with periodontitis (Nakamura *et al.*, 2000). The expression of calprotectin by gingival epithelial cells enhances resistance to *P. gingivalis* infections and is correlated with reduced invasion and binding of *P. gingivalis* to epithelial cells (Nisapakultorn *et al.*, 2001). Calprotectin is a cytosolic calcium-binding protein with broad-spectrum antimicrobial activity (Steinbakk *et al.*, 1990). Calprotectin may kill or inhibit *P. gingivalis* growth within epithelial cells, or may interfere with the internalization process. The antimicrobial peptide human β-defensin (hBD), which is found primarily in association with gingival epithelial surfaces, may restrict intracellular bacterial replication and prevent the physical destruction of host cells. Three human β-defensins—hBD-1, hBD-2, and hBD-3—are expressed in gingival epithelial cells. While hBD-1 expression is constitutive in human epithelial cell, hBD-2 and hBD-3 expression is modulated by pro-inflammatory mediators or bacterial products (Krisanaprakornkit *et al.*, 1998; Dale *et al.*, 2001; Diamond *et al.*, 2001). Exposure of primary cultures of gingival epithelial cells to *P. gingivalis* increases the expression of hBD-2 (Chung *et al.*, 2004). Gingipains are directly involved in the regulation of hBD-2 in cultured gingival epithelial cells (Chung *et al.*, 2004). hBD-2 expression uses several signaling pathways. Gingipains can activate one pathway through the PAR-2 receptor host-signaling pathway, which is dependent on phospholipase C and calcium influx (Krisanaprakornkit *et al.*, 2003; Chung *et al.*, 2004). The regulation of hBD-2 expression also involves the MAPK and NF-κB signaling pathways (Krisanaprakornkit *et al.*, 2002). Neither LPS nor fimbriae appear to be involved in hBD-2 induction by gingival epithelial cells (Chung *et al.*, 2004).

Modulation of MMP Secretion

P. gingivalis can advance into deeper epithelial layers (Papapanou *et al.*, 1994) *via* a paracellular pathway through the degradation of epithelial cell-cell junctional proteins (Katz *et al.*, 2000). However, the intercellular spread of *P. gingivalis* has not been

observed. *P. gingivalis* also penetrates gingival tissue *in vitro* and *in vivo* to initiate tissue destruction (Saglie *et al.*, 1988). *P. gingivalis* degrades basement membrane proteins in a reconstituted basement membrane model, which indicates that it may be able to penetrate the connective tissue (Andrian *et al.*, 2004). The capacity of *P. gingivalis* to breach epithelial cell integrity may allow it to migrate into deeper tissues, which, in turn, may lead to tissue destruction mediated by both bacterial and host proteinases. Stimulated gingival epithelial cells can produce different proteolytic enzymes that contribute to the degradation of intracellular and extracellular host proteins. MMPs, which are zinc-dependent neutral proteinases, are implicated in tissue remodeling and cell migration during the normal turnover of periodontal tissue, and may also be involved in the pathophysiology of periodontitis (Uitto *et al.*, 2003). There is a positive correlation between the presence of high levels of specific MMPs (MMP-1, -8, -9, -13) and the severity of periodontitis (Tervahartiala *et al.*, 2000). Cultured gingival epithelial cells can produce collagenase and gelatinase activities, including collagenase-1 (MMP-1), collagenase-3 (MMP-13), gelatinase A (MMP-2), gelatinase B (MMP-9), and matrilysin (MMP-7), as well as chymotrypsin-like activities (Uitto *et al.*, 2003). Epithelial cells also express MMPs *in vivo* (Tervahartiala *et al.*, 2000; Uitto *et al.*, 2003). These enzymes have the potential to play an important role in the tissue destruction observed in periodontitis, since they can degrade extracellular matrix proteins, including collagen types I, III, and IV, fibronectin, tenascin, elastin, entactin, and proteoglycans. MMP secretion can be induced in cultured gingival epithelial cells by several cytokines as well as by bacterial components such as lipopolysaccharide and phospholipase C (Birkedal-Hansen *et al.*, 1993; Ding *et al.*, 1995; Sorsa *et al.*, 2004). The regulation of MMP-9 production by gingival epithelial cells is disrupted following contact with *P. gingivalis* (Fravalo *et al.*, 1996; DeCarlo *et al.*, 1997, 1998), a phenomenon that may interfere with extracellular matrix repair and re-organization (Grayson *et al.*, 2003). Purified gingipain proteinases of *P. gingivalis* up-regulate MMP-8 and MMP-3 expression in rat mucosal epithelial cells (DeCarlo *et al.*, 1998) and activate latent forms of MMPs such as MMP-1, -3, and -9 (DeCarlo *et al.*, 1997).

(V) CONCLUSIONS

The outcome of the molecular cross-talk between bacteria and host cells has major implications for health and disease. *P. gingivalis* has developed adaptive strategies to invade gingival epithelial cells and overcome the protective defense mechanisms of epithelial cells. *P. gingivalis* adheres to and invades epithelial cells by targeting specific host receptors, modulating host signaling events, and deregulating the host cytokine network. *P. gingivalis*-epithelial cell interactions result in the disruption of tissue homeostasis and the structural and functional integrity of gingival epithelial cells, which may contribute to bacterial persistence and the progression of chronic manifestations of periodontal diseases. Future studies are now focusing on understanding the signaling events that culminate in the epithelial invasion processes of *P. gingivalis*. A 3-D mucosal model has enabled us to study the interactions between epithelial cells and the underlying connective tissue during *P. gingivalis* infections. We and other groups have used human gingival fibroblasts/polymorphonuclear leukocytes and epithelial cell/macrophage cell co-culture models to gain a more comprehensive view of the regulation of local immune mechanisms (Nemoto *et al.*, 2000; Bodet *et al.*, 2005). Further studies involving microbial consortia and cell co-culture models will lead to a better understanding of the complexity of the pathogenic process of mixed infections such as periodontitis.

ACKNOWLEDGMENT

This work was supported by the Canadian Institutes of Health Research.

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