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Molecular aspects of the pathogenesis of periodontitis

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The classical model of periodontal disease pathogenesis, developed by Page & Kornman in 1997 (58), provides a key framework to underpin studies aimed at unraveling the complex interdependent relationships that exist both within the plaque biofilm and between the biofilm and the host response (Fig. 1). Almost two decades later, this classical paradigm still has relevance but advances in knowledge require it to be modified to accommodate new discoveries and learnings in the fields of microbiology and immunology, many of which have been driven by the molecular era. This volume of *Periodontology 2000* addresses several of those issues and contains key narrative reviews by luminaries in their relevant fields, some of which have helped inform changes in the classical model of periodontitis pathogenesis to the one illustrated in Fig. 2.

We now recognize that a pathogenic biofilm is a necessary prerequisite for periodontitis to develop but in itself is insufficient to cause the disease. Disease results from complex interactions between the biofilm and the inflammatory immune response, and it is the latter that is estimated to account for almost 80% of the risk of periodontal tissue damage (25). Periodontitis is a complex disease with multiple component causes, some with their basis in genetics, some caused by epigenetic influences and others that are modifiable because they relate to patient behaviors, medications or environmental factors, all of which conspire to establish and propagate the periodontitis lesion. In addition to such 'patient-specific' risk factors, there are also 'site-specific characteristics' (e.g. anatomical factors), which may favor the development of a lesion. The periodontitis phenotype is characterized by an exaggerated, yet poorly effective and nonresolving, inflammation of the connective tissues supporting the teeth that leads to tissue destruction, rather than a specifically targeted, effective and self-resolving inflammatory immune response. Key changes in our perceptions of the infectious immune condition, which we call periodontitis, include:

- the realization that retaining or attaining clinical health requires a health-promoting biofilm within which symbiotic relationships exist between microorganisms and with the host response. The latter can provide key nutrients via gingival crevicular fluid, and the various proteins and peptides released by biofilm organisms trigger a host response that is both proportionate and resolving (52, 71).
- if the biofilm is not disrupted frequently and is allowed to accumulate, the conditions within it start to favor bacterial species, such as *Fusobacterium nucleatum*, that are capable of sensing and influencing their environment by employing chemical cues. Such 'quorum-sensing' organisms start to emerge and elicit a stronger host response, which, in turn, can lead to the development of gingival inflammation and increase the supply of certain nutrients, such as heme, that encourage the proliferation of traditional pathogens such as *Porphyromonas gingivalis*. This is referred to, in Fig. 2, as 'incipient dysbiosis' because in nonsusceptible individuals it does not progress beyond gingivitis.
- in susceptible patients, incipient dysbiosis can trigger an inappropriate, and frequently excessive, host response, in which excess cytokines, reactive oxygen species (oxidative stress) and matrix metalloproteinases are generated and overwhelm their respective antagonists (e.g. antioxidants and tissue inhibitors of matrix metalloproteinases), resulting in collateral periodontal tissue damage. Damage-associated molecular peptides are released, which further propagate the inflammatory response, and a subsequent failure of innate inflammation resolving mechanisms results in

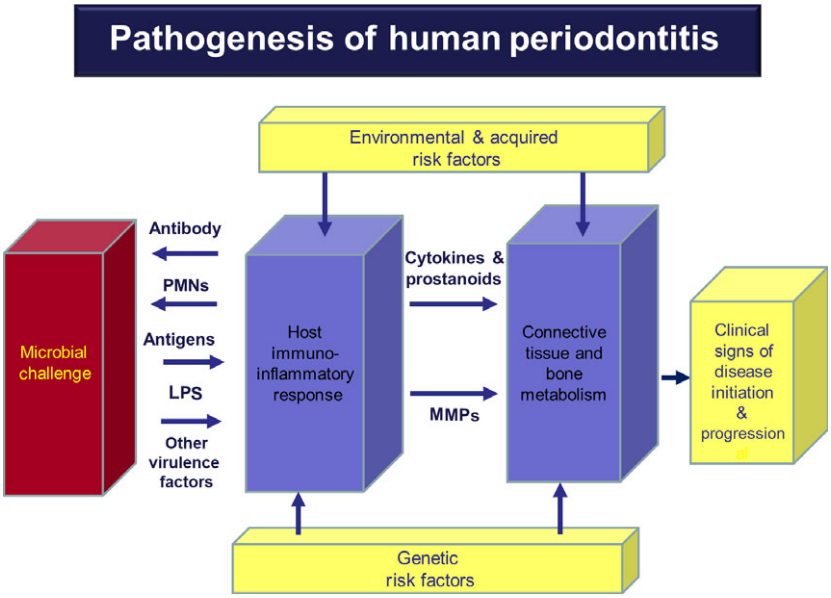

Fig. 1. Classical model of Page & Kornman, showing host–microbe interactions in the pathogenesis of periodontitis (58). LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; PMNs, polymorphonuclear neutrophils.

chronicity of the inflammatory lesion. Viruses appear also to play a role and are capable of priming inflammatory immune cells, as well as subverting various signaling pathways within those cells, in order to create 'dysregulation' in the ordered nature of specific immunity.

- the chronic inflammatory state is characterized by attempts at healing (angiogenesis and fibrosis) arising at the same time as inflammation, creating a rich nutritional environment for sustaining the dysbiosis and thus the pathogenic biofilm. Plasma cells and neutrophils dominate the active lesion, and the latter, being a bridge between both innate and acquired (humoral) immune systems, appear to be particularly destructive in their behavior as a result of dysregulation of chemotactic and microbicidal processes and failure to release pro-resolving lipid mediators, such as lipoxins.
- at the stage of progressing periodontitis, intervention is necessary to remove biofilm to an extent following which health-promoting microbial species can re-establish themselves and help drive a reduction in inflammation, a process that has failed to be activated naturally by pro-resolution pathways and which, when active, also appears to be capable of restoring normal tissue structure and function. The complex interplay between the health-promoting biofilm and the various signaling pathways that drive the host response appear to restore a balanced and well-regulated inflammatory immune repertoire. However, the degree of biofilm reduction necessary to re-establish symbiosis appears to vary from one person to the next. At extreme ends of the normal distribution curve are disease-resistant people who are at low, or even zero, risk of developing periodontitis (disease resistance), and high-risk patients in whom only mild biofilm accumulation appears to exceed the threshold necessary to trigger a destructive host response and subsequent tissue damage.

Oral biofilms

The microbial ecosystem of the oral cavity is the habitat for a multitude of bacterial and viral species. In health there is a symbiotic relationship among the resident microorganisms of the biofilm, including interactions between the different bacterial species and the hosts' inflammatory immune response to that biofilm. However, there remains limited knowledge of the key factors that maintain this stable equilibrium and of the importance and role of oral commensals in protecting against the development of dysbiosis.

Historically, periodontitis was believed to result from an impaired and underactive host response to the oral biofilm, which allowed the biomass to evolve both quantitatively and qualitatively. Such changes affected the composition and virulence expression of certain species in the oral flora, and this was the predominant driving force through which the symbiosis was destroyed. This may still pertain to a degree, but we now recognize that factors which may be inborn or acquired may also drive the dysbiosis and ultimately effect disease progression. In this respect, viruses may play a key role

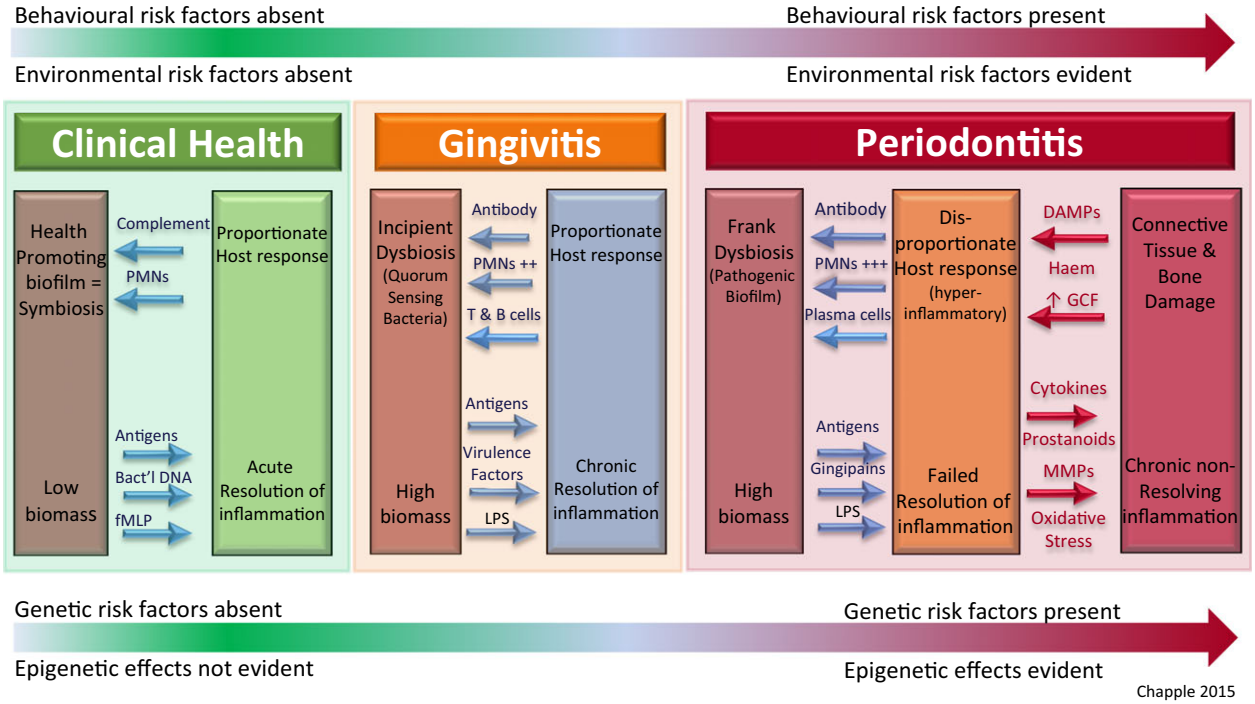

Fig. 2. Contemporary model of host–microbe interactions in the pathogenesis of periodontitis, in which the host response drives an incipient dysbiosis (gingivitis). If the biofilm is not disrupted/removed, frank dysbiosis results and perpetuates a chronic nonresolving and destructive inflammation. DAMPs, damage-associated molecular patterns; fMLP, *N*-formylmethionyl-leucyl-phenylalanine; GCF, gingival crevicular fluid; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; PMNs, polymorphonuclear neutrophils.

in the shift of the whole ecosystem by priming and activating host-response pathways that ultimately become dysfunctional and ineffective. The distinction between pathogens and commensals is becoming more and more difficult because the microbial 'environment' that allows pathogens to thrive is also characterized by bacteria that dominate a healthy biofilm but which may also be necessary for pathogenic species to survive.

Hypotheses that were based on specific periodontal pathogens (47) may also still have relevance, as indeed may the nonspecific hypothesis (48, 68), but there is now a general consensus that these traditional microbiological paradigms are too simplistic and have probably served their time and purpose. What is clear is that the subgingival biofilm is significantly different at clinically healthy sites compared with diseased sites (60).

More recent studies that have examined host–bacteria interactions revealed that commensal bacteria not only protect the host by niche occupation but also appear to create an environment that promotes the development of healthy periodontal tissue structure and function. These data indicate that our host-associated polymicrobial communities, such as those found in the oral cavity, co-evolved with us and have become an integral part of who we are (45). Indeed, Roberts & Darveau (60) argue, in this volume of *Periodontology 2000*, that we should consider the microbiome as a human organ.

Frequently ignored, but potentially vital, inhabitants of periodontal lesions are the ubiquitous human herpesviruses, including cytomegalovirus, Epstein–Barr virus and herpes simplex virus 1. Humans are exposed to herpesviruses following birth; thereafter, those herpesviruses reside within the body in a dormant, nondisease-promoting, state until the local environment is changed or the host becomes immunosuppressed, when they then proliferate and cause clinical pathology. The classic example is herpes simplex virus 1, which resides in the trigeminal ganglion and in susceptible individuals can track down the maxillary or mandibular branches of the trigeminal nerve to cause herpes labialis, when, for example, there is local tissue damage induced by sunlight. Two key issues are relevant here: the susceptible individual; and tissue damage. According to Slots (63), these viruses have been identified in the periodontium in far higher prevalence in patients with aggressive and chronic periodontitis than in subjects with periodontal health. Indeed, several authors in this volume of *Periodontology 2000* describe methods by which viruses

may subvert the molecular signaling pathways that control the highly coordinated immune inflammatory pathways to confer advantage to themselves and, in doing so, may contribute significantly to the pathogenesis of periodontitis. Examples include the activation of type-1 interferon responses in neutrophils (76), the release of proinflammatory cytokines and matrix metalloproteinases and the activation of osteoclast pathways. Slots (63) argues the potential importance of viral–bacterial co-infection as a highly plausible mechanistic contributor to periodontal tissue destruction. Slots (63) also speculates that periodontal herpesviruses may enter the systemic circulation and give rise to medical illnesses and conditions, especially in immunocompromised individuals.

Host response and local inflammation

It is over 100 years since it was first observed that a clinically healthy periodontal state is characterized by a leukocytic infiltrate into the gingival tissues in response to basic microbial challenge. This type of immune surveillance appears to be protective because it elicits continuous migration of leukocytes through the junctional epithelium in a highly regulated manner, in which excessive or insufficient migration may each result in disease.

This state of 'controlled inflammation' may also be beneficial to the biofilm, in which some species have been shown to elicit and activate certain cellular host-response pathways, which by their nature, provide a continuous source of substrates by stimulating leakage from local capillaries for their nutrition. The colonizing microbes on mucosal surfaces, together with dangerous endogenous signaling molecules, such as extracellular ATP or extracellular DNA, activate the inflammasome, resulting in the subsequent secretion of the proinflammatory cytokines interleukin-1beta and interleukin-18. Inflammasome activation is mediated by caspase-1 activity, which also has an important role in the activation of cellular apoptosis. Reactive oxygen species are also required, and indeed periodontitis is characterized by oxidative stress (8), providing further opportunities for inflammasome activation, which may result in positive feedback, impacting upon the host response as well as on the chronicity of the inflammation, as discussed by Yilmaz & Lee (77).

The release and secretion of proinflammatory cytokines activates polymorphonuclear leukocytes (neutrophils), which express various cell-surface receptors that bind to chemotactic stimuli and initiate downstream signaling sequences that lead to complex reactions and events, including cytosolic actin reorganization, shape changes and development of cellular polarity. The release of histamine and the activation of complement components C3a and C5a leads to vasodilatation, increased vascular permeability and slowing of blood flow within the respective capillary beds. The neutrophils then fall out of midstream blood flow and contact the vascular endothelium, where they marginate and roll on the endothelial surface of the capillaries before binding firmly, via intergrin receptors, and moving out of the blood vessels into the tissues via diapedesis.

Neutrophils belong to the fastest-moving mammalian cells, and once in the tissues they migrate along a chemotactic gradient that enables them to locate the site of infection and respond via receptor-mediated phagocytosis and subsequent intracellular killing of the ingested bacteria (9). Recent research has demonstrated that peripheral blood neutrophils from patients with periodontitis have defective chemotactic accuracy, potentially increasing their tissue transit times and thus their potential to cause collateral tissue damage (61).

A major role in this process is played by the lectin site of the leukocyte β_2-integrin receptor (also known as CR3, Mac-1 and CD11b/CD18). Reports suggest that another potentially important receptor (delineated from other myeloid cells), lipopolysaccharide receptor (CD14), is only found in an inactive form. Eventually stored within the neutrophil, CD14 becomes expressed on the outer cell-membrane surface under extreme conditions (73).

Neutrophil movement through the periodontium to maintain healthy periodontal tissues is well documented in both humans and mice (4, 33, 57, 74, 78). Reduced recruitment to the periodontal tissues, or indeed excessive mobilization of cells, as seen in the absence of the regulatory endothelial protein, DEL-1, arises in conditions that lead to periodontitis (15, 53).

Together with neutrophil diapedesis and chemotactic migration toward the site of bacterial infection (i.e. transmigration through the gingival connective tissues into the gingival crevice), local capillaries also release an enhanced amount of serum as a result of the effects of histamine and complement C3a and C5a upon vascular permeability. The serum becomes tissue fluid and carries inflammatory peptides, such as antibodies, complement and other host-mediated defence agents, through the tissues and into the gingival crevice. The increased tissue fluid causes the tissues to swell and also increases the exudation of gingival crevicular fluid.

Some pathogens are able to extend and prolong these inflammatory reactions in order to guarantee a continuous supply of host-derived nutrients. For example, *P. gingivalis* activates complement C5 and macrophages by binding C5a to the C5a receptor, which then leads to intracellular signaling accompanied by Ca^{2+} release and also an 'inside-out' signaling that enables *P. gingivalis* to bind to the cell surface (27–32, 44, 46, 51). Neutrophil activation leads to phagocytosis and intracellular killing of microorganisms, as well as to the release of enzymes, which may contribute to local tissue destruction. Enhanced release of elastase and other proteinases, such as collagenase, results in the depolymerization of tissue collagen fibers, thus increasing local tissue permeability. In this volume of *Periodontology 2000*, Herrmann & Meyle (37) present the most contemporary evidence for neutrophilic inflammation contributing substantially to periodontal pathology.

In diabetes, local inflammatory reactions within the periodontal tissues are modulated by the associated metabolic dysregulation (i.e. tissue responses to inflammatory stimuli are enhanced in poorly controlled diabetes by the binding of glucose, advanced glycation end-products and oxidized low-density lipoproteins to their complementary receptors on the neutrophil surface) (Fig. 3). These hyperinflammatory cellular responses lead to rapid tissue destruction and attachment loss, as highlighted in the review by Sonnenschein & Meyle (66). This specific exposure provides a classical example of how changes in human physiology and the environment can alter the delicate balance between a 'proportionate' host response to the oral microbial challenge, which is associated with clinical health, and a disproportionate host response, which alters the delicate equilibrium between the two and tips the balance toward tissue-destructive sequelae.

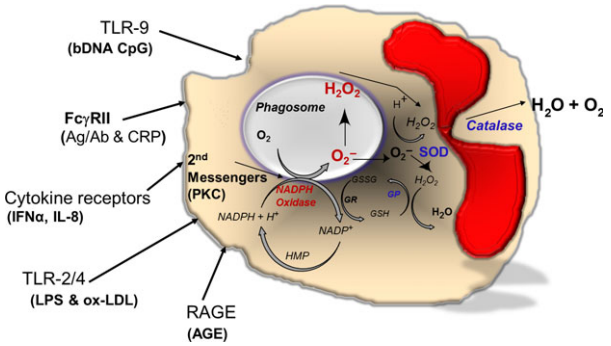

Fig. 3. Activation of the neutrophil nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) generates reactive oxygen species (ROS) via different cell-surface receptor–ligand interactions, followed by removal of ROS by intracellular antioxidant systems. Ab, antibody; Ag, antigen; AGE, advanced glycation end-product; bDNA, bacterial DNA; CpG, Cytosine phosphodiester Guanine; CRP, C-reactive protein; GP, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; HMP, hexose monophosphate; IFN-α, interferon alpha; IL-8, interleukin-8; LPS, lipopolysaccharide; ox-LDL, oxidized low-density lipoprotein; PKC, protein kinase C; RAGE, receptor for advanced glycation end-products; SOD, superoxide dismutase; TLR, toll-like receptor.

Barrier function of the oral epithelium and the role of dendritic cells

The oral gingival epithelium forms a barrier against invading microbes and protects the underlying tissues from infection. However, keratinocytes are not merely physical barrier cells as, upon stimulation with bacterial proteins or after viral infection, they contribute to the local host response by releasing proinflammatory cytokines, such as interleukin-1alpha, interleukin-1-beta, interleukin-6, interleukin-8 and tumor necrosis factor alpha, which act as proinflammatory signaling molecules. Gingival keratinocytes also produce defensins, which act on microbes to effect their killing. Defensins have multiple functions and facets to their behavior, activating immature dendritic cells as well as acting directly as powerful antimicrobial peptides. Their importance, regulation and activities are expertly reviewed by Dommisch & Jepsen in this volume of *Periodontology 2000* (12). Bacterial proteinases, such as gingipains from *P. gingivalis*, counteract these cellular reactions by destroying the epithelial barrier, thus increasing the potential for bacterial invasion as well as leading to the exudation of serum proteins. These interactions are discussed in detail by Groeger & Meyle (24). A broader perspective of oral keratinocytes, their stem cell precursors and advances in knowledge gained from studying the molecular signaling pathways activated and orchestrated by these traditionally 'barrier-type' cells is provided by Calenic and colleagues (6), offering valuable pointers toward future research in this field.

Dendritic cells, which form a family of antigen-presenting cells, are key regulators of immune tolerance and protection. They capture and process antigens and express the costimulatory molecules and cytokines required for antigen presentation to B- and T-lymphocytes. Dendritic cells also play an essential role in 'tolerizing' T-cells to self-antigens, thereby reducing the risk of autoimmune reactions. As such, dendritic

cells play a fundamental role in deciding whether to mount a vigorous immune response against pathogenic bacteria or to tolerate commensal microbes or, indeed, self-antigens (14). When dendritic-cell-mediated immune homeostasis is disrupted, dendritic cells can contribute to the pathogenesis of different destructive inflammatory conditions (5, 11).

Dendritic cells are commonly distinguished by their location in peripheral tissues, secondary lymphoid organs or the blood circulation. Tissue-resident dendritic cells, namely Langerhans cells or interstitial dendritic cells, have relatively long lifespans and play an active role in immune surveillance, promoting host tolerance or immunity. However, nearly 50% of the dendritic cells found in tissues are migratory subsets rather than resident cells. Circulating blood dendritic cells are distinguished from tissue dendritic cells in that they neither show dendrite formation nor express maturation features (80). Blood dendritic cells can be categorized into three general types–plasmacytoid dendritic cells and two types of conventional or myeloid dendritic cells– based upon function and phenotype.

Plasmacytoid dendritic cells are derived from lymphoid progenitors and resemble plasma cells; however, these cells share more commonalities with myeloid dendritic cells. Plasmacytoid dendritic cells strongly express toll-like receptors 7 and 9 and can produce high amounts of interferon alpha in response to C–phosphate–G bacterial DNA motifs (but not in response to bacterial lipopolysaccharide) (72). Therefore, plasmacytoid dendritic cells are thought to recognize predominantly viral antigens (10, 19). Myeloid dendritic cells, on the other hand, are highly phagocytic, antigen-processing cells that recognize both bacterial and viral antigens (50, 67). In this volume of *Periodontology 2000*, El-Awady and colleagues (14) provide a comprehensive overview of dendritic cells and their potential importance in periodontal homeostasis and periodontitis pathogenesis.

Natural killer cells

As discussed by Wilensky and colleagues in this volume of *Periodontology 2000* (75), natural killer cells are a distinct subgroup of cytotoxic lymphocytes that play a major role in the ability of the innate immune system to modulate immune responses (7, 18, 59). They have the ability to kill target cells, without prior sensitization, by direct contact and also indirectly by producing proinflammatory cytokines, especially interferon gamma. They have important functions, not only in the defence against pathogens, but also for the initiation of an adaptive immune response and in the regulation of autoimmune mechanisms. Natural killer cells are able to distinguish between self and foreign antigens and, in contrast to conventional T-cells, natural killer cells are reactive to the major histocompatibility complex class I-like molecule, CD1d (3). Surprisingly, there are limited data within the literature on the role of natural killer cells in the pathogenesis of periodontitis (75).

In animal model studies, mice lacking interferon gamma demonstrated decreased bone loss following infection of the oral cavity with *P. gingivalis*, suggesting that interferon gamma is a central mediator in this process. Local reconstitution of interferon gamma$^{-/-}$ mice, at the site of inflammation, with the interferon gamma gene increases the level of tumor necrosis factor alpha and decreases the level of interleukin-10 (39). Interferon gamma stimulates macrophages to produce proinflammatory cytokines, and plays an important role in the activation and growth enhancement of cytotoxic T-cells, natural killer cells (17) and in the control of immunoglobulin isotype switching (16, 65). In addition, it inhibits most of the activities induced by T-helper 2 cytokines, providing important pointers to the potential importance of natural killer cell activities in the pathogenesis of inflammatory periodontal diseases.

T- and B-cell subsets

The T-helper-cell population is characterized by different cell subsets, such as T-helper 1, T-helper 2 and T-helper 17 cells, regulatory T-cells and follicular helper cells. As reviewed by Gonzales (21), each of these subsets is characterized by specific functions and gene-expression patterns, and also by differing degrees of plasticity between the subsets.

The key cytokine involved in T-helper type 1 differentiation is interleukin-12 (1, 41). Binding of interleukin-12 to its receptor on $CD4^+$ cells triggers the activation of the janus kinase 2 and tyrosine kinase 2 signaling pathways, leading to the phosphorylation and activation of signal transducer and activator of transcription 4 (49). Phosphorylated signal transducer and activator of transcription 4 plays a critical role during T-helper 1 differentiation by promoting expression of the transcription factor, T-bet, and recent studies have defined unique roles for both signal transducer and activator of transcription 4 and T-bet in regulating expression of the interferon gamma gene within committed T-helper 1 cells (69). Additionally, interferon gamma enhances expression of both T-bet and

interleukin-12 receptor beta-2, reinforcing the interleukin-12-mediated T-helper 1 commitment (2, 54).

The primary signal that drives T-helper 2 differentiation from naive precursors is influenced by interleukin-4 (20, 54). T-helper 2 differentiation involves the integration of signals, both from the T-cell receptor and from interleukin-4 signaling via signal transducer and activator of transcription 6, which culminates in the induction of the GATA3 transcription factor (22, 23, 36, 38). GATA3 subsequently promotes transcription at the T-helper 2 cytokine locus containing the interleukin-4, interleukin-5 and interleukin-13 genes. This pathway also acts acutely to inhibit expression of the interleukin-12 receptor beta-2 subunit (55, 56). Consequently, induction of GATA3 serves to block T-helper 1 development whilst positively regulating the T-helper 2 commitment. T-helper 2 cells produce interleukin-4, interleukin-5, interleukin-9, interleukin-10 and interleukin-13, and provide optimal help for humoral immune responses, including IgE and IgG1 isotype switching, and mucosal immunity, through the production of mast cell and eosinophil growth and differentiation and by facilitating IgA synthesis (21).

The role of T-helper 17 in periodontal disease has been analyzed in recent studies and is discussed in this volume of *Periodontology 2000* by Zenobia & Hajishengallis (79). The presence of T-helper 17 cells in gingival and alveolar bone samples from healthy patients and patients with chronic periodontitis has been investigated by analysis of mRNA expression for interleukin-17, transforming growth factor-beta, interleukin-1beta, interleukin-6 and interleukin-23 in the gingiva, or interleukin-17 and RANKL in alveolar bone. The production of interleukin-17, tumor necrosis factor beta, interleukin-1beta, interleukin-6 and interleukin-23 proteins was investigated by immunohistochemistry, and the presence of T-helper 17 cells in the inflamed gingiva was confirmed by immunofluorescence confocal microscopy for CD4 and interleukin-17 co-localization. The results demonstrated elevated levels of interleukin-17, tumor necrosis factor beta, interleukin-1beta, interleukin-6 and interleukin-23 mRNA and protein in diseased tissues, as well as the presence of T-helper 17 cells in gingiva from patients with periodontitis.

MicroRNAs in the etiopathogenesis of periodontal disease

The complex interactions taking place during the host response to oral bacterial infections are further complicated by the detection and the role of microRNAs (small, noncoding RNA molecules that negatively regulate protein expression) in periodontal tissues (43). Aberrant expression of microRNAs triggers the dysregulation of multiple cellular processes involved in both innate and adaptive immune responses, leading either to ineffective countering of microbial challenges or to excessive catabolic responses. Both types of microRNA-induced dysregulation facilitate the development of chronic inflammatory diseases. The (microRNA/microRNA) -complex is loaded on the Argonaute 2 protein during assembly of the microRNA-induced silencing complex, which shuts down target mRNAs (26).

Currently only a few studies have investigated the role and importance of microRNAs in states of gingival health and disease, and no clear conclusion can be drawn. An important caveat for all available studies, to date, is that they assessed microRNA expression in whole gingival biopsy samples, which contain a mixture of cellular components, the proportions of which may vary significantly. This emerging field is comprehensively appraised in the review by Kebschull & Papapanou (43).

The chemokine stromal-cell derived factor-1 (also known as CXCL12), which is strongly up-regulated in human periodontitis, along with its receptor CXCR4 (35, 42), is a target of the periodontal health-associated miR-141. The reduced amount of miR-141 in the diseased state is inversely related to the increased expression of these proteins in disease (40).

Dendritic cell signaling bridges innate and adaptive immunity (for review, see (14) in this volume of *Periodontology 2000*). Dendritic cells detect pathogens and their components using their surface receptors, and produce cytokines that mediate the cellular response. In periodontal infections, dendritic cell signaling is considered a critical step in the regulation of immune responses. The pathways controlling dendritic cell functions are tightly regulated by microRNAs (64). Silencing of c-Fos expression in dendritic cells by miR-155 was shown to be critical for dendritic cell maturation and function, including their ability to trigger a cellular inflammatory response (13). miR-451, which was strongly over-expressed in inflamed gingival tissues, was shown to reduce cytokine secretion by infected dendritic cells using a negative-feedback loop (62).

Whilst research into microRNA signaling in periodontal diseases is in its infancy, emerging data support a role of microRNA signaling in the regulation of innate and adaptive immunity in periodontitis. The importance and role of these small molecules in the pathogenesis of periodontitis remains an interesting target of future research.

Periodontally driven systemic inflammation and pro-resolution pathways

The potential for components of the periodontal biofilm to impact upon the systemic circulation, specifically the cardiovascular system through interactions with vascular endothelium, and also upon diabetes control via hyperglycemia and dyslipidemia, are discussed by Hasturk and Kantarci in this volume of *Periodontology 2000* (34). The authors present two models for the development of systemic inflammation arising secondary to periodontitis. One model involves the dissemination of periodontal pathogens to distant sites, where they may trigger local inflammation. In the second, and most plausible, model [according to a recent European/American consensus workshop (70)], periodontal bacteremia triggers an acute-phase response by the liver, involving release of C-reactive protein and production of interleukin-6, and also activates peripheral blood leukocytes (neutrophils) to release oxygen radicals, thus creating a peripheral oxidative stress response. The subsequent activation of local cytokine networks by these processes creates a biologically plausible explanation for what is essentially 'metastatic inflammation' arising secondary to periodontal bacteraemia. This low-grade peripheral inflammation arising in periodontitis may contribute, in the longer term, to pancreatic beta-cell damage as well as to vascular endothelial damage.

The recent identification of small fatty acids released endogenously during an acute inflammatory response as a feedback mechanism to actively start resolving inflammation has led to a new field of discovery in host immunity. Such molecules, known as lipoxins, also have exogenous dietary-derived analogs called resolvins, obtained from omega-3 fatty acid metabolites docosahexaenoic acid and eicosapentaenoic acid, which can be metabolized in a staged process involving cyclooxygenase-2 that has been acetylated by aspirin or via microbial or mammalian cytochrome p450, to form the E-resolvins, E1 and E2, respectively. These mediators have similar functions in actively switching off the inflammatory response and, in doing so, resolving inflammation before it becomes chronic in nature and leads to scarring. Hasturk & Kantarci (34) describe these processes and also recent research demonstrating that resolving inflammation creates an environment in which human tissue regeneration, as opposed to repair, is facilitated, restoring normal tissue structure and function, at least in animal models. The future for exogenous application of such pro-resolving mediators appears to hold great promise, and indeed there may also be systemic health benefits of pro-resolving approaches for managing chronic inflammatory diseases.

Conclusions

Here we have presented a contemporary model of periodontitis pathogenesis based upon a circular relationship between the periodontal biofilm and the inflammatory immune response. Implicit in the model is that the transition from health to gingivitis, and ultimately to periodontitis, is associated with evolution of a health-promoting biofilm, to one of incipient dysbiosis and then to one of frank dysbiosis, and at the same time the host's inflammatory response transits from being proportionate and pro-resolving, to proportionate/nonresolving and then to disproportionate/nonresolving. Unlike the classical paradigm of a pathogenic microflora inducing inflammation, we now recognize that inflammation also contributes to the biofilm structure and function and there is a need for metagenomic studies to start defining what functional characteristics of the biofilm render it pathogenic as opposed to health promoting. At the same time, pathogenic roles for viruses are emerging, either as priming agents of host immune cells or as co-infectors alongside bacteria, conspiring together to deregulate host-defence systems. It is also becoming clear that the hosts' periodontal armamentarium against dysbiosis includes diverse cell types, epithelial cells, dendritic cells, natural killer cells, T- and B-lymphocytes and neutrophils, all of which carefully orchestrate an appropriate response to the biofilm and its components. There are likely to be a multitude of pathways to dysregulation of local host immunity within the periodontium that may arise when such a complex series of highly coordinated signaling events is necessary to maintain tissue homeostasis. The key features of immune disruption in periodontitis include excessive inflammation that fails to resolve and becomes chronic and self-destructive in nature, generating an environment that favors pathogenic bacteria. Future research needs to identify ways of restoring a balance between the inflammatory immune response and the biofilm, resolving the chronic inflammatory lesion and re-establishing a symbiotic relationship within the biofilm and between the biofilm and the host.

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