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Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva

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Abstract

Objectives: The primary aetiologic factor of periodontal disease is the bacterial biofilm. Gram-positive and Gram-negative bacteria possess a plethora of structural or secreted components that may cause direct destruction to periodontal tissues or stimulate host cells to activate a wide range of inflammatory responses. These responses are intended to eliminate the microbial challenge, but may often cause further tissue damage.

Methods: This review has been divided into three parts: (a) bacterial virulence factors, which includes basic information on bacterial virulence factors, and the principle inflammatory responses that host cells elicit against these factors, (b) main receptors and signalling pathways, which includes basic information about the main receptors that interact with the bacterial virulence factors, the nature of these interactions, and the activated signalling pathways that lead to inflammatory responses, and (c) initiation of inflammation, which includes a model by which the virulence factors may interact with host cells and lead to inflammatory responses in the gingiva.

Findings and Conclusions: Bacterial components/virulence factors may be involved in modulating inflammatory responses and include: lipopolysaccharides (LPS), peptidoglycans, lipotechoic acids, fimbriae, proteases, heat-shock proteins, formyl-methionyl peptides, and toxins. Potential host cell receptors involved in recognizing bacterial components and initiating signalling pathways that lead to inflammatory responses include: Toll-like receptors (TLRs), CD14, nucleotide-binding oligomerization domain proteins (Nod) and G-protein-coupled receptors, including formyl-methionyl peptide receptors and protease-activated receptors. Of the above bacterial and host molecules, evidence from experimental animal studies implicate LPS, fimbriae, proteases, TLRs, and CD14 in periodontal tissue or alveolar bone destruction. However, evidence verifying the involvement of any of the above molecules in periodontal tissue destruction in humans does not exist.

Key words: inflammation; toll like receptors; bacterial virulence; lipopolysaccharide; CD 14

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The primary aetiologic factor of periodontal disease is the bacterial biofilm. Several hundred different bacterial species colonize the sulcus and the periodontal pockets. Some of them are protective for the host, but others may be pathogenic initiating inflammatory processes in the gingiva, leading to gingivitis and in some cases periodontitis. Gram-positive and Gram-negative bacteria possess a plethora of structural or secreted components that may cause direct destruction to periodontal tissues, or stimulate host cells to activate a wide range of inflammatory responses. These responses are intended to eliminate the microbial challenge but may often cause further tissue damage. This review sets out to address the range of microbial and host interactions which result in inflammation of the gingiva.

Lipopolysaccharides (LPS), peptidoglycans (PGNs), lipotechoic acids (LTA), fimbriae, proteases, heat-shock proteins (HSPs), formyl-methionyl-leucyl-phenylalanine (fMLP), and toxins are probably the most studied bacterial

components that may be deleterious for the host. Some of these components are common to bacteria and others are specific to either Gram positive or Gram negative. Variations of these bacterial components may also be seen among various species, or even among different strains of the same species. Despite their structural heterogeneity, most of these molecules seem to have conserved motifs known as pathogen-associated molecular patterns (PAMPs), which are recognized by a relatively small number of host cells receptors called pattern recognition receptors (PRRs). Hence, the same PRR may recognize the same bacterial component from different species and sometimes, different bacterial components.

As these molecules can directly or indirectly induce periodontal destruction it is very tempting to consider them as virulence factors. This could be true for proteases and toxins, which are developed as a microbial adaptation to the unique environment within the host, but not for conserved molecules such as the PAMPs (LPS, PGN, LTA, etc.). Virulence factors are produced by pathogens to allow them to interact and survive within the host by assisting in invasion, colonization, adjusting to new nutrient sources and avoiding the ever surveillant host immune responses. On the other hand, PAMPs did not evolve to interact with the host immune system, but are considered to perform essential physiologic functions for the bacteria. As PAMPs are conserved in nature, host cells detect these PAMPs through PRRs and mount an inflammatory and or immune response to kill them. Inevitably, the response to both PAMPs and virulence factors may lead to host tissue destruction. While acknowledging that there are differences, for simplicity, we will refer to PAMPs along with proteases and toxins, etc., collectively as microbial virulence factors (Medzhitov 2001).

This review has been divided into three parts: (a) bacterial virulence factors, which includes basic information on bacterial virulence factors, and the principle inflammatory responses that host cells elicit against these factors, (b) main receptors and signalling pathways, which includes basic information about the main receptors that interact with the bacterial virulence factors, the nature of these interactions, and the activated signalling pathways that lead to inflammatory responses, and (c) initiation of inflammation, which includes a model by which the virulence factors may interact with host cells in vivo and lead to inflammatory responses in the gingiva. As some of the virulence factors have various activities and as inflammatory responses are, by nature, complex processes, we will focus only on initial events that are crucial in setting up the inflammatory response, such as the increase in vascular permeability, expression, and/or secretion of adhesion molecules, chemoattractants, and pro-inflammatory cytokines, which result in leucocyte recruitment and function.

Bacterial Virulence Factors

LPS

The outer membrane of Gram-negative bacteria consists of LPS. This is an amphipathic molecule that can be divided structurally in three parts: (a) the O-polysaccharide (or O-antigen), (b) the core polysaccharide, and (c) the lipid A. The O-antigen is a long, linear polysaccharide consisting of 50–100 repeating saccharide units of four to eight sugars per unit. These units may differ even between bacterial strains of the same species, which is demonstrated by the hundreds of serotypes of Gram-negative bacteria. The O-antigen is the outmost part of LPS expressed on bacteria and therefore is the major antigen targeted by host antibody responses. The core polysaccharide is less variable and is divided to the outer and inner core. The outer core typically consists of common hexose sugars (glucose, galactose, etc.), while the inner core, which is proximal to lipid A contains a high proportion of unusual sugars (3-deoxy-D-manno-octulosonic acid and L-glycero-D-manno heptose). Both inner and outer core sugar residues can be substituted with charged groups (phosphate, pyrophosphate, etc.), which maintain a close association with Ca^{2+} and Mg^{2+} ions that are required for holding the outer membrane together. Finally, the lipid A is the highly hydrophobic and endotoxically active part of LPS. It is composed of a β-D-GlcN-(1–6)-α-D-GlcN (GlcN: glucosamine) disaccharide carrying two phosphoryl groups at positions 1 and 4′. These groups can be further substituted with phosphate, ethanolamine, ethanolamine phosphate, etc. To this structure are attached up to four acyl chains by ester or amid linkage, which can then in turn be substituted by further fatty acids that vary in length and number among species. The chemical structure of *Escherichia coli* lipid A is considered to be close to that optimally recognized by human cellular LPS responses. Deviations from *E. coli* lipid A structure are typically seen to result in molecules of lower biological activity (Rietschel et al. 1994). LPS exerts its biological functions by signalling via Toll-like receptor-4 (TLR-4). One of the few exceptions includes the LPS of the periodontopathogen *Porphyromonas gingivalis*, which has been shown to signal through TLR-2. This difference has been attributed to the shape of *P. gingivalis* LPS (Netea et al. 2002) and is considered responsible for some quantitative and qualitative variations in gene expression induced by this LPS compared with that of LPS of other bacteria (Hirschfeld et al. 2001).

Among other properties, LPS from Gram-negative bacteria is well known for its ability to induce a wide range of pro-inflammatory responses. Specifically, LPS (*E. coli*) can stimulate macrophages/monocytes to produce pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumour necroses factor-α (TNF-α), IL-6, interferon-γ (IFN-γ), IL-12, IFN inducible protein-10 (IP-10), chemotactic cytokines, like monocyte chemotactic protein-5 (MCP-5), IL-8, MIP-1α and MIP-2, prostaglandin E_2 (PGE_2), and NO. *P. gingivalis* LPS is less potent and induces less IL-1β, TNF-α, IL-6, IP-10, MIP-1, and MIP-2, while it produces very few, if at all, IFN-γ, MCP-5, IL-12, and NO (Shapira et al. 1998, Hirschfeld et al. 2001). Interestingly, one study by Shapira et al. (1998) showed that LPS of *P. gingivalis* A7436, although stimulating lower levels of NO, induced similar high levels of TNF-α from human monocytes as other potent LPS. This demonstrates that different strains of *P. gingivalis* may have different potentials in activating host cells. LPS from other periodontal pathogens, such as *Actinobacillus actinomycetemcomitans* and *Prevotella intermedia* may also stimulate IL-1β, TNF-α, IL-6, IL-10 from human whole blood, and NO in an in vitro murine macrophage system (Blix & Helgeland 1998, Schytte Blix et al. 1999, Hashimoto et al. 2003, Nakamura et al. 2004). Moreover, *A. actinomycetemcomitans* LPS can significantly upregulate the expression of the β-2 integrins CD11a/CD18 and CD11b/CD18 as

Table 1. Acronyms and short description of bacterial and host molecules included in the text

Caspase-activating and recruitment domain	CARD	Domain of Nod receptor
c-jun N-terminal kinase	JNK	Kinase
Extracellular signal-regulated kinase	ERK1/2	Kinase
G-protein-coupled receptors	GPCR	G-protein-coupled receptors
Granulocyte/macrophage colony-stimulating factor	GM-CSF	Chemotactic/growth factor
Human β-defensins	hBD	Cytotoxic polypeptide
IκB kinase	IKK	Kinase
IL-1 receptor-associated kinase	IRAK	Kinase
Immunoglobulin	Ig	Antibody
Interferon-γ	INF-γ	Cytokine activating Th1 inflammatory responses
Interferon inducible protein-10	IP-10	Interferon inducible protein
Interleukin	IL	Inflammatory cytokines
Intracellular adhesion molecule	ICAM	Molecules mediating adhesion with leucocytes
Leucocyte function-associated antigen-1	LFA-1	Leucocyte adhesion molecule
Lipopolysaccharide binding protein	LBP	Receptor/transfer of LPS
Macrophage inflammatory protein	MIP	Chemotactic factor
Major histocompatibility complex	MHC	Antigen presentation molecule
Matrix metalloproteinase	MMP	Matrix metalloproteinase
Mitogen-activated protein kinase	MAPK	Kinase
Monocyte chemotactic protein	MCP	Chemotactic factor
Myeloid differentiation factor 88	MyD88	Adaptor protein
Nuclear factor-κB	NF-κB	Transcription factor
Nucleotide-binding oligomerization domain	Nod	Receptor for virulent factors
Peptidoglycan recognition proteins	PGRP	Receptor for virulent factors
Phospho-38 MAPK	p38	Kinase
Prostaglandin E_2	PGE_2	Lipid mediator
Protease-activated receptor	PAR	Receptor for virulent factors
Protein kinase c	PKC	Kinase
Receptor-interacting protein 2	Rip2	Adaptor protein
TNF receptor-associated factor 6	TRAF-6	Adaptor protein
Toll-like receptor	TLR	Receptor for virulent factors of infectious agents
Tumour necrosis factor-α	TNF-α	Inflammatory cytokine/induction of apoptosis
Vascular cell adhesion molecule-1	VCAM-1	Mediates adhesion with leucocyte

well as L-selectin on granulocytes and monocytes (Blix et al. 1999) (Table 1).

In addition, LPS induces the production of selectins from endothelial cells, which could promote diapedesis of circulating leucocytes into the inflamed area (Darveau et al. 1995). In a mouse model of acute inflammation LPS was able to enhance E-, P-selectin, and MCP-1 expression (Reife et al. 1995). In contrast, *P. gingivalis* LPS does not induce E-selectin from human umbilical vein endothelial cells (HUVEC), and it may even inhibit E-selectin expression induced by other LPS. As expected, in the acute inflammation model mentioned above, stimulation with *P. gingivalis* LPS showed reduced expression of MCP-1 and E-, P-selectin. As a consequence of the low E-selectin expression on endothelial cells, neutrophil-binding assays have demonstrated that *P. gingivalis* LPS attenuates adhesiveness of neutrophils with HUVEC (Darveau et al. 1995, Reife et al. 1995, Cunningham et al. 1999, Coats et al. 2003).

LPS from a variety of periodontal pathogens, such as *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis*, *Bacteroides oralis*, *Fusobacerium nucleatum*, etc., may also interact with human gingival fibroblasts and induce the expression of MCP-1, IL-1β, IL-6, IL-8, and intercellular adhesion molecule-1 (ICAM-1) (Takada et al. 1991, Tamura et al. 1992, Hanazawa et al. 1993, Masaka et al. 1999). Moreover, LPS may interact with two more cell types present in periodontal tissues, mast cells, and dendritic cells. Although studies involving the effects of LPS of periodontal bacteria on these cells are limited in number we should acknowledge the possibility of these interactions. LPS has been shown to be able to induce de novo synthesis and secretion of IL-1β, TNF-α, IFN-γ, IL-6, and IL-10 from mast cells, and thus contribute to the initiation of a pro-inflammatory response (Supajatura et al. 2002). As far as dendritic cells are concerned, a study by Jotwani et al. (2003) revealed that LPS could pulse human monocyte-derived dendritic cells to release Th1-biasing cytokines, like IFN-γ, IL-2, IL-12, IP-10, but also IL-1β, TNF-α, IL-6, and IL-10. In contrast no IL-12 or IP-10 and lower levels of TNF-α and IL-10 were induced by *P. gingivalis* LPS. These are interesting observations as they introduce the concept that LPS not only plays a critical role in the organization of the initial steps of inflammatory responses, but also participates in the transition towards the more efficient adaptive immune response. This is further emphasized by the fact that LPS, via the TLR-4 signalling, enhances also the expression of co-stimulatory molecules, like CD80/CD86 and major histocompatibility complx class II (MHC-II), which are important for T-cell activation (Medzhitov 2001). Finally, LPS molecules may interact and activate the complement system, which could initiate the release of chemoattractants of leucocytes, and enhance bacterial phagocytosis.

PGN

Gram-positive bacteria have a thick multilayered cell wall consisting mainly of PGN, which surrounds the cytoplasmic membrane. PGN is a mesh-like exoskeleton that provides rigidity and shape to bacteria, and at the same time is sufficiently porous to allow diffusion of metabolites. It is often covalently linked to teichoic acid (TA). On the other hand, Gram-negative bacteria have only a thin layer of PGN found in the periplasmic space, which is not bound to TA. PGN is a mesh made up of ropelike linear polysaccharide chains cross-linked by peptides. The polysaccharide consists of repeating disaccharide of N-acetylglucosamineand N-acetylmuramic acid (NAM). The peptide subunit (or stem peptide) consists of four to five amino acids connected to some NAMs. The third amino acid, usually a Lysine, cross-links the PGN chains either directly via amino acids of a different stem peptide, or indirectly via glycine bridges. The composition of the peptide subunits and the number of cross-links varies considerably among different bacterial species. PGN is constantly synthesized and degraded, and during degradation a complex mixture of solu-

ble portions are released and may activate host cells. Signalling of PGN is conferred mainly via TLR-2, but PGN can also be recognized by complement, the intracellular nucleotide-binding oligomerization domain receptors Nod1 and Nod2 and several other recently discovered receptors, like PGN recognition proteins.

PGN has been found to be able to induce the release of TNF-α in human monocytes with similar kinetics to that induced by LPS. The same study also revealed that PGN enhanced the production of IL-1β and IL-6 (Mattsson et al. 1993). Another study, using a gene array approach, verified these observations, and also showed that the chemokines IL-8 and MIP-1α were more strongly activated (Wang et al. 2000). In synergy with LTA, PGN may also induce NO formation from macrophages (Kengatharan et al. 1998). Despite the results from these in vitro studies, it has been argued that PGN is not an important initiator of inflammatory responses as cellular responses to this wall component require concentrations, which are several, logs higher than the concentrations of LPS required for activating macrophages. Of course more accurate answers about the role of PGN in periodontal tissues would be best given using PGN in in vivo models but these are rarely studied.

In combination with LTA, PGN enhances adhesiveness of endothelial cells for granulocytes. This corresponds with increased expression of ICAM-1 on the cell surface and release of the chemokine IL-8 (van Langevelde et al. 1999). On the other hand, PGN does not stimulate directly vascular cell adhesion molecule-1 (VCAM-1) or IL-6 in endothelial or epithelial cells, but PGN-induced IL-1β and TNF-α from monocytes could confer these responses (Jin et al. 1998). Similarly, gingival epithelial cells do not produce IL-8 after LPS, PGN, or LTA stimulation, but this can be reverted when cells are pretreated with IFN-γ (Uehara et al. 2001, 2002). IL-8 production is also enhanced after stimulation of human gingival fibroblasts with PGN (Hatakeyama et al. 2003). Moreover, PGN has been shown to activate mast cells, but in a different way than LPS. Specifically, it induces degranulation and secretion of preformed mediators, like histamine, TNF-α, prostaglandins, and cytokines that promote TH2-type responses (IL-4, IL-5, IL-10, etc.), rather than secretion of de novo synthesized pro-inflammatory cytokines (Supajatura et al. 2002). Release of histamine may result in the increase of vascular permeability, which could lead to an increase in inflammatory mediators in the inflamed area, whereas, the secretion of TH2-type cytokines is another example of how PAMPs may modulate the next step of the immune response, adaptive immunity. Finally, PGN can activate the complement system leading to similar results as for activation with LPS.

LTA

LTA is present only in Gram-positive bacteria. It traverses the cell wall and is anchored to the cytoplasmic membrane via its lipid portion. LTA consists of a diacyglycerol-containing glycolipid anchor and a covalently coupled polymeric structure. These polymers are chemically modified ribose or glycerol connected by phosphates. Sugars, alanine, choline may be attached to the ribose or glycerol providing antigenic determinants. Differences in the polymeric backbone and the lipid anchor are present among various Gram-positive bacterial LTA. Signalling of this amphiphilic molecule is mediated through TLR-2.

LTA induces the release of IL-1β, TNF-α, IL-6, from human monocytes although there is a controversy about the ability of LTA from *Staphylococcus aureus* and *Streptococcus pneumoniae* to do so (Bhakdi et al. 1991). TNF-α, IL-6, IFN-γ, IL-8, IL-10 secretion is also enhanced in human blood after stimulation with LTA, and, as mentioned earlier, in synergy with PGN it can activate NO production from macrophages (Kengatharan et al. 1998, von Aulock et al. 2004). From in vivo studies it has been demonstrated that LTA can also induce neutrophil infiltration (Leemans et al. 2002).

When challenged with LTA endothelial cells do not secrete IL-6 or IL-8, but they enhance the expression of E-selectin and neutrophil adhesion as shown in HUVEC (Kawamura et al. 1995, Hermann et al. 2002). On gingival epithelial cells LTA does not have any significant effect unless the cells are primed first with IFN-γ, which may lead to increased IL-8 secretion (Pollanen et al. 2000, Uehara et al. 2001, 2002). Finally, LTA can interact and activate the complement leading to release of chemoattractants and enhancement of phagocytosis (Loos et al. 1986).

Fimbriae

A large number of bacteria, especially Gram negative, have on their surfaces numerous thin, straight appendages referred to as fimbriae (originally called pili). Two major classes of fimbriae have been described: (a) the type-specific fimbriae, which are involved in interactions with other bacteria and mammalian cells (adhesions) and, in the adherence to soft and hard cell surfaces, and (b) the F- or sex-pili, which are involved in bacterial conjugation. Fimbriae in most bacteria have approximately the same size (3–25 μm long) and are shorter than sex-pili. Their distribution and numbers vary significantly with some organisms having 10 fimbriae per cell and others having up to 1000 (Holt et al. 1999). The most studied fimbriae of the periodontal bacteria are those of *P. gingivalis*. The strain ATCC 33277 possesses two types of fimbrial structures; one consisting of the 67 kDa fimbrillin protein encoded by the *mfa1* gene (major fimbriae), and the other consisting of the 41 kDa fimbrillin protein encoded by the *fimA* gene (minor fimbriae). Fimbriae are the principle molecules by which periodontal bacteria attach to other bacterial species, epithelial cells, gingival fibroblasts, extracellular matrix proteins and salivary pellicle coated tooth surfaces (Goulbourne & Ellen 1991, Watanabe et al. 1992, Amano et al. 1994, Hamada et al. 1994, Amano 2003). Following attachment some bacteria (i.e. *P. gingivalis*) may invade various host cells (epithelial cells, endothelial cells, fibroblasts) depending upon the fimbriae present on their surface (Lamont et al. 1995, Sandros et al. 1996, Weinberg et al. 1997, Deshpande et al. 1998, Dorn et al. 1999).

Fimbriae (from *P. gingivalis*) have also been shown to be able to modulate inflammatory responses from host cells in vitro. This is considered to occur via activation of the TLR-2 and leucocyte function-associated antigen-1 (LFA-1) receptors, although, recently, it was reported that fimbriae can activate TLR-4, through interaction with additional LPS-related molecules (Asai et al. 2001, Ogawa et al. 2002, Hajishengallis et al. 2004). Specifically, several studies have demonstrated that bacterial fimbriae are able to induce the production

of IL-1α, IL-1β, IL-6, and TNF-α from gingival fibroblasts, epithelial cells and macrophages/monocytes (Hedges et al. 1992, Ogawa et al. 1994, 2002, Hamada et al. 2002). Moreover, Nassar et al. (2002) and Asai et al. (2001) showed that fimbriae enhanced the expression of MCP-1 from HUVEC, and of IL-8 from both HUVEC and human gingival epithelial cells. Besides their ability to stimulate the induction of chemotactic cytokines from host cells, these fimbriae and related synthetic peptides have been shown to enhance the chemotactically induced migration of human peripheral blood monocytes (Ogawa & Hamada 1994). Finally, Khlgatian et al. (2002) using wild-type and mutant bacteria that did not express fimbriae showed that these molecules activated the expression of the cell adhesion proteins ICAM-1, VCAM-1, P- and E-selectin from HUVEC.

Proteases

Proteases (or proteinases) are enzymes that are capable of hydrolysing peptide bonds of proteins. These putative in vivo virulence factors are classified based upon their catalytic functions. They are produced by a broad range of bacteria and are considered to be important for nutrient acquisition and protection against inflammatory responses (Holt et al. 1999).

Two of the most studied bacterial proteases are the gingipains of *P. gingivalis*. Arginine-gingipain (Rgp) and Lysine-gingipain (Kgp) are cysteine proteases that specifically cleave after arginine and lysine, respectively. They are produced in large amounts and are associated with both the cell surface and the secretory vesicles of the bacterium (Potempa et al. 1995). With respect to the physiology of the bacterium, as *P. gingivalis* is an asaccharolytic bacterium it depends on Rpg and Kpg to acquire nutrients, such as peptides and amino acids from environmental proteins. Furthermore, proteolytic activities of Rpg and Kpg contribute to processing/maturation of various cell surface proteins, like fimbriae, haemagglutinins, etc. (Zhang et al. 1999).

Besides these properties, Rpg and Kpg have been shown to be able, at least in vitro, to modulate the initiation of inflammatory responses in various ways. First, gingipains cleave pro-inflammatory cytokines that are induced by host cells after activation with bacteria and their products. Zhang et al. (1999) and Madianos et al. (1997) demonstrated that *P. gingivalis* proteases degraded IL-8 secreted by oral epithelial cells, and this was associated with inhibition of transepithelial migration of neutrophils. IL-8 and MCP-1 secretion from HUVEC was also inhibited after treatment with *P. gingivalis*, and this was attributed to the bacterial proteases (Nassar et al. 2002). Moreover, Fletcher et al. (1997) also showed that IL-1b and IL-6, added to the culture medium of *P. gingivalis* were degraded by these proteases. On the other hand, Lourbakos et al. (2001) and Chung et al. (2004) demonstrated that Rpg (the RpgB variant) enhanced the expression of IL-6, and human β-defensins (hBDs) from oral epithelial cells by activating the G-protein-coupled protease-activated receptors-1 and -2 (PAR-1 and PAR-2). Finally, Tada et al. (2003) showed that gingipains reduced the expression of ICAM-1 from human oral epithelial cells, and degraded these molecules on the cell membranes and consequently disrupted the polymorphonuclear (PMN)–oral epithelial cell interaction.

Along with the degradation of pro-inflammatory cytokines and chemoattractants, proteases from *P. gingivalis* and *P. intermedia* can also cleave LBP and CD14 on human monocytes and human gingival fibroblasts, leading to LPS hyporesponsiveness (Sugawara et al. 2000, Tada et al. 2002, Deschner et al. 2003, Duncan et al. 2004). Moreover, they can cleave complement factors, such as C3 and C5, to C3a and C5a, respectively, which may imply that bacterial proteases could generate chemotactic activity (i.e. C5a) from plasma proteins (Sundqvist et al. 1984, 1988). Importantly, C3a and C5a can also act on endothelial cells to induce adhesion molecule expression and increase vascular permeability. The latter may also occur by bacterial proteases after induction of bradykynin release. Specifically, Rgp and Kgp can release bradykynin either via prekallikrein activation and/or high-molecular-weight kininogen cleavage (Amano et al. 1994). Despite the recruitment of leucocytes, gingipains are able to inactivate the leucocyte C5a receptor on neutrophils, degrade immunoglobulins (Ig) IgG, IgA, and IgM and disrupt the bactericidal activity of PMNs, thus protecting the bacteria from host responses (Sundqvist et al. 1985, Grenier et al. 1989, Nakayama et al. 1995, Jagels et al. 1996). Interestingly, Lourbakos et al. (1998) found that Rpg may also activate neutrophils by activating the PAR-2 receptors on these cells. This was based on the increase of neutrophil intracellular Ca ions that was observed after protease stimulation.

HSPs

Prokaryotic and eukaryotic cells respond to a broad range of environmental stresses (temperature, pH, redox potential, etc.) by inducing or accelerating the synthesis of specific proteins known as stress proteins, including HSPs. These proteins have a high degree of homology and the genes coding for them are among the most conserved in nature. HSPs are grouped into families based on their molecular weight: small HSP, GroES-homologue proteins or HSP10 ($\sim$ 10 kDa), DnaJ-homologue proteins or HPS40 ($\sim$ 40 kDa), GroEL-homologue proteins or HPS60 ($\sim$ 60 kDa), DnaK-homologue proteins or HSP70 ($\sim$ 70 kDa), HptG-homologue proteins or HSP90 ($\sim$ 90 kDa), and Clp ATP-dependent proteases (HSP100) (Goulhen et al. 2003). HSPs that belong to the same family share strong amino acid sequence identity. They act as molecular chaperones in the assembly and folding of proteins, and as proteases when damaged or toxic proteins have to be degraded. Thus, they may protect cells from the damaging effects associated with stressful conditions.

Many HSPs of oral micro-organisms particularly periodontopathogens have been identified and some of their properties – including location, cytotoxicity, and amino acid sequence homology with other HSPs – have been reported (Goulhen et al. 2003). As these proteins are immunodominant antigens in many human pathogens, studies have recently focused on the potential contributions of HSPs to oral disease. The cytotoxicity of some bacterial HSPs may contribute to tissue destruction, whereas the presence of common epitopes in host proteins and microbial HSPs may lead to autoimmune responses (Goulhen et al. 2003). A series of reports have demonstrated that bacterial HSPs are expressed in periodontal tissues during periodontal disease and that they may form immunocomplexes with specific antibodies (Ando et al. 1995, Tabeta et al. 2000, Shelburne et al. 2002, Yamazaki et al. 2002). Moreover, serum from these

patients contained antibodies that reacted with *A. actinomycetemcomitans*, *F. nucleatum*, and *P. nigrescens* HSP60 and/or HSP70.

The fact that HSPs are not exclusively intracellular proteins, but may also be located on the cell surface, in surface-associated material, and on outer membrane vesicles produced by bacteria (Goulhen et al. 1998), allows them not only to interact with antibodies, but also with PRR on host cells. HSPs (HSP60) are considered to signal via CD14/TLR-4 and stimulate the expression of pro-inflammatory cytokines (Ueki et al. 2002). Hence, *Campylobacter rectus* GroEL activates the production of both IL-6 and IL-8 by human gingival fibroblasts without affecting their viability (Hinode et al. 1998). Moreover, at low concentrations, *C. rectus* GroEL can also stimulate the production of IL-6 by a confluent monolayer of human gingival epithelial cells (Tanabe et al. 2003). Interestingly, HSPs may also be produced by host cells, which could also stimulate an inflammatory response. Ueki et al. (2002) demonstrated that human HSP60, but not *P. gingivalis* or *A. actinomycetemcomitans* GroEL, induced TNF-α by THP-1 cells. Immunohistochemical analysis also showed that HSP60 was abundantly expressed in periodontal lesions, and, therefore, the authors postulated that periodontopathic bacteria stimulate host cells in the periodontium to upregulate the expression of HSP60, which may in turn stimulate macrophages and possibly other cells to produce pro-inflammatory cytokines.

fMLP

Formyl-methionyl peptides are derived from the NH_2-terminal regions of newly synthesized proteins of prokaryotic cells. For some proteins, including those found in the cytoplasm, the formyl-methionyl peptides are cleaved post-translationally and are not found in the mature protein. In the case of membrane and secretory proteins, which possess NH_2-terminal signal peptides, these NH_2-terminal extensions are cleaved by a signal peptidase following appropriate polypeptide transport and, therefore, could be released into the extracellular space. The signal peptide is encoded in the protein in the form of a short-lived sequence extension and is believed to direct the transport of newly synthesized polypeptides across the appropriate membrane following their synthesis. fMLP is one such bacterial peptide and is mainly considered a strong chemoattractant and a powerful activator of PMN and mononuclear phagocytes (Panaro & Mitolo 1999). The receptor for fMLP is a G-protein-coupled receptor, and its activation upon ligand binding, among others, activates the mitogen-activated protein kinase (MAPK) phospho38 (p38) and extracellular signal-regulated kinase1/2 (ERK1/2) signalling pathways. This may result in the observed release of pro-inflammatory cytokines such as TNF-α (Balazovich et al. 1996). fMLP is also reported to up-regulate the expression of CD11a, CD11b, and CD18, which are important for granulocyte adhesion to endothelial cells, as well as, diapedesis and migration (Volz 1993, Derian et al. 1995).

Toxins

There are two main categories of bacterial toxins: (a) the Arginine–Threonine–X (RTX) toxins, and (b) the cytolethal distending toxins (CDT).

The RTX toxins are named by their C-terminal amino acids repeats and are produced by a number of Gram-negative bacteria. They are encoded by four genes (rtxC, A, B, and D) and can be divided into broad-spectrum hemolysins, and leucotoxins, such as leucotoxin A (LtxA), that display both cell and species specificity. Following membrane insertion, RTX toxins form channels (pores) and kill cells either by osmotic lysis or by induction of apoptosis (Lally et al. 1999). The most studied RTX toxins from periodontal pathogens is the LtxA of *A. actinomycetemcomitans*. This leucotoxin kills lymphoid and myeloid cells from humans and some nonhuman primates, and the basis of this cell/species selectivity is due to the toxin binding to human cells via the β_2-integrin LFA-1 (Lally et al. 1997). It is possible to block the activity of LtxA by antibodies to CD18 or CD11a, the two subunits of LFA-1 (Lally et al. 1997, Johansson et al. 2000). LFA-1 is not endocytosed and so the interaction with the toxin does not result to internalization. Hence, LtxA–LFA-1 interaction appears to result in the destruction of host immune cells rather than facilitate microbial internalization (Schnur & Newman 1990). At low concentrations LtxA promotes neutrophil degranulation, including the release of matrix metalloproteinase-8 (MMP-8), but inhibits phagocytosis. Such effects may be a result of the LtxA increasing intracellular Ca^{2+} ions (Taichman et al. 1991). At high concentrations LtxA can cause cell lysis or apoptosis (Lally et al. 1999). Interestingly, IL-1β and TNF-α may increase the cytotoxic effect of LtxA by enhancing the expression of LFA-1 (Yamaguchi et al. 2004). RTX toxins are secreted within membranous vesicles that are released from the outer membrane of the bacteria (Kato et al. 2002).

The CDT toxins cause the arrest of the mammalian cell cycle in the G_2 phase. The action of CDT appears to be caused by its DNase activity, once it enters into target cells (De Rycke & Oswald 2001). They may also induce distension, actin rearrangement, and apoptosis depending upon the type of cell. *A. actinomycetemcomitans* produces CDT, which is encoded by three genes *cdt*A, *cdt*B, and *cdt*C. *cdt*B is the active component able to block the proliferation of T lymphocytes. Interestingly, Aficusa et al reported that Cdts, either alone or in combination, could also stimulate human PBMCs to synthesize IL-1β, IL-6, IL-8 and IFN-γ, but not TNF-α, IL-12, granulocyte/macrophage colony-stimulating factor (GM-CSF) and the anti-inflammatory IL-10 (Akifusa et al. 2001). The nature of the receptors and of the signalling pathways utilized by CDT to stimulate human leucocytes to synthesize pro-inflammatory cytokines is unknown.

Hence, toxins may modulate inflammatory responses by killing/arresting inflammatory cells and by stimulating pro-inflammatory cytokines and chemoattractants.

From all the above, it is clear that virulent factors from periodontal bacteria can affect the function of a variety of cells found in periodontal tissues. Although the information generated from these in vitro studies are important to understand the possible mechanisms by which these virulent factors may modulate inflammatory responses in the gingiva, it is the in vivo experiments that verify these mechanisms. Compared with in vitro studies, only few in vivo experiments have been performed. Specifically, TLR-4-deficient mice demonstrate reduced inflammatory responses (IL-1, IL-12) and bone destruction after challenge with a mixed anaerobic infection which included the periodontal pathogens *P. intermedia* and

F. nucleatum (Hou et al. 2000). Moreover, in a mouse model of experimental periodontitis where *P. gingivalis* LPS solution was applied to the buccal region of the mice, IL-6 production in the gingiva and bone resorption were significantly inhibited by pretreatment with anti-CD14 antibody. This further emphasizes the role of LPS in inflammation as CD14 is a protein that transfers LPS to its receptor as will be described latter (Wang et al. 2002). The effects of LPS and proteases in rat gingiva were also studied in a periodontitis model. LPS-induced elongation of rete ridge, apical migration of junctional epithelium, increased numbers of B cells and bone resorption. Proteases led to an increase in the number of infiltrating PMN leucocytes and the combination of LPS with proteases augmented the effects of LPS (Ekuni et al. 2003). Another study of experimental periodontitis in non human primates revealed also that immunization with a purified *P. gingivalis* cysteine protease was able to alter/reduce the microbiological and clinical signs of gingivitis/periodontitis (Moritz et al. 1998) the role of fimbriae in periodontal disease has also been studied. Mutant strains of *P. gingivalis* that possess inactive fimbriae are less able to cause periodontal bone loss as shown in a gnotobiotic rat model of periodontal disease (Malek et al. 1994). Finally, *A. actinomycetemcomitans* LPS elicited the induction of expression of the co-stimulatory molecules B7-1 and B7-2 on gingival macrophages, which was required for TH1-mediated inflammatory bone resorption in experimental periodontal disease in rats (Kawai et al. 2000).

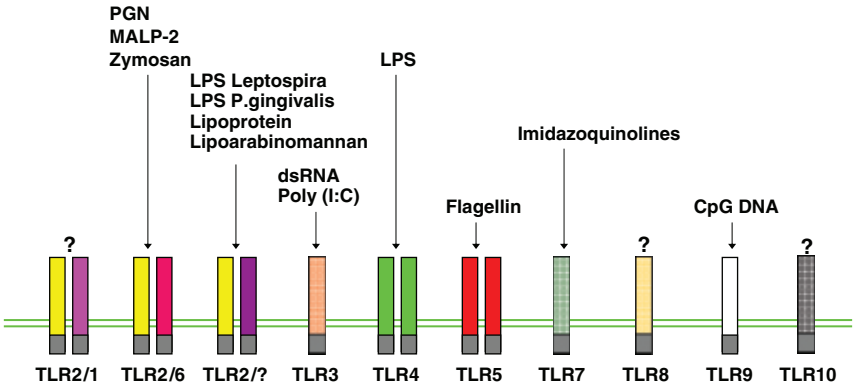

Fig. 1. Depiction of Toll-like receptors (TLRs) and their ligands. Note hetero- and homodimerizations.

Main Receptors and Signalling Pathways

TLRs

TLRs are transmembrane PRRs found on the surface of cells involved in immune responses. To date, at least 10 different TLRs have been characterized, and all of them share a similar structure (Fig. 1).

This includes leucine-rich repeats in the extracellular domain and a cytoplasmic domain, which has significant homology with the IL-1 receptor signalling domain, termed the Toll/IL-1 homologous region. A broad variety of PAMPs interacts with high specificity with these receptors. LPS, which activates TLR-4, and PGN and LTA, which activate TLR-2, bind first to lipopolysaccharide binding protein (LBP) and are then transported to CD14. CD14 is a soluble or membrane bound protein that transfers the complex to the receptor. TLR4 also needs another protein MD-2 to be activated. Once activated, TLR2/4 recruit myeloid differentiation factor 88 (MyD88), which associates with the serine-threonine protein kinase IL-1 receptor-associated kinase (IRAK) and TNF receptor-associated factor-6 (TRAF-6) adaptor protein. Oligomerization of TRAF-6 activates a group of MAPK kinase kinases, which directly or indirectly leads to activation of IκB kinase 1 (IKK1) and IKK2. These kinases phosphorylate IκB on serine residues thus targeting IκB for degradation and releasing nuclear factor-κB (NF-κB), which translocates to the nucleus and induces de novo synthesis of inflammatory and immune response genes (Fig. 2). TLRs may also activate the three MAPK (p38, ERK1/2 and c-jun N-terminal kinase) and protein kinase C, but the exact pathways have not been elucidated yet. Interestingly, TLR-4 may also activate NF-κB, MAPK, and IFN-inducible genes in a MyD88-independent way, while recent studies have demonstrated that, depending upon the stimulus (TLR-2 or TLR-4) NF-κB, the transcription factors activated by MAPK, and the promoters of various genes are differentially activated (not consistent data).

This is also true in macrophages between *P. gingivalis* and *E. coli* LPS. Finally, TLR-2 and TLR-4 also differ in the fact that TLR-4 forms homodimers, while TLR-2 forms heterodimers with TLR-1 or TLR-6. Thus, although there are general similarities between TLR-2 and TLR-4 pathways, it is not surprising that quantitative and/or qualitative differences in gene expression are present. Nevertheless, the general pattern shows that TLRs induce the expression of pro-inflammatory, anti-inflammatory, chemokine and lymphocyte-associated cytokine genes, which allow for the initiation of inflammatory processes and of co-stimulatory molecules, which are important for the switch from innate to adaptive immune responses.

Nucleotide-binding oligomerization domain proteins (Nod)

Nod1 and Nod2 belong to a recently discovered family of proteins the nucleotide-binding site and leucine-rich repeat (NBS-LRR) proteins. In contrast to TLRs, which are mainly integral membrane proteins, Nod proteins are cytosolic and are involved in intracellular recognition of microbes and of their products. Nod1 and Nod2 molecules have a series of LRRs at their C-terminal, which is the domain that senses the microbial ligand. This domain is connected to the NBS, which is important for the oligomerization of the receptor, which is necessary for the signal transduction induced by the N-terminal caspase-activating and recruitment domain (CARD) (Chamaillard et al. 2003).

The microbial motifs sensed by these two molecules have been characterized. Both, Nod1 and Nod2 recognize bacterial PGN, although through distinct motifs within this structure. Nod1 senses a naturally occurring muropeptide of PGN that presents a unique amino acid at its terminus called diaminopilemic acid (DAP) [MurNAc-L-Ala-γ-D-Glu-meso-DAP]. As this structure is uniquely found in PGN of Gram-nega-

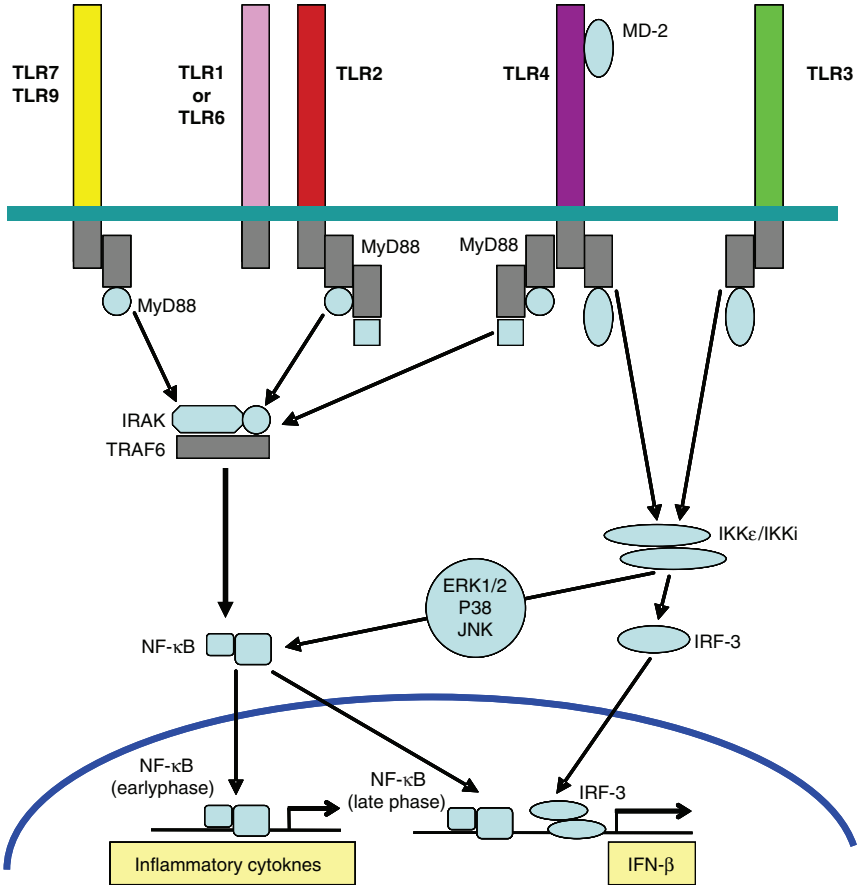

Fig. 2. Toll-like receptor (TLR) signalling pathways. Modified from Beutler (2004) and Takeda and Akira (2005).

tive bacteria, Nod1 is designated as a sensor of Gram-negative bacteria. In contrast to Nod1, Nod2 has been implicated as a general sensor for both Gram-positive and Gram-negative bacteria as it recognizes the minimal motif in all PGNs, the muramyl dipeptide MurNAc-L-Ala-D-isoGln (Philpott & Girardin 2004).

Sensing of PGN by the LRR domain of the Nod receptors, leads to their oligomerization, which seems adequate to induce signal transduction. Although Nod1 has one CARD while Nod2 has two, the signalling pathways triggered appear to be very similar. Specifically, oligomerization is sufficient to induce the recruitment of receptor-interacting protein 2 (Rip2) through homophilic CARD-CARD interaction. Rip2 is an adapter protein sharing homology with IRAK, which by interacting with Nods leads to activation of the NF-κB pathway through the recruitment of the IKK complex to its central domain (Inohara et al. 2000, Ogura et al. 2001b).

Nod1 and Nod2 are expressed in epithelial cells lining mucosal surfaces, but Nod2 is predominantly expressed in cells of the myeloid lineage. It has been suggested that these receptors may interact with PGN after the latter has been released within the cell during bacterial invasion (Girardin et al. 2001). Interestingly, pathogens that are not invasive, like *Helicobacter pylori*, may also activate cytosolic Nods (Philpott et al. 2002). Finally, the mechanism by which Nod receptors in professional phagocytic cells may be activated following bacterial internalization has not been elucidated.

Mutations in the gene encoding Nod2 were recently shown to be associated with inflammatory disorders, such as Crohn's disease (Ogura et al. 2001a). This finding along with the fact that Nods activate the NF-κB pathway, and, subsequently, pro-inflammatory responses may imply that these receptors may be important for the host's protection against bacterial infections. The role of these receptors in setting up of inflammatory responses in the gingiva during periodontal disease has not been elucidated yet.

G-protein-coupled receptors (GPCR) and hBDs

GPCR are receptors composed of seven transmembrane domains with loops spanning both the intracellular and extracellular faces of the cell membrane. They have a wide range of ligands, such as sensory stimuli, nucleotides, ions, lipids, hormones, chemokines, complement molecules, etc. Two such receptors that may interact with bacterial virulence factors are the fMLP receptor and the PARs.

Activation of the fMLP receptor is initiated after binding with its ligand. This induces a conformational change in the receptor. Heterodimeric G-proteins, consisting of α, β and γ subunits, interact selectively with the cytoplasmic face of the activated receptor. Hence, the Gα subunit releases guanine di-phosphate (GDP) (inactive form) and subsequently binds to guanine tri-phosphate GTP, resulting in activation and dissociation of the heterotrimer into α and $\beta\gamma$ constituents, which activate downstream effectors. Specifically, the fMLP receptor may activate the MAPK p38 and ERK1/2 signalling pathways leading to pro-inflammatory cytokine production. Also these receptors may induce degranulation via activation of inositol triphosphate and diacylglyserol.

PARs are GCPRs that mediate cellular responses to extracellular proteinases. Various proteases, such as thrombin and trypsin-like serine proteases, bind to PAR's N-terminal domain and cleave it (after Arg41) to generate a new N-terminus for the receptor. The first six amino acids of this new N-terminus, SFLLRN, may act as a ligand and bind intramolecularly to the heptahelical bundle of the receptor and induce transmembrane signalling and G protein activation (Coughlin & Camerer 2003).

Currently, four PARs have been identified (PAR-1–4). PAR-1 is expressed by platelets, fibroblasts, endothelial cells, and neurons, whereas PAR-2 is expressed by epithelial cells, endothelial cells, smooth muscle cells, T cells, neutrophils, and neurons. PAR-1, -3, -4 are activated by thrombin, and PAR-2 is activated by trypsin and a number of trypsin-like serine proteases. PARs are considered to mediate cellular responses to tissue injury, like expression of IL-8, GM-CSF, ICAM-1, VCAM-1, platelet activating factor, IL-6, nitric oxide, etc that may serve to recruit platelets and

leucocytes to sites of injury and promote access of plasma proteins to the extravascular space. PAR-1 may also activate degranulation of mast cells, which may further activate PAR-2 via the released tryptase. Unfortunately, little is known about the role of PARs in periodontal tissues. Hou et al. (1998) demonstrated that thrombin, which is formed at sites of coagulation and inflammation, stimulates PAR-1 from human gingival fibroblasts to induce IL-6. Moreover, Lourbakos et al. (2001) showed that *P. gingivalis* proteases (RgpB) stimulated PARs in the oral epithelial cell line KB, and interestingly, this activation lead to secretion of the pro-inflammatory cytokine IL-6 . Finally, Chung et al. (2004) reported that *P. gingivalis* gingipains induce hBDs partially by activation of PAR-2.

hBDs are found in three forms and belong to a family of cationic polypeptides of fewer than 100 amino acids, the defensins, and are mainly found in various types of epithelial cells (Dunsche et al. 2001). They use their positive charge to enter the membranes where they form pores. Permeabilization of the target membranes is the crucial step in defensin-mediated antimicrobial activity and cytotoxicity. When their concentration is high, as in inflamed tissues, defensins may also induce pro-inflammatory signals from host cells, i.e upregulation of IL-8, IL-1, TNF-α (Van Wetering et al. 1997, Chaly et al. 2000), while various defensins have also been reported to have chemotactic activity for monocytes, T cells and dendritic cells (Territo et al. 1989, Yang et al. 2000). It has been suggested that the expression of hBD-2 and -3 is reduced in inflamed gingival tissues, although hBD-2 mRNA can be induced in human gingival epithelial cells following exposure to *A. actinomycetecomitans* (Dunsche et al. 2002, Noguchi et al. 2003, Lu et al. 2004).

Complement system

The complement system is made up of a large number of distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses. Although these proteins are not receptors of bacterial virulence factors themselves, they can be activated on the surface of a pathogen. Most of these proteins are proteases that are themselves activated by proteolytic cleavage through a triggered-enzyme cascade. In this way, the activation of a small number of complement proteins at the start of the pathway is hugely amplified by each successive enzymatic reaction, resulting in the rapid generation of a disproportionately large complement response. In the early phases of an infection, the complement cascade can be activated through any one, or more, of three pathways: the classical, the mannan-binding lectin, or the alternative. All three pathways merge to a common protease called C3 convertase, which cleaves C3 to C3b and C3a. The C3b molecule acts as an opsonin by covalently binding to the pathogen and thereby targeting it for destruction by phagocytes equipped with receptors for C3b. C3b can also bind to C3 convertase to form C5 convertase, which in turn can produce C5a and initiate the activation of a membrane-attack complex that creates pores in the membrane of some pathogens leading to their death. On the other hand, the C3a, and C5a fragments may act on specific receptors on host cells to produce local inflammatory responses. Specifically, the can induce smooth muscle contraction and increase of vascular permeability, and furthermore, they enhance the expression of adhesion molecules on endothelial cells. They can also activate mast cells leading to release of histamine and TNF-α, which have also similar effects. Hence, C3a and C5a help recruit leucocytes in the inflamed area and enhance phagocytosis. Signalling of C3a and C5a occurs via G-protein-coupled receptors, described earlier, and hence the action of these molecules is analogous to that of chemokines (Janeway et al. 2001). Although the role of the complement system in inflammatory responses is very important, to date, little is known about its interactions with periodontal bacteria.

Initiating Inflammation

From all the above, it is clear that the host has developed receptors and systems to recognize the presence of bacteria. As the number of different bacteria in various infections may be high, this recognition occurs through molecular patterns that are common on most bacteria independently of their species. This will activate host cells to set up an inflammatory response in order to combat the bacteria, and the whole process is part of the innate immunity. On the other hand, bacteria have also developed mechanisms, sometimes unique for each species, to interfere with the organization of this inflammatory response and hence to avoid their clearance. If the host is not able to control the infection, it activates more efficient mechanisms that target the infectious agents with higher specificity. These mechanisms comprise the adaptive immune system, and not surprisingly depend, in part, upon the efficient organization of the initial events of the inflammatory response.

Periodontal disease (gingivitis and periodontitis), is an inflammatory process of the gingiva and supporting structures of the teeth induced by a microbial biofilm. Based on in vitro and in vivo studies, as described, and histological assessments of inflamed and healthy gingival tissues (Page & Schroeder 1976), a model of the initial events that may occur when bacteria set up inflammatory responses in the gingiva can be proposed (Fig. 3). Of course, this model should not be considered as a ''new'' hypothesis, but as a summary of the existing knowledge.

Different host mechanisms, such as the regular shedding of epithelial cells, the washing effect of the saliva and the gingival crevicular fluid (GCF), and most importantly the phagocytic action of neutrophils that migrate continuously through the junctional epithelium into the sulcus/pocket, are all able to maintain a normal, non-irritating environment for the host bacterial flora. Once this equilibrium is disturbed and more pathogenic bacteria populate the periodontal niche the host becomes challenged. The first cells to be challenged are the epithelial cells (Fig. 3a). The intact sulcular and junctional epithelium presents normally an effective physical barrier against these bacteria, and the inflammatory response that the host may mount will focus on eliminating the infection and keeping the bacteria from entering the host tissues. Even if this is generally true, a number of periodontal parhogens, like *P. gingivalis* and *A. actinomycetemcomitans,* have been shown to invade epithelial cells in vitro, while *A. actinomycetemcomitans* can be found in the connective tissue of gingiva from periodontitis patients (Kinane et al. 1998, Hajishengallis et al. 2004). Bacterial components (i.e. LPS, PGN, etc.) and products (proteases, peptides, etc.) that are either shed or secreted can also

diffuse through the epithelium and act as virulence factors for the host inside and outside of the gingiva.

Bacteria that populate the sulcus/pockets may use their fimbriae to attach to epithelial cells and this interaction will activate epithelial cells to secrete IL-1β, TNF-α, IL-6, and IL-8. Activation, leading to IL-8 release, can also occur when primed epithelial cells interact with LPS, PGN, and LTA, while HSP may enhance the induction of IL-6. At the same time, virulence factors that have diffused in the connective tissue will stimulate directly or indirectly host cells that reside in this area, such as leucocytes, fibroblasts, mast cells, endothelial cells, dendritic cells and lymphocytes (Fig. 3b). Hence, LPS,

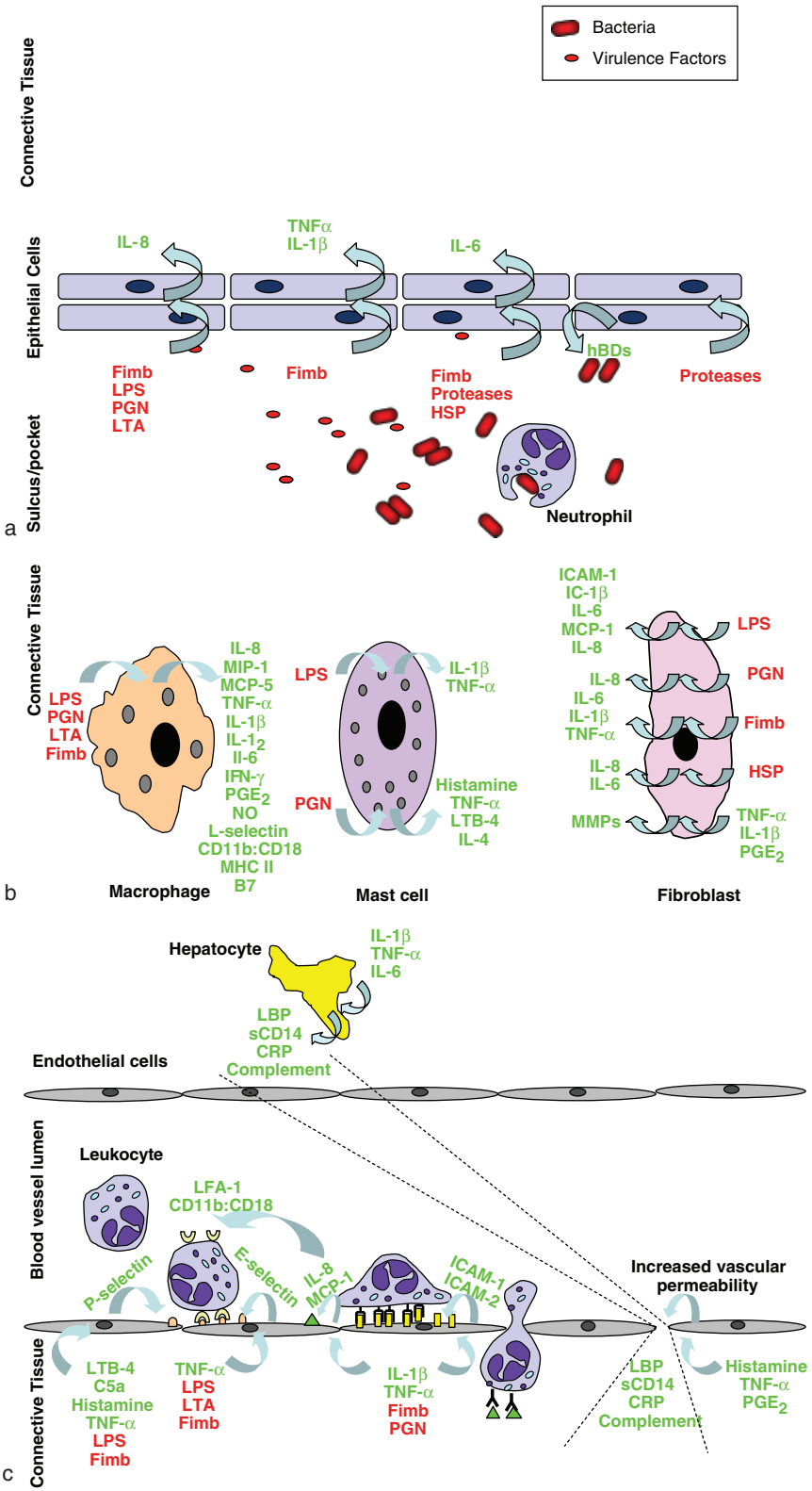

Fig. 3. Initiation of inflammatory responses by bacteria: a schematic representation including basic bacterial (red) and host (green) components. (a) Epithelial cells are the first cells to be challenged by bacteria in the sulcus/pocket. This interaction triggers the first steps of the inflammatory response and leads to cell activation in the connective tissue compartment and the recruitment of neutrophils in the crevice. Bacterial adhesion through fimbriae activates epithelial cells to secrete IL-1β, TNF-α, IL-6, and IL-8. Activation, leading to IL-8 release, can also occur when primed epithelial cells interact with LPS, PGN, and LTA. (b) Bacterial factors diffused in the connective tissue, as well as, inflammatory mediators produced by epithelial cells stimulate host cells resident in the area, such as monocytes/macrophages, fibroblasts and mast cells, to produce and release pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-12), chemotactic molecules (MIP-1α, MIP-2, MCP-1, MCP-5, and IL-8), prostaglandins (PGE_2), histamine, leukotrienes, as well as, matrix metalloproteinases that degrade collagen from the connective tissue compartment. In addition, macrophages express also co-stimulatory molecules (B7) and MHC class II molecules, and dendritic cells engulf bacteria and their products and process them for antigen presentation at the local lymph nodes. Hence, while the inflammatory response is getting organized, the host prepares also for the adaptive immune response. (c) Mediators, such as, IL-1β, TNF-α, and histamine released from activated host cells, participate along with bacterial factors in the activation of endothelial cells which express surface molecules, such as P- and E-selectins and ICAMs that are important for leucocyte extravasation. Leucocytes then migrate through the tissues against a concentration gradient of chemoattractants derived either from the host (IL-8, MCP-1, etc.), or from bacteria (fMLP, fimbriae) towards the focus of infection, where they start phagocytosing bacteria and their virulence factors. TNF-α, PGE_2, and histamine increase vascular permeability, which leads to efflux of plasma proteins and fluid in the connective tissue, and subsequently into the crevice, consisting part of the gingival crevicular fluid. Finally, locally produced cytokines, such as IL-1β, TNF-α, and IL-6 may enter the circulation and activate hepatocytes to synthesize acute-phase proteins, such as LBP, sCD14, complement proteins, and C-reactive protein, which help the host eliminate the infection.

PGN, LTA, and fimbriae will interact with TLRs on macrophages and induce the release of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-12), chemotactic molecules (MIP-1α, MIP-2, MCP-5, and IL-8), and PGE_2. Pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and chemoattractants (MCP-1 and IL-8) will also be secreted from fibroblasts after TLR interaction with LPS, PGN, fimbriae, and HSP. On the other hand, mast cells will secrete de novo synthesized IL-1β, TNF-α, and IL-6 when TLR-4 are stimulated with LPS, and will release histamine, leukotrienes, and TNF-α when TLR-2 are activated by PGN.

Some of these mediators, such as IL-1β, TNF-α, and histamine that are released from activated host cells, will participate along with the virulence factors in the activation of endothelial cells (Fig. 3c). Initially, LPS, fimbriae or the host-derived TNF-α, histamine, C5a, and LTB-4 will induce the expression of P-selectin on endothelial cells. This will be followed by the expression of E-selectin induced by LPS, LTA, fimbriae, and TNF-α. Finally, ICAM-1, -2, IL-8, and MCP-1 will be up-regulated after stimulation with PGN, fimbriae, IL-1β, and TNF-α. All these molecules found on the surface of endothelial cells are important for leucocyte extravasation. Specifically, P- and E-selectins interact with glycoproteins on leucocytes and allow the cells to adhere reversibly to the vessel wall, so that circulating leucocytes appear to ''roll'' along the activated endothelium. IL-8 and other chemokines, bound to proteoglycans on the surface of endothelial cells, trigger a conformational change to the leucocyte integrins LFA-1 and CD11b:CD18 on the rolling leucocyte, which greatly increases its adhesive properties. Hence, leucocytes attach firmly to ICAM-1 and rolling is arrested. Next, the leucocytes squeeze between the endothelial cells and enter the connective tissue in a process known as diapedeses. Finally, leucocytes migrate through the tissues under the influence of chemoattractants derived either from the host (i.e. IL-8, MCP-1, etc.), or from bacteria (i.e. fMLP, fimbriae, etc.). Chemokines such as IL-8 are produced at the site of infection and bind to proteoglycans of the extracellular matrix. They form a matrix-associated concentration gradient along which the leucocytes can migrate to the focus of infection. Importantly, the first leucocytes to appear are neutrophils, followed by macrophages. When these leucocytes arrive at the area of infection they will start phagocytosing bacteria and their virulence factors. Interaction with LPS, PGN, LTA, and fMLP will make these cells more efficient phagocytes as it will increase NO production and the expression of complement receptors (CD11b:CD18).

It is obvious that for the complement receptors to work, complement proteins must be present in the tissues or in the sulcus/pocket. IL-1β, TNF-α, and IL-6, produced as seen from host cells, may reach the liver via the circulation and activate hepatocytes. This will result, among others, to the synthesis of plasma proteins called acute-phase proteins (Fig. 3c). Such proteins are LBP, sCD14, complement proteins, and C-reactive proteins, which opsonize bacteria for phagocytosis.

TNF-α, PGE_2, and histamine, released also by host cells, are vasoactive molecules and increase vascular permeability. This leads to an increase in the accumulation of exudative fluid and proteins (including acute-phase proteins), in the connective tissue, which is further infiltrated with migrating leucocytes. This infiltrate will consist part of the GCF, which flows through the connective tissue into the sulcus/pocket. In order to make space for the infiltrate, fibroblasts activated by IL-1β, TNF-α, and PGE_2 will secrete matrix metalloproteinases that degrade collagen from the connective tissue compartment.

Meanwhile, macrophages express also co-stimulatory molecules and MHC-II molecules, and dendritic cells engulf bacteria and their products and process them for antigen presentation at the local lymphnodes. Hence, while the inflammatory response is getting organized the host prepares also for a more effective immune response, the adaptive immune response.

Hence, so far, we have seen that host cells recognize bacteria and their products and set up an inflammatory response that although meant to eliminate the infection may, in fact, cause tissue destruction.

The bacteria, in order to survive the inflammatory response, will use their virulence factors to modulate this response. Specifically, periodontal bacteria release proteases that can cleave LBP and CD14, which may disable the ability of host cells to recognize the presence of bacteria and initiate an inflammatory response. Furthermore, these bacterial proteases can also cleave pro-inflammatory cytokines like IL-1β and IL-6, chemoattractants such as IL-8 and MCP-1, and adhesion molecules like ICAM-1, which may impair the host from recruiting leucocytes in the infected areas. Also, these proteases can cleave complement receptors on neutrophils attenuating their efficiency in phagocytosis. Finally, some periodontal bacteria produce toxins that are cytotoxic for leucocytes or inhibit phagocytosis. In both cases the bacteria acquire an advantage over the host that may be sufficient to sustain them in the periodontal niche.

The ability of the inflammatory response, initiated by the host, to clear the infection will determine the future of this complex process. If the infection is not eliminated and contained, more periodontal destruction will occur, and the host will launch the more effective adaptive immune response. If on the other hand the infection is resolved, the inflammatory process ceases and repair mechanisms are activated in order to repair damage and bring the tissues to a healthy state free of inflammation. Such ideal conditions are extremely rare and may be achieved only in experimental settings. Hence, in the ''real world'', histological signs of inflammation such as neutrophils in the crevice are present even in the ''healthy'' gingiva (Kinane et al. 1998).

References

Akifusa, S., Poole, S., Lewthwaite, J., Henderson, B. & Nair, S. P. (2001) Recombinant *Actinobacillus actinomycetemcomitans* cytolethal distending toxin proteins are required to interact to inhibit human cell cycle progression and to stimulate human leukocyte cytokine synthesis. *Infection and Immunity* **69**, 5925–5930.

Amano, A. (2003) Molecular interaction of *Porphyromonas gingivalis* with host cells: implication for the microbial pathogenesis of periodontal disease. *Journal of Periodontology* **74**, 90–96.

Amano, A., Sojar, H. T., Lee, J. Y., Sharma, A., Levine, M. J. & Genco, R. J. (1994) Salivary receptors for recombinant fimbrillin of *Porphyromonas gingivalis*. *Infection and Immunity* **62**, 3372–3380.

Ando, T., Kato, T., Ishihara, K., Ogiuchi, H. & Okuda, K. (1995) Heat shock proteins in the human periodontal disease process. *Microbiology and Immunology* **39**, 321–327.

Asai, Y., Ohyama, Y., Gen, K. & Ogawa, T. (2001) Bacterial fimbriae and their peptides activate human gingival epithelial cells

through Toll-like receptor 2. *Infection and Immunity* **69**, 7387–7395.

Balazovich, K. J., Suchard, S. J., Remick, D. G. & Boxer, L. A. (1996) Tumor necrosis factor-alpha and FMLP receptors are functionally linked during FMLP-stimulated activation of adherent human neutrophils. *Blood* **88**, 690–696.

Beutler, B. (2004) Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* **430**, 257–63.

Bhakdi, S., Klonisch, T., Nuber, P. & Fischer, W. (1991) Stimulation of monokine production by lipoteichoic acids. *Infection and Immunity* **59**, 4614–4620.

Blix, I. J. & Helgeland, K. (1998) LPS from *Actinobacillus actinomycetemcomitans* and production of nitric oxide in murine macrophages J774. *European Journal of Oral Sciences* **106**, 576–581.

Blix, I. J., Helgeland, K., Kahler, H. & Lyberg, T. (1999) LPS from *Actinobacillus actinomycetemcomitans* and the expression of beta2 integrins and L-selectin in an ex vivo human whole blood system. *European Journal of Oral Sciences* **107**, 14–20.

Chaly, Y. V., Paleolog, E. M., Kolesnikova, T. S., Tikhonov, I. I., Petratchenko, E. V. & Voitenok, N. N. (2000) Neutrophil alpha-defensin human neutrophil peptide modulates cytokine production in human monocytes and adhesion molecule expression in endothelial cells. *European Cytokine Network* **11**, 257–266.

Chamaillard, M., Girardin, S. E., Viala, J. & Philpott, D. J. (2003) Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation. *Cell Microbiology* **5**, 581–592.

Chung, W. O., Hansen, S. R., Rao, D. & Dale, B. A. (2004) Protease-activated receptor signaling increases epithelial antimicrobial peptide expression. *Journal of Immunology* **173**, 5165–5170.

Coats, S. R., Reife, R. A., Bainbridge, B. W., Pham, T. T. & Darveau, R. P. (2003) Porphyromonas gingivalis lipopolysaccharide antagonizes *Escherichia coli* lipopolysaccharide at toll-like receptor 4 in human endothelial cells. *Infection and Immunity* **71**, 6799–6807.

Coughlin, S. R. & Camerer, E. (2003) Participation in inflammation. *The Journal of Clinical Investigation* **111**, 25–27.

Cunningham, M. D., Bajorath, J., Somerville, J. E. & Darveau, R. P. (1999) Escherichia coli and *Porphyromonas gingivalis* lipopolysaccharide interactions with CD14: implications for myeloid and nonmyeloid cell activation. *Clinical Infectious Diseases* **28**, 497–504.

Darveau, R. P., Cunningham, M. D., Bailey, T., Seachord, C., Ratcliffe, K., Bainbridge, B., Dietsch, M., Page, R. C. & Aruffo, A. (1995) Ability of bacteria associated with chronic inflammatory disease to stimulate E-selectin expression and promote neutrophil adhesion. *Infection and Immunity* **63**, 1311–1317.

Derian, C. K., Santulli, R. J., Rao, P. E., Solomon, H. F. & Barrett, J. A. (1995) Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. *Journal of Immunology* **154**, 308–317.

De Rycke, J. & Oswald, E. (2001) Cytolethal distending toxin (CDT): a bacterial weapon to control host cell proliferation? *FEMS Microbiology Letters* **203**, 141–148.

Deschner, J., Singhal, A., Long, P., Liu, C. C., Piesco, N. & Agarwal, S. (2003) Cleavage of CD14 and LBP by a protease from *Prevotella intermedia*. *Archives of Microbiology* **179**, 430–436, Epub 2003 May 1.

Deshpande, R. G., Khan, M. B. & Genco, C. A. (1998) Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis*. *Infection and Immunity* **66**, 5337–5343.

Dorn, B., Dunn, W. & Progulske-Fox, A. (1999) Invasion of human coronary artery cells by periodontal pathogens. *Infection and immunity* **67**, 5792–5798.

Duncan, L., Yoshioka, M., Chandad, F. & Grenier, D. (2004) Loss of lipopolysaccharide receptor CD14 from the surface of human macrophage-like cells mediated by *Porphyromonas gingivalis* outer membrane vesicles. *Microbial Pathogenesis* **36**, 319–325.

Dunsche, A., Acil, Y., Dommisch, H., Siebert, R., Schroder, J. M. & Jepsen, S. (2002) The novel human beta-defensin-3 is widely expressed in oral tissues. *European Journal of Oral Sciences* **110**, 121–124.

Dunsche, A., Acil, Y., Siebert, R., Harder, J., Schroder, J. M. & Jepsen, S. (2001) Expression profile of human defensins and antimicrobial proteins in oral tissues. *Journal of Oral Pathology and Medicine* **30**, 154–158.

Ekuni, D., Yamamoto, T., Yamanaka, R., Tachibana, K. & Watanabe, T. (2003) Proteases augment the effects of lipopolysaccharide in rat gingiva. *Journal of Periodontal Research* **38**, 591–596.

Fletcher, J., Reddi, K., Poole, S., Nair, S., Henderson, B., Tabona, P. & Wilson, M. (1997) Interactions between periodontopathogenic bacteria and cytokines. *Journal of Periodontal Research* **32** (Part 2), 200–205.

Girardin, S. E., Tournebize, R., Mavris, M., Page, A. L., Li, X., Stark, G. R., Bertin, J., DiStefano, P. S., Yaniv, M., Sansonetti, P. J. & Philpott, D. J. (2001) CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive Shigella flexneri. *EMBO Reports* **2**, 736–742.

Goulbourne, P. A. & Ellen, R. P. (1991) Evidence that *Porphyromonas* (Bacteroides) *gingivalis* fimbriae function in adhesion to *Actinomyces viscosus*. *Journal of Bacteriology* **173**, 5266–5274.

Goulhen, F., Grenier, D. & Mayrand, D. (2003) Oral microbial heat-shock proteins and their potential contributions to infections. *Critical Reviews in Oral Biology and Medicine* **14**, 399–412.

Goulhen, F., Hafezi, A., Uitto, V. J., Hinode, D., Nakamura, R., Grenier, D. & Mayrand, D. (1998) Subcellular localization and cytotoxic activity of the GroEL-like protein isolated from *Actinobacillus actinomycetemcomitans*. *Infection and Immunity* **66**, 5307–5313.

Grenier, D., Mayrand, D. & McBride, B. C. (1989) Further studies on the degradation of immunoglobulins by black-pigmented Bacteroides. *Oral of Microbiology and Immunology* **4**, 12–18.

Hajishengallis, G., Sojar, H., Genco, R. J. & DeNardin, E. (2004) Intracellular signaling and cytokine induction upon interactions of *Porphyromonas gingivalis* fimbriae with pattern-recognition receptors. *Immunological Investigations* **33**, 157–172.

Hamada, N., Watanabe, K., Arai, M., Hiramine, H. & Umemoto, T. (2002) Cytokine production induced by a 67-kDa fimbrial protein from *Porphyromonas gingivalis*. *Oral Microbiology and Immunology* **17**, 197–200.

Hamada, N., Watanabe, K., Sasakawa, C., Yoshikawa, M., Yoshimura, F. & Umemoto, T. (1994) Construction and characterization of a fimA mutant of *Porphyromonas gingivalis*. *Infection and Immunity* **62**, 1696–1704.

Hanazawa, S., Kawata, Y., Takeshita, A., Kumada, H., Okithu, M., Tanaka, S., Yamamoto, Y., Masuda, T., Umemoto, T. & Kitano, S. (1993) Expression of monocyte chemoattractant protein 1 (MCP-1) in adult periodontal disease: increased monocyte chemotactic activity in crevicular fluids and induction of MCP-1 expression in gingival tissues. *Infection and Immunity* **61**, 5219–5224.

Hashimoto, M., Asai, Y., Tamai, R., Jinno, T., Umatani, K. & Ogawa, T. (2003) Chemical structure and immunobiological activity of lipid A from *Prevotella intermedia* ATCC 25611 lipopolysaccharide. *FEBS Letters* **543**, 98–102.

Hatakeyama, J., Tamai, R., Sugiyama, A., Akashi, S., Sugawara, S. & Takada, H. (2003) Contrasting responses of human gingival and periodontal ligament fibroblasts to bacterial cell-surface components through the CD14/Toll-like receptor system. *Oral Microbiology and Immunology* **18**, 14–23.

Hedges, S., Svensson, M. & Svanborg, C. (1992) Interleukin-6 response of epithelial cell lines to bacterial stimulation in vitro. *Infection and Immunity* **60**, 1295–1301.

Hermann, C., Spreitzer, I., Schroder, N. W., Morath, S., Lehner, M. D., Fischer, W., Schutt, C., Schumann, R. R. & Hartung, T. (2002) Cytokine induction by purified lipoteichoic acids from various bacterial species – role of LBP, sCD14, CD14 and failure to induce IL-12 and subsequent IFN-gamma release. *European Journal of Immunology* **32**, 541–551.

Hinode, D., Yoshioka, M., Tanabe, S., Miki, O., Masuda, K. & Nakamura, R. (1998) The GroEL-like protein from *Campylobacter rectus*: immunological characterization and interleukin-6 and -8 induction in human gingival fibroblast. *FEMS Microbiological Letters* **167**, 1–6.

Hirschfeld, M., Weis, J. J., Toshchakov, V., Salkowski, C. A., Cody, M. J., Ward, D. C., Qureshi, N., Michalek, S. M. & Vogel, S. N. (2001) Signaling by toll-like receptor 2 and 4 agonists results in differential gene expres-

sion in murine macrophages. *Infection and Immunity* **69**, 1477–1482.

Holt, S. C., Kesavalu, L., Walker, S. & Genco, C. A. (1999) Virulence factors of *Porphyromonas gingivalis*. *Periodontology 2000* **20**, 168–238.

Hou, L., Ravenall, S., Macey, M. G., Harriott, P., Kapas, S. & Howells, G. L. (1998) Protease-activated receptors and their role in IL-6 and NF-IL-6 expression in human gingival fibroblasts. *Journal of Periodontal Research* **33**, 205–211.

Hou, L., Sasaki, H. & Stashenko, P. (2000) Toll-like receptor 4-deficient mice have reduced bone destruction following mixed anaerobic infection. *Infection and Immunity* **68**, 4681–4687.

Inohara, N., Koseki, T., Lin, J., del Peso, L., Lucas, P. C., Chen, F. F., Ogura, Y. & Nunez, G. (2000) An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. *Journal of Biological Chemistry* **275**, 27823–27831.

Jagels, M. A., Travis, J., Potempa, J., Pike, R. & Hugli, T. E. (1996) Proteolytic inactivation of the leukocyte C5a receptor by proteinases derived from *Porphyromonas gingivalis*. *Infection and Immunity* **64**, 1984–1991.

Janeway, C. A., Travers, P., Walport, M. & Shlomchik, M. (2001) *Immunobiology. The Immune System in Health and Disease*, 5th edition. New York: Garland Publishing.

Jin, Y., Gupta, D. & Dziarski, R. (1998) Endothelial and epithelial cells do not respond to complexes of peptidoglycan with soluble CD14 but are activated indirectly by peptidoglycan-induced tumor necrosis factor-alpha and interleukin-1 from monocytes. *The Journal of Infectious Diseases* **177**, 1629–1638.

Johansson, A., Claesson, R., Hanstrom, L., Sandstrom, G. & Kalfas, S. (2000) Polymorphonuclear leukocyte degranulation induced by leukotoxin from *Actinobacillus actinomycetemcomitans*. *Journal of Periodontal Research* **35**, 85–92.

Jotwani, R., Pulendran, B., Agrawal, S. & Cutler, C. W. (2003) Human dendritic cells respond to *Porphyromonas gingivalis* LPS by promoting a Th2 effector response in vitro. *European Journal of Immunology* **33**, 2980–2986.

Kato, S., Kowashi, Y. & Demuth, D. R. (2002) Outer membrane-like vesicles secreted by *Actinobacillus actinomycetemcomitans* are enriched in leukotoxin. *Microbial Pathogenesis* **32**, 1–13.

Kawai, T., Eisen-Lev, R., Seki, M., Eastcott, J. W., Wilson, M. E. & Taubman, M. A. (2000) Requirement of B7 costimulation for Th1-mediated inflammatory bone resorption in experimental periodontal disease. *Journal of Immunology* **164**, 2102–2109.

Kawamura, N., Imanishi, N., Koike, H., Nakahara, H., Phillips, L. & Morooka, S. (1995) Lipoteichoic acid-induced neutrophil adhesion via E-selectin to human umbilical vein endothelial cells (HUVECs). *Biochemical and Biophysical Research Communications* **217**, 1208–1215.

Kengatharan, K. M., De Kimpe, S., Robson, C., Foster, S. J. & Thiemermann, C. (1998) Mechanism of Gram-positive shock: identification of peptidoglycan and lipoteichoic acid moieties essential in the induction of nitric oxide synthase, shock, and multiple organ failure. *The Journal of Experimental Medicine* **188**, 305–315.

Khlgatian, M., Nassar, H., Chou, H. H., Gibson, F. C. III & Genco, C. A. (2002) Fimbria-dependent activation of cell adhesion molecule expression in *Porphyromonas gingivalis*-infected endothelial cells. *Infection and Immunity* **70**, 257–267.

Kinane, D. F., Berglundh, T. & Lindhe, J. (1998) *Host–Parasite Interactions in Periodontal Disease. Clinical Periodontology and Implant Dentistry*, 4th edition, Chapter 5. Oxford: Blackwell Munksgaard.

Lally, E. T., Hill, R. B., Kieba, I. R. & Korostoff, J. (1999) The interaction between RTX toxins and target cells. *Trends in Microbiology* **7**, 356–361.

Lally, E. T., Kieba, I. R., Sato, A., Green, C. L., Rosenbloom, J., Korostoff, J., Wang, J. F., Shenker, B. J., Ortlepp, S., Robinson, M. K. & Billings, P. C. (1997) RTX toxins recognize a beta2 integrin on the surface of human target cells. *The Journal of Biological Chemistry* **272**, 30463–30469.

Lamont, R. J., Chan, A., Belton, C. M., Izutsu, K. T., Vasel, D. & Weinberg, A. (1995) *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infection and Immunity* **63**, 3878–3885.

Leemans, J. C., Vervoordeldonk, M. J., Florquin, S., van Kessel, K. P. & van der Poll, T. (2002) Differential role of interleukin-6 in lung inflammation induced by lipoteichoic acid and peptidoglycan from *Staphylococcus aureus*. *American Journal of Respiratory and Critical Care Medicine* **165**, 1445–1450.

Loos, M., Clas, F. & Fischer, W. (1986) Interaction of purified lipoteichoic acid with the classical complement pathway. *Infection and Immunity* **53**, 595–599.

Lourbakos, A., Chinni, C., Thompson, P., Potempa, J., Travis, J., Mackie, E. J. & Pike, R. N. (1998) Cleavage and activation of proteinase-activated receptor-2 on human neutrophils by gingipain-R from *Porphyromonas gingivalis*. *FEBS Letters* **435**, 45–48.

Lourbakos, A., Potempa, J., Travis, J., D'Andrea, M. R., Andrade-Gordon, P., Santulli, R., Mackie, E. J. & Pike, R. N. (2001) Arginine-specific protease from *Porphyromonas gingivalis* activates protease-activated receptors on human oral epithelial cells and induces interleukin-6 secretion. *Infection and Immunity* **69**, 5121–5130.

Lu, Q., Jin, L., Darveau, R. P. & Samaranayake, L. P. (2004) Expression of human beta-defensins-1 and -2 peptides in unresolved chronic periodontitis. *Journal of Periodontal Research* **39**, 221–227.

Madianos, P. N., Papapanou, P. N. & Sandros, J. (1997) *Porphyromonas gingivalis* infection of oral epithelium inhibits neutrophil transepithelial migration. *Infection and Immunity* **65**, 3983–3990.

Malek, R., Fisher, J. G., Caleca, A., Stinson, M., van Oss, C. J., Lee, J. Y., Cho, M. I., Genco, R. J., Evans, R. T. & Dyer, D. W. (1994) Inactivation of the *Porphyromonas gingivalis* fimA gene blocks periodontal damage in gnotobiotic rats. *Journal of Bacteriology* **176**, 1052–1059.

Masaka, T., Hayashi, J. & Ishikawa, I. (1999) Soluble CD14-dependent intercellular adhesion molecular-1 induction by *Porphyromonas gingivalis* lipopolysaccharide in human gingival fibroblasts. *Journal of Periodontology* **70**, 772–778.

Mattsson, E., Verhage, L., Rollof, J., Fleer, A., Verhoef, J. & van Dijk, H. (1993) Peptidoglycan and teichoic acid from *Staphylococcus epidermidis* stimulate human monocytes to release tumour necrosis factor-alpha, interleukin-1 beta and interleukin-6. *FEMS Immunology and Medical Microbiology* **7**, 281–287.

Medzhitov, R. (2001) Toll-like receptors and innate immunity. *Nature Reviews Immunology* **1**, 135–145.

Moritz, A. J., Cappelli, D., Lantz, M. S., Holt, S. C. & Ebersole, J. L. (1998) Immunization with *Porphyromonas gingivalis* cysteine protease: effects on experimental gingivitis and ligature-induced periodontitis in *Macaca fascicularis*. *Journal of Periodontology* **69**, 686–697.

Nakamura, T., Nitta, H. & Ishikawa, I. (2004) Effect of low dose *Actinobacillus actinomycetemcomitans* lipopolysaccharide pretreatment on cytokine production by human whole blood. *Journal of Periodontal Research* **39**, 129–135.

Nakayama, K., Kadowaki, T., Okamoto, K. & Yamamoto, K. (1995) Construction and characterization of arginine-specific cysteine proteinase (Arg-gingipain)-deficient mutants of *Porphyromonas gingivalis*. Evidence for significant contribution of Arg-gingipain to virulence. *Journal of Biological Chemistry* **270**, 23619–23626.

Nassar, H., Chou, H. H., Khlgatian, M., Gibson, F. C. III, Van Dyke, T. E. & Genco, C. A. (2002) Role for fimbriae and lysine-specific cysteine proteinase gingipain K in expression of interleukin-8 and monocyte chemoattractant protein in *Porphyromonas gingivalis*-infected endothelial cells. *Infection and Immunity* **70**, 268–276.

Netea, M. G., van Deuren, M., Kullberg, B. J., Cavaillon, J. M. & Van der Meer, J. W. (2002) Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? *Trends in Immunology* **23**, 135–139.

Noguchi, T., Shiba, H., Komatsuzawa, H., Mizuno, N., Uchida, Y., Ouhara, K., Asakawa, R., Kudo, S., Kawaguchi, H., Sugai, M. & Kurihara, H. (2003) Syntheses of prostaglandin E2 and E-cadherin and gene expression of beta-defensin-2 by human gingival epithelial cells in response to *Actinobacillus actinomycetemcomitans*. *Inflammation* **27**, 341–349.

Ogawa, T., Asai, Y., Hashimoto, M. & Uchida, H. (2002) Bacterial fimbriae activate human peripheral blood monocytes utilizing TLR2,

CD14 and CD11a/CD18 as cellular receptors. *European Journal of Immunology* **32**, 2543–2550.

Ogawa, T. & Hamada, S. (1994) Hemagglutinating and chemotactic properties of synthetic peptide segments of fimbrial protein from *Porphyromonas gingivalis*. *Infection and Immunity* **62**, 3305–3310.

Ogawa, T., Uchida, H. & Hamada, S. (1994) Porphyromonas gingivalis fimbriae and their synthetic peptides induce proinflammatory cytokines in human peripheral blood monocyte cultures. *FEMS Microbiology Letters* **116**, 237–242.

Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L., Chen, F. F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R. H., Achkar, J. P., Brant, S. R., Bayless, T. M., Kirschner, B. S., Hanauer, S. B., Nunez, G. & Cho, J. H. (2001a) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411**, 603–606.

Ogura, Y., Inohara, N., Benito, A., Chen, F. F., Yamaoka, S. & Nunez, G. (2001b) Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *Journal of Biological Chemistry* **276**, 4812–4818.

Page, R. C. & Schroeder, H. E. (1976) Pathogenesis of inflammatory periodontal disease. A summary of current work. *Laboratory Investigation* **34**, 235–249.

Panaro, M. A. & Mitolo, V. (1999) Cellular responses to FMLP challenging: a mini-review. *Immunopharmacology and Immunotoxicology* **21**, 397–419.

Philpott, D. J., Belaid, D., Troubadour, P., Thiberge, J. M., Tankovic, J., Labigne, A. & Ferrero, R. L. (2002) Reduced activation of inflammatory responses in host cells by mouse-adapted *Helicobacter pylori* isolates. *Cellular Microbiology* **4**, 285–296.

Philpott, D. J. & Girardin, S. E. (2004) The role of Toll-like receptors and Nod proteins in bacterial infection. *Molecular Immunology* **41**, 1099–1108.

Pollanen, M. T., Salonen, J. I., Grenier, D. & Uitto, V. J. (2000) Epithelial cell response to challenge of bacterial lipoteichoic acids and lipopolysaccharides in vitro. *Journal of Medical Microbiology* **49**, 245–252.

Potempa, J., Pike, R. & Travis, J. (1995) The multiple forms of trypsin-like activity present in various strains of *Porphyromonas gingivalis* are due to the presence of either Arg-gingipain or Lys-gingipain. *Infection and Immunity* **63**, 1176–1182.

Reife, R. A., Shapiro, R. A., Bamber, B. A., Berry, K. K., Mick, G. E. & Darveau, R. P. (1995) *Porphyromonas gingivalis* lipopolysaccharide is poorly recognized by molecular components of innate host defense in a mouse model of early inflammation. *Infection and Immunity* **63**, 4686–4694.

Rietschel, E. T., Kirikae, T., Schade, F. U., Mamat, U., Schmidt, G., Loppnow, H., Ulmer, A. J., Zahringer, U., Seydel, U., DiPadova, F., Schreier, M. & Brade, H. (1994) Bacterial endotoxin: molecular relationships of structure to activity and function. *The FASEB Journal* **8**, 217–225.

Sandros, J., Madianos, P. N. & Papapanou, P. N. (1996) Cellular events concurrent with *Porphyromonas gingivalis* invasion of oral epithelium in vitro. *European Journal of Oral Sciences* **104**, 363–371.

Schnur, R. A. & Newman, S. L. (1990) The respiratory burst response to histoplasma capsulatum by human neutrophils. Evidence for intracellular trapping of superoxide anion. *Journal of Immunology* **144**, 4765–4772.

Schytte Blix, I. J., Helgeland, K., Hvattum, E. & Lyberg, T. (1999) Lipopolysaccharide from *Actinobacillus actinomycetemcomitans* stimulates production of interleukin-1beta, tumor necrosis factor-alpha, interleukin-6 and interleukin-1 receptor antagonist in human whole blood. *Journal of Periodontal Research* **34**, 34–40.

Shapira, L., Champagne, C., Van Dyke, T. E. & Amar, S. (1998) Strain-dependent activation of monocytes and inflammatory macrophages by lipopolysaccharide of *Porphyromonas gingivalis*. *Infection and Immunity* **66**, 2736–2742.

Shelburne, C. E., Gleason, R. M., Germaine, G. R., Wolff, L. F., Mullally, B. H., Coulter, W. A. & Lopatin, D. E. (2002) Quantitative reverse transcription polymerase chain reaction analysis of *Porphyromonas gingivalis* gene expression in vivo. *Journal of Microbiological Methods* **49**, 147–156.

Sugawara, S., Nemoto, E., Tada, H., Miyake, K., Imamura, T. & Takada, H. (2000) Proteolysis of human monocyte CD14 by cysteine proteinases (gingipains) from *Porphyromonas gingivalis* leading to lipopolysaccharide hyporesponsiveness. *Journal of Immunology* **165**, 411–418.

Sundqvist, G. K., Bengston, A. & Carlsson, J. (1988) Generation and degradation of the complement fragment C5a in human serum by *Bacteroides gingivalis*. *Oral Microbiology and Immunology* **3**, 103–107.

Sundqvist, G. K., Carlsson, J., Herrmann, B. F., Hofling, J. F. & Vaatainen, A. (1984) Degradation in vivo of the C3 protein of guinea-pig complement by a pathogenic strain of *Bacteroides gingivalis*. *Scandinavian Journal of Dental Research* **92**, 14–24.

Sundqvist, G. K., Carlsson, J., Herrmann, B. F. & Tarnvik, A. (1985) Degradation of human immunoglobulins G and M and complement factors C3 and C5 by black-pigmented Bacteroides. *Journal of Medical Microbiology* **19**, 85–94.

Supajatura, V., Ushio, H., Nakao, A., Akira, S., Okumura, K., Ra, C. & Ogawa, H. (2002) Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *Journal of Clinical Investigation* **109**, 1351–1359.

Tabeta, K., Yamazaki, K., Hotokezaka, H., Yoshie, H. & Hara, K. (2000) Elevated humoral immune response to heat shock protein 60 (hsp60) family in periodontitis patients. *Clinical and Experimental Immunology* **120**, 285–293.

Tada, H., Sugawara, S., Nemoto, E., Imamura, T., Potempa, J., Travis, J., Shimauchi, H. & Takada, H. (2003) Proteolysis of ICAM-1 on human oral epithelial cells by gingipains. *Journal of Dental Research* **82**, 796–801.

Tada, H., Sugawara, S., Nemoto, E., Takahashi, N., Imamura, T., Potempa, J., Travis, J., Shimauchi, H. & Takada, H. (2002) Proteolysis of CD14 on human gingival fibroblasts by arginine-specific cysteine proteinases from *Porphyromonas gingivalis* leading to down-regulation of lipopolysaccharide-induced interleukin-8 production. *Infection and Immunity* **70**, 3304–3307.

Taichman, N. S., Iwase, M., Lally, E. T., Shattil, S. J., Cunningham, M. E. & Korchak, H. M. (1991) Early changes in cytosolic calcium and membrane potential induced by *Actinobacillus actinomycetemcomitans* leukotoxin in susceptible and resistant target cells. *Journal of Immunology* **147**, 3587–3594.

Takada, H., Mihara, J., Morisaki, I. & Hamada, S. (1991) Induction of interleukin-1 and -6 in human gingival fibroblast cultures stimulated with Bacteroides lipopolysaccharides. *Infection and Immunity* **59**, 295–301.

Takeda, K. & Akira, S. (2005) Toll-like receptors in innate immunity. *International Immunology* **17**, 1–14.

Tamura, M., Tokuda, M., Nagaoka, S. & Takada, H. (1992) Lipopolysaccharides of *Bacteroides intermedius* (*Prevotella intermedia*) and *Bacteroides* (Porphyromonas) *gingivalis* induce interleukin-8 gene expression in human gingival fibroblast cultures. *Infection and Immunity* **60**, 4932–4937 (Erratum in: *Infection and Immunity* 1993 61, 368).

Tanabe, S., Hinode, D., Yokoyama, M., Fukui, M., Nakamura, R., Yoshioka, M., Grenier, D. & Mayrand, D. (2003) *Helicobacter pylori* and *Campylobacter rectus* share a common antigen. *Oral Microbiology and Immunology* **18**, 79–87.

Territo, M. C., Ganz, T., Selsted, M. E. & Lehrer, R. (1989) Monocyte-chemotactic activity of defensins from human neutrophils. *The Journal of Clinical Investigation* **84**, 2017–2020.

Uehara, A., Sugawara, S. & Takada, H. (2002) Priming of human oral epithelial cells by interferon-gamma to secrete cytokines in response to lipopolysaccharides, lipoteichoic acids and peptidoglycans. *Journal of Medical Microbiology* **51**, 626–634.

Uehara, A., Sugawara, S., Tamai, R. & Takada, H. (2001) Contrasting responses of human gingival and colonic epithelial cells to lipopolysaccharides, lipoteichoic acids and peptidoglycans in the presence of soluble CD14. *Medical Microbiology and Immunology* **189**, 185–192.

Ueki, K., Tabeta, K., Yoshie, H. & Yamazaki, K. (2002) Self-heat shock protein 60 induces tumour necrosis factor-alpha in monocyte-derived macrophage: possible role in chronic inflammatory periodontal disease. *Clinical and Experimental Immunology* **127**, 72–77.

van Langevelde, P., Ravensbergen, E., Grashoff, P., Beekhuizen, H., Groeneveld, P. H. & van Dissel, J. T. (1999) Antibiotic-induced cell wall fragments of *Staphylococcus aureus* increase endothelial chemokine secre-

tion and adhesiveness for granulocytes. *Antimicrobial Agents and Chemotherapy* **43**, 2984–2989.

Van Wetering, S., Mannesse-Lazeroms, S. P., Van Sterkenburg, M. A., Daha, M. R., Dijkman, J. H. & Hiemstra, P. S. (1997) Effect of defensins on interleukin-8 synthesis in airway epithelial cells. *The American Journal of Physiology* **272**, L888–L896.

Volz, A. (1993) Regulation of CD18 expression in human neutrophils as related to shape changes. *Journal of Cell Science* **106**, 493–501.

von Aulock, S., Schroder, N. W., Traub, S., Gueinzius, K., Lorenz, E., Hartung, T., Schumann, R. R. & Hermann, C. (2004) Heterozygous toll-like receptor 2 polymorphism does not affect lipoteichoic acid-induced chemokine and inflammatory responses. *Infection and Immunity* **72**, 1828–1831.

Wang, P. L., Oido-Mori, M., Fujii, T., Kowashi, Y., Kikuchi, M., Suetsugu, Y., Tanaka, J., Azuma, Y., Shinohara, M. & Ohura, K. (2002) Effect of anti-CD14 antibody on experimental periodontitis induced by *Porphyromonas gingivalis* lipopolysaccharide. *Japanese Journal of Pharmacology* **89**, 176–183.

Wang, Z. M., Liu, C. & Dziarski, R. (2000) Chemokines are the main proinflammatory mediators in human monocytes activated by *Staphylococcus aureus*, peptidoglycan, and endotoxin. *The Journal of Biological Chemistry* **275**, 20260–20267.

Watanabe, K., Yamaji, Y. & Umemoto, T. (1992) Correlation between cell-adherent activity and surface structure in *Porphyromonas gingivalis*. *Oral Microbiology and Immunology* **7**, 357–363.

Weinberg, A., Belton, C. M., Park, Y. & Lamont, R. J. (1997) Role of fimbriae in *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infection and Immunity* **65**, 313–316.

Yamaguchi, N., Kubo, C., Masuhiro, Y., Lally, E. T., Koga, T. & Hanazawa, S. (2004) Tumor necrosis factor alpha enhances *Actinobacillus actinomycetemcomitans* leukotoxin-induced HL-60 cell apoptosis by stimulating lymphocyte function-associated antigen 1 expression. *Infection and Immunity* **72**, 269–276.

Yamazaki, K., Ohsawa, Y., Tabeta, K., Ito, H., Ueki, K., Oda, T., Yoshie, H. & Seymour, G. J. (2002) Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. *Infection and Immunity* **70**, 2492–2501.

Yang, D., Chen, Q., Chertov, O. & Oppenheim, J. J. (2000) Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. *Journal of Leukocyte Biology* **68**, 9–14.

Zhang, J., Dong, H., Kashket, S. & Duncan, M. J. (1999) IL-8 degradation by *Porphyromonas gingivalis* proteases. *Microbial Pathogenesis* **26**, 275–280.

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