J Clin Periodontol 2011; 38 (Suppl. 11): 49–59 doi: 10.1111/j.1600-051X.2010.01678.x

Journal of Clinical Periodontology

How has neutrophil research improved our understanding of periodontal pathogenesis?

Gabriel Nussbaum[1] and Lior Shapira[2]

[1]Institute of Dental Sciences, Faculty of Dental Medicine, Hebrew University-Hadassah Medical Center, Jerusalem, Israel; [2]Department of Periodontology, Faculty of Dental Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Nussbaum G, Shapira L: How has neutrophil research improved our understanding of periodontal pathogenesis? J Clin Periodontol 2011; 38 (Suppl. 11): 49–59. doi: 10.1111/j.1600-051X.2010.01678.x.

Abstract

Background: Neutrophils are the predominant cells responsible for host defence against bacterial infection. Loss of neutrophil defence, due either to deficient number or function, strongly predisposes to bacterial infections such as periodontitis. Yet, the neutrophil oxidative and proteolytic arsenal has also been implicated in perpetrating periodontal tissue damage in periodontitis.

Aim: In this review, we focus on recent developments that shed light on these two aspects of neutrophil function in periodontitis.

Methods: Primary search: using PubMed search for ''neutophil'', ''periodontal'', and ''periodontitis''. Secondary search: using references from the articles found in the first stage.

Results: Early histological studies showed that infiltrating neutrophils form a wall of cells abutting the junctional epithelium in periodontal inflammatory lesions. The chronic standoff between these neutrophils and the bacterial community suggests that bacterial evasion of neutrophil clearance is a major characteristic of periodontitis. Indeed, not all functional neutrophil deficiencies increase the risk of periodontitis, an observation that points the way towards identification of particular anti-bacterial pathways essential for protection against periodontal pathogens. The net result in the majority of periodontitis patients who exhibit normal neutrophil number and function, is that neutrophils accumulate in the periodontal tissue where they are available to participate in tissue destruction. Diminished neutrophil clearance further contributes to the persistence of activated neutrophils in the periodontal tissue.

Conclusions: Data on the role of neutrophils in the pathogenesis of periodontitis are mixed. Neutrophils are a critical arm of the defence against periodontitis, but bacterial evasion of the neutrophil microbicidal machinery coupled with delayed neutrophil apoptosis may transform the neutrophil from defender to perpetrator. At this stage of knowledge, attempts to induce host modulation through neutrophil suppression or activation are premature.

Key words: inflammation; neutrophils; periodontitis; periodontal pathogenesis

Accepted for publication 7 November 2010

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest. This work was supported in part by a grant to G. N. from the Israel Science Foundation (Grant No. 1488/08). This supplement was supported by an unrestricted grant from Colgate.

Were a highly intelligent and discriminatory Martian to be charged with evaluating the role of neutrophils in periodontal pathogenesis using PubMed as a tool, she would be faced with three mountains of evidence. In the first, she would find a plethora of studies demonstrating that neutropenia predisposes to aggressive and chronic periodontitis. In the second heap would sit studies that demonstrate that individuals with periodontitis, although they may have normal neutrophil counts, display subtle abnormalities of neutrophil function, such as diminished chemotaxis, phagocytosis, and bactericidal activity. Stopping at this point, our Martian would report that normal numbers of properly functioning neutrophils are required for humans to protect themselves from periodontitis. The third pile of studies, however,

would muddle these clear waters. Here, she would discover that periodontally diseased gingiva are packed with neutrophils that form a wall abutting the pocket epithelium, and that these neutrophils migrate into the crevicular pocket where they encounter bacterial plaque. Not only do these neutrophils migrate efficiently but they also appear hyperactive, primed to release enhanced levels of bactericidal molecules such as oxygen radicals, inflammatory mediators such as cytokines, and matrix-degrading enzymes. These exact factors are considered responsible for the tissue destruction and osteoclast activation that are the hallmarks of periodontitis, leading authors of these studies to conclude that primed or hyperactive neutrophils are the perpetrators of periodontitis, rather than the protectors.

Aggravated, our Martian would most certainly abandon her mission.

This review addresses the *dual role of neutrophils* – as defenders and perpetrators – in periodontal pathogenesis. Like the Martian above, the authors of this review are frustrated by the often conflicting and contradictory conclusions that can be drawn from the myriad of studies of neutrophil function in periodontitis. Undoubtedly, differences in experimental methodology and disease definitions explain some of the inconsistent conclusions. Others stem from biological variation over time – as disease progresses a defender may transform into a perpetrator. Nevertheless, we wonder what definitive statements can be made about neutrophil function in the pathogenesis of periodontitis. Clinicians practicing evidence-based medicine and dentistry are accustomed to a hierarchical ranking of evidence in support of, or opposition to healthcare interventions. To borrow that image, we would like to classify the evidence supporting the roles of neutrophils in periodontal disease pathogenesis. To start, we will review neutrophil deficiency states because these ''experiments of nature'' provide the most conclusive evidence of the neutrophil's role in periodontitis. The observation that not all neutrophil disorders predispose to periodontitis, together with recent discoveries related to how periodontal pathogens evade neutrophil-mediated destruction, can help to shed light on the mechanism by which neutrophils contribute to disease pathogenesis.

A brief introduction to the Neutrophil

Neutrophils are highly specialized cells equipped with machinery for the destruction of microorganisms. Neutrophil responses to infection can be divided to ''3 R's'' – Recruitment, Response, and Resolution. Acquired or genetic defects in one or more of these mechanisms may impair the protective capacity of the neutrophil.

Recruitment

Neutrophils circulating in the blood stream are able to adhere to endothelial cells, a process that involved a highly regulated interplay between adhesion molecules and their ligands. This *adhesion* process precedes the transition of the neutrophils to the affected tissues. Neutrophils are attracted to the site of microbial infection in response to specific chemoattractant molecules derived from the microbe or from the responding host, a process known as *chemotaxis*.

Response

Phagocytosis and intracellular killing

The neutrophil recognizes host-derived molecules that are bound to the bacterial surface (opsonins – IgG, C3b) and engulfs microorganisms via invagination of the plasma membrane, which encloses the microbe in a phagosome. Phagosomes undergo fusion with other organelles to form phagolysosomes, which are specialized structures for killing microbes through acidification of the contents and release of anti-bacterial molecules from neutrophil granules. To kill microbes, neutrophils can use both oxygen-dependent and oxygen-independent means. Oxygen-dependent killing depends on generation of reactive oxygen intermediates (ROIs,) through the action of the NADPH oxidase system. Oxygen-independent killing relies upon the armamentarium of microbicidal molecules contained in the three sets of neutrophil cytoplasmic granules: primary (azurophilic granules), secondary (specific), and gelatinase-containing. One, or a combination of means – toxic oxygen and nitrogen radicals, cationic peptides that disrupt the microbial membrane, and enzymes that dissolve the invading pathogen – lead to microbial demise.

Extracellular killing

The same set of tools available to kill microbes intracellularly can be released by neutrophils to the extracellular space in an effort to kill microbes. These potent oxidative and enzymatic molecules can cause considerable collateral damage to the surrounding connective tissue. Recently, neutrophils were shown to entrap microbes in the extracellular space through release of a web of fibres containing DNA, histones, and granule-derived bactericidal molecules. These neutrophil extracellular traps (NETs) may serve to limit secondary damage to surrounding tissue by preventing random diffusion of granule contents (Brinkmann et al. 2004). Vitkov et al. (2009) showed that NETs are abundant on the gingival surface and in the gingival crevicular exudates in patients with chronic periodontitis.

Resolution

Successful resolution of inflammation is a prerequisite for restoration of healthy tissue. Because acute inflammation is characterized by an abundance of infiltrating neutrophils, their elimination, and the restoration of normal numbers of tissue leucocytes, is a critical step in the progression towards tissue repair. Therefore, defective clearance of *normal* neutrophils from the periodontium would theoretically predispose to non-healing chronic inflammation, such as that found in periodontitis.

Neutrophils and the periodontal inflammatory lesion

The periodontal lesion is characterized by large proportions of inflammatory cells and vascular structures. Neutrophils predominate in the early periodontal lesions that characterize gingivitis; however, the relative proportion of neutrophils in the inflammatory infiltrate decreases during the transition to periodontitis (Kinane et al. 2008), in which plasma cells and lymphocytes are dominant. It was reported that plasma cells occupied 31% of the periodontititis lesion volume, while the proportion of lymphocytes varied between 5% and 10%. Macrophages and neutrophils were found in densities of 1–2% and fibroblasts in 5% (Berglundh & Donati 2005). Neutrophils may be especially important during the transition from gingivitis to periodontitis. Furthermore, they may play a key role in the pathogenesis of periodontitis in the gingival crevice and in the epithelium, where

they are the dominant inflammatory cell. The early, non-specific neutrophil response to dental plaque organisms (both pathogenic and non-pathogenic) allows pathogenic bacteria, such as *Porphyromonas gingivalis*, to proliferate and invade, setting the stage for the transition from gingivitis to periodontitis.

Neutrophil disorders predispose to periodontitis

Neutrophils are the most important phagocytic cells, protecting the host against bacterial infection and invasion. Disorders that affect neutrophil number or function strongly predispose individuals to infection. Neutrophil disorders can be divided to those that affect neutrophil number, neutrophil chemotaxis, and various physiological functions of the neutrophil once it has migrated to the site of inflammation. The study of naturally occurring neutrophil disorders can relate biochemical pathways to the resulting clinical manifestations of disease. Such functional disorders of neutrophils predispose to recurrent cutaneous, respiratory, and periodontal infections. The fact that quantitative or qualitative defects in neutrophil function may lead to severe periodontal breakdown, supports the central role of these cells in protecting the periodontium in health, when a constant bacterial burden is present. Identification of these biochemical and molecular defects that lead to neutrophil dysfunctions and their physiological consequences has improved our understanding of how the activated neutrophil is attracted and adheres to inflammatory sites, and how it produces toxic products that destroy bacteria.

The association of severe periodontitis with a decrease in the number of circulating neutrophils is well documented in the literature. Situations such as neutropenia, agranulocytosis (Hou & Tsai 1988, Lamster et al. 1987, Saglam et al. 1995, Zubery et al. 1991, Watanabe 1990), cyclic neutropenia (Rylander & Ericsson 1981, Scully et al. 1982, Prichard et al. 1984, Baer & Iacono 1994, Pernu et al. 1996, da Fonseca & Fontes 2000), chronic benign neutropenia (Reichart & Dornow 1978), chronic idiopathic neutropenia (Baehni et al. 1983, Kamma et al. 1998), and familial benign chronic neutropenia (Deasy et al. 1980, Stabholz et al. 1990, Kirstila et al. 1993, Porter et al. 1994), are all associated with periodontitis. In neutropenic patients, the number of neutrophils sometimes can be restored with external administration of haematopoietic colony-stimulating factors, such as granulocyte colony-stimulating factor. In these patients, the success in elevating the number of circulating neutrophils is usually correlated with improved antibacterial responses in the periodontium, and improved clinical outcomes (Weston et al. 1991, Briars et al. 1996, Hasturk et al. 1998, Ozer et al. 2000). These interventional studies support the central role of the neutrophil in protecting the host from periodontal destruction (Holmstrup & Glick 2002).

In animals, experimental neutrophil depletion supports the protective role of neutrophils in periodontitis. Long-term methotrexate-induced neutropenia (7–9 weeks) significantly increased alveolar bone resorption in ligature-induced periodontal lesions in rats (Yoshinari et al. 1994). However, methotrexate is a general inhibitor of folate metabolism and will inhibit all rapidly dividing cells. Increased periodontal lesion size may be due to other cell populations, or other effects such as an anti-cytokine effect (Verzeletti et al. 2007). Indeed, at shorter time periods (4 weeks), treatment with methotrexate did not affect ligature-induced periodontitis possibly because the protective effect of reducing inflammatory cytokines offset the detrimental effect of neutrophil depletion (Verzeletti et al. 2007). A more specific strategy to deplete neutrophils experimentally would be to administer anti-Gr1 monoclonal antibodies (clone RB6, Stephens-Romero et al. 2005); however, this approach has two limitations: depletion is transient, requiring daily treatment, and Gr1 is expressed by many non-granulocytic cell types at different stages of differentiation such as monocytes and fibroblasts (Daley et al. 2008).

Individuals with normal neutrophil counts, but who manifest functional neutrophil impairments, are also predisposed to periodontitis. One of the most striking examples is the leucocyte adhesion deficiency syndrome (LAD) (Meyle 1994). During the recruitment process, circulating neutrophils are locally captured and begin to roll along the vessel wall in the infected area, using the selectin family of adhesion molecules and their respective ligands. Signals from the affected vessel wall induce adhesion of the neutrophil to the vessel wall. The adhesion process is dependent on integrins, transmembrane receptors specialized for anchoring cells to the surrounding matrix or to other cells. The neutrophils spread and begin to crawl along the vessel wall until they find their optimal site for extravasation into tissue. Disorders of the recruitment cascade of white blood cells are collectively termed LAD. Clinically, there are irregularities of adherence-dependent leucocyte function, and affected patients have recurrent bacterial infections.

Three different LAD variants (LAD I–III) have been described (Nussbaum et al. 2010). In LAD-I, mutations in the $\beta 2$ integrin gene (CD18/CD11) result in a profound defect in leucocyte adhesion giving rise to serious infections in early life and high infant lethality. In LAD-II, a defective sugar transporter leads to disturbed glycosylation of selectin ligands that primarily affects leucocyte capture and rolling. Infections in LAD-II are often milder and patients usually survive into adulthood. LAD-III resembles the clinical presentation of LAD-I and was initially described as a variant of LAD-I, but the precise molecular basis of LAD-III has recently been identified. Molecular characterization of leucocytes isolated from LAD-III patients revealed that $\beta 1$, $\beta 2$, and $\beta 3$ integrins are normally expressed, but they are inactive. The first indication for the integrin-binding protein Kindlin-3 as the responsible gene mutated in LAD-III came from studies in Kindlin-3 knockout mice. Recently, mutations in the human Kindlin-3 gene have been identified in LAD III patients (Moser et al. 2009, Svensson et al. 2009).

Most of the data available for periodontitis and LAD are from LAD-I patients. Characteristic features of LAD patients include rapidly progressive attachment and bone loss in the primary and permanent dentitions, leading to premature tooth loss (Page et al. 1987, Waldrop et al. 1987, Cox & Weathers 2008, Dababneh et al. 2008). The LAD syndrome emphasizes the importance of normal neutrophil recruitment in order to establish competent host defence in the periodontium.

Another early onset inherited form of periodontitis is found in the Papillon–Lefevre syndrome. The genetic defect of this syndrome was mapped to the gene for cathepsin C (Hart et al. 1999, Toomes et al. 1999), a lysosomal protease of neutrophils, which is an important activator of neutrophil-derived

serine proteases. Surprisingly, these individuals are particularly prone to periodontitis, and do not appear to suffer from an increased incidence of other infectious diseases. This observation suggests that neutrophil-derived cathepsin C is an essential element in the pathway of normal physiological defence in periodontitis. Although mice deficient in cathepsin C have defects in serine protease activation in multiple cellular compartments, the role of cathepsin C for human serine protease activation is largely undefined. Neutrophils from patients with Papillon–Lefevre syndrome have no defect in their ability to kill *Staphylococcus aureus* and *Escherichia coli*, indicating that serine proteases are not the primary tool used by neutrophils to clear common bacterial pathogens (Pham et al. 2004). However, it is possible that serine-protease-dependent mechanisms are essential for neutrophils to kill some periodontal pathogens such as *Actinobacillus actinomycetemcomitans* (de Haar et al. 2006), and cathepsin C appears to be worthy of future study.

Patients with Kostmann syndrome have an inherited disorder of impaired bone marrow myelopoiesis and a severe congenital neutropenia (Kostmann 1956). Clinically, patients with Kostmann syndrome experience recurrent infections, and all types of severe periodontitis. On the molecular level, the syndrome was found to be associated with deficiencies in neutrophil antimicrobial peptides LL-37 and α-defensins (Putsep et al. 2002). As expected, Kostmann patients show normal levels of LL-37 after a bone marrow transplant, and no further periodontal destruction (Putsep et al. 2002). In contrast, treatment of patients with Kostmann syndrome with recombinant granulocyte-colony stimulating factor restored the levels of circulating and bone marrow neutrophils, but these patients still experienced recurring periodontal infections due to insufficient levels of LL-37 (Carlsson et al. 2006). The above data emphasize the importance of neutrophil-derived cationic peptides in the protection of the host against periodontal breakdown.

In most experimental animal studies, defective neutrophil recruitment or function increases host susceptibility to experimentally induced alveolar bone loss. A recent report of experimentally induced alveolar bone loss in IL-17 receptor-deficient mice highlights the critical role of neutrophils as protectors of the periodontium. IL-17 is an inflammatory cytokine important for the host response to infection, and the downstream genes most highly regulated by IL-17 receptor signalling include granulocyte colony-stimulating factor and the chemokines that recruit neutrophils: KC, CXCL1, CXCL5, and CXCL2 (Yu & Gaffen 2008). This positions IL-17 as a critical factor for neutrophil homeostasis and recruitment. However, because IL-17 is also a proinflammatory cytokine associated with tissue damage (Gaffen et al. 2006), the effect of deficient IL-17 signalling in periodontitis could theoretically have gone either way. IL-17 receptor-deficient mice develop significantly more alveolar bone loss compared with controls, and demonstrate significantly reduced neutrophil recruitment to the periodontal tissue (Yu et al. 2007). Thus, the potential benefit of deficient proinflammatory cytokine signalling is more than offset by the lack of efficient neutrophil recruitment.

Perhaps the most prominent example of a neutrophil defect increasing experimental periodontitis in animals is the defect in lysosomal-associated membrane protein-2 (LAMP-2). Phagosomal maturation and formation of the phagolysosome are impaired in neutrophils of LAMP-2-deficient mice (Saftig et al. 2008). Strikingly, these mice develop spontaneous periodontal bone destruction early in life despite the infiltration of high numbers of neutrophils to the periodontium (Beertsen et al. 2008). Importantly, the neutrophil oxidative burst is normal in LAMP-2-deficient mice, suggesting that the inability to clear bacteria through non-oxidative mechanisms is the primary factor predisposing to experimental periodontitis in these animals (Beertsen et al. 2008). The accumulation of abundant, but ineffective neutrophils in the periodontium of LAMP-2-deficient mice resembles the familiar picture of human periodontitis where a wall of neutrophils accumulates adjacent to the junctional epithelium. Spontaneous periodontal destruction in LAMP-2-deficient mice suggests that when neutrophils are present and unable to kill bacteria, their weaponry (such as ROI generation) may mistakenly wreak havoc on host tissue. Future human studies may correlate deficiencies in neutrophil phagosome maturation with human periodontitis.

In summary, genetic defects in neutrophil number or function underscore the major defensive role of neutrophils in periodontitis. However, because the vast majority of periodontitis occurs in otherwise healthy individuals without any known neutrophil dysfunction, can these rare cases instruct us where to look for the role of neutrophils in chronic periodontitis?

Neutrophil disorders in periodontitis pathogenesis in otherwise healthy subjects.

As described above, there is no doubt that low numbers of neutrophils or severely impaired neutrophil function have a strong effect on the ability of the host to cope with infections, including periodontal infection. However, the role of intrinsic or acquired neutrophil defects in the periodontal environment of otherwise healthy subjects is much less straightforward. Most of the data on neutrophil disorders in otherwise healthy subjects were accumulated on young patients with early onset forms of periodontitis, i.e. aggressive periodontits. Early studies demonstrated that peripheral neutrophils of patients with aggressive forms of periodontitis exhibited reduced chemotaxis in response to chemotactic signals in vitro and in vivo (recently reviewed in (Ryder 2010). This phenomenon was described as a neutrophil disorder, and was suggested to be part of the pathogenesis. In addition, some early studies also showed impaired phagocytosis and neutrophil killing in aggressive periodontitis patients. However, other studies carried out on different populations contradict these findings, showing normal neutrophil chemotaxix in aggressive periodontitis patients (Takahashi et al. 2001), or no correlation between the described impairment in neutrophil function and disease severity (Kinane et al. 1989a, b). Furthermore, some studies even found elevated neutrophil chemotactic responses in aggressive periodontitis patients (Ryder 2010). Contradictory results were also described regarding the nature of the impairments in neutrophil functions. Some studies found that periodontal treatment was not able to reverse the observed defects, suggesting that they are intrinsic (Meng et al. 2007), while other studies show reversal by treatment (Ryder 2010), suggesting that the observed neutrophil behaviour was due to exposure to inflammatory mediators or bacterial products. The majority of these studies analysed the response of periph-

eral neutrophils, which may be different from neutrophils of the periodontal pocket. Peripheral neutrophils may be influenced by transient systemic illnesses such as viral infections unrelated to the periodontal status of the patient. Another possibility is that due to genetic variations in individuals and populations, effective neutrophil responses to particular periodontal pathogens may be impaired in specific individuals. This may be considered an ''intrinsic defect'' of the host on one hand, but on the other hand it may be an ''evasion mechanism'' of the pathogen. Early studies demonstrated reduced phagocytosis of some strains of *P. gingivalis* and *A. actinomycetemcomitans* by neutrophils from subsets of aggressive periodontitis patients (Eick et al. 2000). In support of the ''evasion model'', incubation of neutrophils with *A. actinomycetemcomitans* decreased the expression of the chemotactic receptor fMLP-R (Ashkenazi et al. 1992). Molecular studies have shown that some aggressive periodontitis patients have a specific polymorphism in the Fc γ receptor, an important neutrophil receptor for phagocytosis of opsonized bacteria [reviewed in (Stabholz et al. 2010)]. This polymorphism can be an ''Achilles heel'' of the neutrophil – neutrophils bearing this polymorphism are less efficient in the killing of specific pathogens, and specific bacteria may capitalize on this weakness to evade clearance. On the other hand, genetic hyperreactivity of neutrophils was also suggested to contribute to disease pathogenesis. Nicu et al. (2007) showed that neutrophils of periodontitis patients that are homozygous for the131H variant of the Fc γ-receptor RIIa, have more severe bone loss than those with the H/R or R/R genotype. Furtheremore, in vitro functional studies indicate a hyperreactivity of the H/H-neutrophil in response to *A. actinomycetemcomitancs*, which may be one of several pathways leading to more severe periodontal breakdown. Nibali et al. (2010) could not demonstrate statistical differences in the same genetic polymorphism between aggressive periodontitis and healthy subjects, although they demonstrated ''hyperactivity'' of neutrophils of aggressive patients compared with controls. A further possibility is that peripheral neturophil hyperactivity results from the inflammatory state of the patients, rather than causing it.

In conclusion, the evidence that specific variants in neutrophil function contribute to periodontitis (especially aggressive periodontitis) in otherwise healthy individuals is low. Early studies had demonstrated differences in chemotaxis and ROI production between periodontitis patients and controls, and considered them as ''neutophil defects''. However, today these results are interpetated as ''neutrophil hyperresponsiveness''. The hyperresponsiveness is probably due to circulating factors (Dias et al. 2011), which may be due to individual differences or environmental effects. It is possible that in some cases genetic polymorphisms related to neutrophil function may help subsets of periodontal pathogens to evade the neutrophil response or may lead to neutrophil hyperactivity; however, this most likely only contributes to disease pathogenesis in a minority of aggressive or chronic periodontitis cases (Nicu et al. 2007).

Not all neutrophil defects predispose to periodontitis.

Given the fact that many periodontal pathogens are facultative or obligate anaerobes, one may imagine that the neutrophil oxidative burst would be a highly efficient way to clear infection. However, clinically, chronic granulomatous disease (CGD) patients who suffer from defective neutrophil oxygen-dependent bactericidal activity, are not more susceptible to periodontitis. In patients with CGD, mutations in the phagocyte oxidase (phox) system render the neutrophils unable to generate an oxidative burst to kill microbes through oxidative damage (Giannopoulou et al. 2008, Holland 2010). Phagocyte oxidase is an electron transporter that accepts electrons from NADPH inside the cell and transports them across the membrane to attach them to oxygen. This reaction leads to the generation of superoxide and downstream ROIs. The neutrophils of CGD patients are therefore unable to kill catalase-positive organisms due to the lack of production of reactive oxygen species that normally eliminate these microbes. Two studies found that CGD patients have more severe gingivitis, but do not demonstrate more attachment loss, compared with normal individuals (Winkelstein et al. 2000, Martire et al. 2008). A study of 368 patients with CGD revealed that only nine were diagnosed with gingivitis and/or mild periodontitis (Winkelstein et al. 2000). Furthermore, no cases of periodontitis were described for 221 Japanese patients with CGD (Hasui 1999). Equally, no periodontitis cases were found over a 25-year observation period in the dental records from a series of paediatric patients with CGD (Soler-Palacin et al. 2007).

Air-sensitive bacteria, to be pathogens, require protection from oxygen. The apparently normal or even decreased rate of periodontitis in patients with CGD, suggest that certain pathogens have the ability to utilize the ROIs as a virulence factor. For example, *P. gingivalis*, a major periodontal pathogen, possesses several mechanisms to resist oxidative killing such as superoxide dismutase (Nakayama 1994) and the non-haeme iron protein rubrerythrin (Sztukowska et al. 2002). Using neutrophils from NADPH oxidase-null mice, Mydel et al. (2006). demonstrated that oxidative killing is not an important means of *P. gingivalis* clearance because mutant and wild-type neutrophils eliminated *P. gingivalis* similarly. Moreover, they found that oxidase-null mice were significantly more resistant to infection with *P. gingivalis* in subcutaneous chambers, supporting the concept of the proverbial ''double-edged sword'' – the oxidative burst, devoid of its protective role in bacterial clearance, may damage the tissue and pathogenic bacteria with the appropriate evading mechanism can grow and induces periodontal pathology (Mydel et al. 2006). Of note, the ''pathogenic'' neutrophil response, in this setting, is composed of normal neutrophils responding in a presumably appropriate manner to infection.

In conclusion, although most studies have shown that patients with neutropenias and overt neutrophil functional defects suffer from advanced periodontitis, patients with CGD are a stark exception to the rule. They may suffer from more gingivitis, but they do not seem to be predisposed to periodontitis (and in fact, they may be less susceptible). Knockout mice that mimic the CGD defect support this observation. We conclude that reduced neutrophil recognition or response will predispose to periodontitis, but defects in particular bactericidal mechanisms that are not required to clear periodontal pathogens may weaken the collateral damage of the neutrophil response, and paradoxically protect the periodontium. As outlined below, periodontal pathogens have evolved mechanisms allowing them to

escape from neturophil-mediated clearance. Thus, normal neutrophils unable to clear bacteria can accumulate in the periodontal tissue where they may perpetrate tissue damage.

Bacterial evasion of killing by neutrophils

If periodontitis occurs in the setting of normal neutrophil number and function, the lack of bacterial clearance may be attributable to bacterial mechanisms that allow for persistence in the face of active host responses. We will now review several lines of evidence supporting this hypothesis.

The primary aetiologic factor of periodontitis is polymicrobial infection, and it is highly conceivable that some known and further-to unknown periodontal pathogens are involved. Some periodontal pathogens have developed mechanisms to modulate the host innate response and interfere with neutrophil function. For example, it was shown that *A. actinomycetemcomitans* are able to kill neutrophils by secretion of a potent toxin termed leukotoxin (Korostoff et al. 1998, Johansson et al. 2000). In addition, it was recently shown that the majority of phagocytized *A. actinomycetemcomitans* remained viable after exposure to neutrophils, and uptake of antibody-opsonized bacteria resulted in the rapid cell death, regardless of strain-specific serotype or leukotoxin production (Permpanich et al. 2006). These results suggest that *A. actinomycetemcomitans* possess more than one mechanism to evade neutrophil killing. Secreted leukotoxin has the additional effect of protecting other periodontal pathogens by killing neutrophils.

Anti-microbial proteins and peptides are critical components of the non-oxidative bactericidal machinery of neutrophils. Neutrophils are an important source of the antimicrobial peptides, such as defensins, bacterial/permeability-increasing protein-like proteins, LL-37, lactoferrin, cationic proteins, and others, and these molecules are expressed early in the response to bacterial stimuli (Kinane et al. 2007). The biology of the antimicrobial proteins was extensively studied and reviewed (Bals 2000, Boman 2003, Ganz 2003, Dale & Fredericks 2005, Kinane et al. 2007); these molecules damage the bacterial cell membrane by permeabilization or rupture. The cathelicidin LL-37 was found to be expressed in human tongue, buccal mucosa (Frohm Nilsson et al. 1999), and saliva (Murakami et al. 2002), and neutrophils are the most likely source of production (Dale & Fredericks 2005). In addition, the neutrophil α-defensins, HNP-1–3, which are active against some periodontal pathogens, were detected in the junctional epithelium and the gingival crevicular fluid (McKay et al. 1999, Dale et al. 2001). However, *P. gingivalis* is resistant to killing by the content of neutrophilic granules, and *P. gingivalis* culture supernatants inactivated cathepsin G, elastase, bacterial-permeability increasing factor, and defensins (Odell & Wu 1992). Furthermore, an 11 kDa fragment of cathelicidin was found in the GCF, suggesting the ability of protease-positive bacteria (*P. gingivalis*, *Tannerella. forsythia*, and *Treponema denticola* and most likely many others) to degrade LL-37, thereby evading killing and protecting other bacteria. The resistance to neutrophil non-oxidative killing mechanisms may therefore be an important virulence factor for protease-positive bacteria.

Toll-like receptors (TLRs) are a group of pattern-recognition receptors that govern responses to a vast range of bacterial, viral, fungal, and protozoan molecules (Akira & Takeda 2004). Individual TLR proteins selectively control the response to specific microbial structures; for example, TLR4 is required to respond to enterobacterial lipopolysaccharide (LPS), TLR2 for the response to bacterial lipopeptides, and TLR9 for the response to hypomethylated CpG oligonucleotides (Hemmi et al. 2000, Takeda et al. 2003). TLRs are expressed on a diverse range of immune and non-immune cell types and receptor expression can vary in response to signals from the environment and differentiation status of the cell (Gribar et al. 2008). TLR expression is increased in inflamed periodontal tissue and TLR signalling has been shown experimentally to play a major role in the induction of the inflammatory destruction of periodontal tissues (Burns et al. 2006). Neutrophils also express TLRs and respond to TLR ligands expressed by periodontal pathogens (Hayashi et al. 2003); however, chronically persistent pathogens have learned to subvert TLR-mediated clearance. *P. gingivalis*, for example, is rapidly cleared following subcutaneous infection in TLR2-deficient mice as compared with their wild-type counterparts (Burns et al. 2006, Burns et al. 2010). Neutrophil phagocytosis is down-regulated in the presence of an inflammatory TLR2-driven response, enabling bacteria to benefit from the increased nutrient supply afforded by inflammation, while escaping clearance (Burns et al. 2006, Burns et al. 2010). Neutrophil CD14/TLR2 activation by *P. gingivalis* fimbriae has also been shown to induce CD11b-CD18 inside–outside signalling through activation of PI3K, which may attenuate the inflammatory response (Harokopakis et al. 2006). In addition to inducing a conformational change leading to active CD11b-CD18, *P. gingivalis* fimbria are also recognized by the activated CD11b-CD18 complex, resulting in down-regulation of the active form of IL-12. Because IL-12 is a critical mediator of intracellular killing, this recognition pathway may lead to intracellular persistence (Hajishengallis et al. 2005, Hajishengallis & Harokopakis 2007). Similar to the case of *A. actinomycetemcomitans*, the inhibition of IL-12 activity could also benefit other pathogenic bacteria residing in the subgingival biofilm.

The persistent neutrophil as perpetrator of tissue damage

Once at an infected site, appropriate neutrophil decision making can be summed up in the following manner: search out and destroy microbes, call reinforcements when needed, and commit suicide once the situation is under control. In the periodontal sulcus, where bacteria are always present, there is a chronic activation of neutrophils by the bacteria and their products. The longer they stay alive, the more chance for tissue damage. There is no need to hypothesize that tissue damage in the periodotium is due to a ''hyperactivated'' state of the neutrophil (Kantarci et al. 2003) – even normal neutrophils that persist in the tissue are sufficient to induce damage because neutrophils that sense infection but fail to encounter a bacterium within a short period, will fire off their arsenal into the extracellular space (Nathan 2006). Persistent neutrophils in established gingivitis may cause enough damage to create conditions that allow pathogenic bacteria, such as *P. gingivalis*, to grow and invade, driving the transition from gingivitis to periodontitis. It was shown that *P. gingivalis* has the potential to prevent neutrophil

migration through the gingival epithelium (Madianos et al. 1997), thereby the activated neutrophils may accumulate in the connective tissue compartment of the periodontium and induce tissue damage. There is also evidence that circulating neutrophils of periodontitis patients are already in a ''primed'' or ''hyperresponsive'' state (Wright et al. 2008, Dias et al. 2011) in response to bacterial stimuli, and so their potential to cause tissue damage by secretion of ROI is enhanced. Suicide of activated neutrophils is therefore a crucial step in the resolution of inflammation and the prevention of further damage caused by necrotic cell lysis and the release of cytotoxic granule proteins (Kobayashi et al. 2002, Kobayashi et al. 2003, Theilgaard-Monch et al. 2004).

Suicide by apoptosis has an enormous benefit over necrotic death. Apoptotic cells down-regulate inflammation by sending signals to the macrophages that engulf them, to secrete anti-inflammatory cytokines and regulatory molecules (Fadok et al. 1998, Huynh et al. 2002). In contrast, necrotic neutrophils promote inflammation by acting as danger signals that activate macrophages through pattern recognition receptors (Bianchi 2007). Macrophages that engorge themselves on apoptotic neutrophils contain and dispose of the potent unexploded granule contents, a further advantage of apoptotic neutrophil death (Nathan 2006). Thus, diminished apoptosis would be expected to have two effects: first, it would provide a pool of neutrophils available to release their destructive contents upon stimulation. Second, diminished apoptosis would also reduce the collection of regulatory macrophages that control inflammation. The signals that control neutrophil fate are inflammatory signals: TLR activation, for the most part, has been shown in multiple studies to delay apoptosis (Parker et al. 2005). Specifically, *P. gingivalis* LPS has been shown to delay neutrophil apoptosis (Preshaw et al. 1999, Murray & Wilton 2003) possibly through cytokine-induced increases in anti-apoptotic proteins such as Bcl-x(L) or reduction pro-apoptotic proteins such as Bax (Ocana et al. 2008). Furthermore, *P. gingivalis* proteolytic enzymes hamper the recognition of apoptotic neutrophils by macrophages (Guzik & Potempa 2008). Therefore, periodontal pathogens themselves may ensure the presence of high numbers of viable neutrophils and reduced numbers of anti-inflammatory macrophages, setting the stage for neutrophil-mediated tissue damage.

Although persistence of neutrophils in the tissue may be a contributing factor for periodontitis, the level of destruction may be dependent on the response of the persistent neutrophil. Only 10–15% of the population is prone to develop severe periodontitis, and this may be due to genetic variations between individuals, some of which may be related to neutrophil functions, as described previously.

Neutrophils and bone resorption

In periodontitis, cytokines and growth factors produced by cells in the inflamed periodontal tissue can influence osteoclast differentiation and function, providing a link between inflammation and the process of bone destruction. These factors may elicit their effects directly, by acting on the osteoclast precursor or mature cell, or indirectly, via another cell type, to modulate receptor activator of NF-κB (RANK) ligand (RANKL) and osteoprotegerin (OPG) expression (Walsh et al. 2005). The imbalance in bone remodelling that favours resorption is caused by various cytokines in the inflammatory tissue, such as RANKL, tumour necrosis factor-α (TNF-α), interleukin (IL)-1, and prostaglandin E_2 (PGE_2), IL-11 and IL-17. Conversely, anti-resorptive factors, such as transforming growth factor-β, IL-12, and IL-18 may lead to inhibition of osteoclast differentiation and function (Walsh et al. 2005). Although neutrophils may secrete much lower amounts of cytokines on a per cell basis as compared with macrophages, the sheer number of neutrophils in the periodontium during the transition state between established gingivitis and periodontitis may make up for this difference (Nathan 2006). Furthermore, neutrophils may be an important source of osteoclastogenic cytokines such as IL-17, which is produced exclusively by activated Th17 cells and neutrophils, and stimulates osteoclastic bone resorption via osteoblasts by inducing the expression of RANKL (Kitami et al. 2010). In addition, neutrophils were found to be a major source for prostaglandins, potent mediators of bone resorption (Pouliot et al. 2000). Microarray analysis of circulating and wound neutrophils found that arrival to the site of inflammation initiates the secretion of inflammatory chemokines, matrix metalloproteinases (MMPs), and cytokines, including up-regulation of many signalling molecules that are known to be involved in bone resorption, such as IL-1β and TNF-α (Theilgaard-Monch et al. 2004). Therefore, persistent neutrophils provide an additional pool of osteoclastogenic factors and tissue-destructive mediators associated with periodontitis.

Neutrophils may have a more direct role in osteoclastogenesis than thought previously. Recently, it was shown that activation of neutrophil TLR4 by LPS up-regulated the expression of membrane RANKL on the surface of human and mouse neutrophils (Chakravarti et al. 2009). Human monocyte-derived osteoclasts, co-cultured with activated neutrophils, were able to stimulate bone resorption in vitro. In addition, the transfection of neutrophil-like cells with RANKL antisense RNA reduced osteoclastogenesis, and OPG, the RANKL decoy receptor, suppressed osteoclast activation by neutrophils from these human and animal sources (Chakravarti et al. 2009). This recent study suggests a direct role for neutrophils in the process of bone resorption, an observation that may have special relevance for peri-implantitis, where the inflammatory infiltrate and bone are in direct contact (Heitz-Mayfield & Lang 2010).

Neutrophils and tissue regeneration

Because of the constant irritation of the periodontal tissues, these tissues are always active and there is a balance between destruction and repair. Regeneration of the periodontal tissues is part of this balance, and also an important part of the desired outcome of periodontal therapy. Neutrophils were once ignored as cells unimportant in the process of tissue regeneration, but there is now an increasing body of evidence that neutrophils can affect tissue regeneration by the release of neutrophil-derived factors that promote angiogenesis. Neutrophils produce angiogenic factors such as vascular endothelial growth factor (VEGF) (Mueller et al. 2000), and depletion of neutrophils from mice (using anti-GR-1 antibodies) hampers the normal angiogenenic process of the endometrium (Heryanto et al. 2004). Neutrophils are a known source of

MMPs and MMP-activating enzymes, considered part of the destructive arsenal. However, neutrophil MMP-9 was found to induce angiogenesis via an FGF2/FGFR2 pathway (Ardi et al. 2009), and it was also demonstrated that neutrophils from MMP-9-deficient mice could not induce tissue regeneration in an ischaemia model (Heissig et al. 2010). Ultimately, inflammation and tissue repair are interdependent processes and molecular signals promiscuously signal in both systems. Thus, neutrophil degranulation may be a critical step in jump-starting angiogenesis and tissue repair through the secretion of factors such as VEGF, and enzymes that release angiogenic factors concealed within the web of extracellular matrix components. Future research is required in order to define the neutrophil pathways involved in promoting repair *versus* inducing tissue damage during periodontal tissue regeneration.

Possible implications for therapy

It is tempting to speculate about the benefit of suppressing neutrophil recruitment or activation in patients with periodontitis. Any attempt at host modulation through neutrophil suppression must take into consideration the most definitive evidence that supports neutrophils as essential protectors in the periodontium. The simplest reading of the literature would suggest, however, that normally activated neutrophils, when persistent in the periodontium and unable to clear bacteria, do wreak hazard upon healthy tissue. During periodontal therapy, surgical resection of the inflammatory infiltrate in the diseased connective tissue improves healing, potentially due to the elimination of cells contributing to destruction, and possibly by allowing for fresh neutrophils with regenerative capacities to arrive (Zitzmann et al. 2005). The essential question for therapy is how to control neutrophil-mediated damage without mimicking neutrophil deficiency states due to lack of migration, adhesion, or bactericidal activity. One approach is to block specific neutrophil pathways that do not appear essential for clearance of periodontal pathogens but do appear to contribute to tissue damage such as the phox pathway mutated in CGD patients. Of course, one is treading on dangerous ground given the implications of such an approach for the ability to ward off often fatal infections outside the periodontium. Blocking the activity of enzymes released by neutrophils that cause tissue destruction, such as collagenase, elastase, and MMPs does not seem feasible due to the redundancy in enzyme activity and the involved pathways (Nathan 2006). A more sophisticated approach might be to promote resolution by inducing neutrophil apoptosis (or at least restoring ''normal'' kinetics of apoptosis) in the periodontium. An added benefit of such an approach would be the creation of a population of macrophages laden with apoptotic neutrophils that could serve to suppress inflammation long term. In the last year, it was suggested that some natural lipid agonists, Resolvins, have a potential to actively induce resolution of persistent inflammation followed by clinical resolution of the disease. This issue is discussed in details in this issue (Van Dyke 2011). However, a greater understanding of the pathways controlling neutrophil apoptosis in the periodontal tissue is required in order to design new therapeutic interventions.

Yet another approach is to screen chemical libraries for compounds that limit neutrophil-mediated tissue damage while preserving beneficial antibacterial activity. Towards this goal, Han et al. (2005) screened for compounds that blocked neutrophil phox activation by soluble inflammatory mediators but not by phorbol esters. This approach yielded a new molecule, neucalcin, which blocked the TNFα-induced rise in intracellular calcium but did not block neutrophil degranulation or phos activation following phagocytosis of bacteria, and did not affect bactericidal activity (Han et al. 2005, Han et al. 2006). Although the exact target of neucalcin is not known, such approaches that distinguish between inflammation and bactericidal activity may yield new therapeutic entities to control persistent periodontal inflammation.

References

Akira, S. & Takeda, K. (2004) Toll-like receptor signalling. *Nature Reviews Immunology* **4**, 499–511.

Ardi, V. C., Van den Steen, P. E., Opdenakker, G., Schweighofer, B., Deryugina, E. I. & Quigley, J. P. (2009) Neutrophil MMP-9 proenzyme, unencumbered by TIMP-1, undergoes efficient activation in vivo and catalytically induces angiogenesis via a basic fibroblast growth factor (FGF-2)/FGFR-2 pathway. *Journal of Biological Chemistry* **284**, 25854–25866.

Ashkenazi, M., White, R. R. & Dennison, D. K. (1992) Neutrophil modulation by *Actinobacillus actinomycetemcomitans*. I. Chemotaxis, surface receptor expression and F-actin polymerization. *Journal of Periodontal Research* **27**, 264–273.

Baehni, P. C., Payot, P., Tsai, C. C. & Cimasoni, G. (1983) Periodontal status associated with chronic neutropenia. *Journal of Clinical Periodontology* **10**, 222–230.

Baer, P. N. & Iacono, V. J. (1994) Cyclic neutropenia: report of a case with a 15-year follow up. *Periodontal Clinical Investigation* **16**, 14–19.

Bals, R. (2000) Epithelial antimicrobial peptides in host defense against infection. *Respiratory Research* **1**, 141–150.

Beertsen, W., Willenborg, M., Everts, V., Zirogianni, A., Podschun, R., Schroder, B., Eskelinen, E. L. & Saftig, P. (2008) Impaired phagosomal maturation in neutrophils leads to periodontitis in lysosomal-associated membrane protein-2 knockout mice. *Journal of Immunology* **180**, 475–482.

Berglundh, T. & Donati, M. (2005) Aspects of adaptive host response in periodontitis. *Journal of Clinical Periodontology* **32**, 87–107.

Bianchi, M. E. (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. *Journal of Leukocyte Biology* **81**, 1–5.

Boman, H. G. (2003) Antibacterial peptides: basic facts and emerging concepts. *Journal of Internal Medicine* **254**, 197–215.

Briars, G. L., Parry, H. F. & Ansari, B. M. (1996) Dominantly inherited severe congenital neutropenia. *Journal of Infection* **33**, 123–126.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., Weinrauch, Y. & Zychlinsky, A. (2004) Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535.

Burns, E., Bachrach, G., Shapira, L. & Nussbaum, G. (2006) Cutting Edge: TLR2 is required for the innate response to *Porphyromonas gingivalis*: activation leads to bacterial persistence and TLR2 deficiency attenuates induced alveolar bone resorption. *Journal of Immunology* **177**, 8296–8300.

Burns, E., Eliyahu, T., Uematsu, S., Akira, S. & Nussbaum, G. (2010) TLR2-dependent inflammatory response to *Porphyromonas gingivalis* is MyD88 independent, whereas MyD88 is required to clear infection. *Journal of Immunology* **184**, 1455–1462.

Carlsson, G., Wahlin, Y. B., Johansson, A., Olsson, A., Eriksson, T., Claesson, R., Hanstrom, L. & Henter, J. I. (2006) Periodontal disease in patients from the original Kostmann family with severe congenital neutropenia. *Journal of Periodontology* **77**, 744–751.

Chakravarti, A., Raquil, M. A., Tessier, P. & Poubelle, P. E. (2009) Surface RANKL of Toll-like receptor 4-stimulated human neutrophils activates osteoclastic bone resorption. *Blood* **114**, 1633–1644.

Cox, D. P. & Weathers, D. R. (2008) Leukocyte adhesion deficiency type 1: an important consideration in the clinical differential diagnosis of prepubertal periodontitis. A case report and review of the literature. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **105**, 86–90.

da Fonseca, M. A. & Fontes, F. (2000) Early tooth loss due to cyclic neutropenia: long-term follow-up of one patient. *Special Care Dentistry* **20**, 187–190.

Dababneh, R., Al-Wahadneh, A. M., Hamadneh, S., Khouri, A. & Bissada, N. F. (2008) Periodontal manifestation of leukocyte adhesion deficiency type I. *Journal of Periodontology* **79**, 764–768.

Dale, B. A. & Fredericks, L. P. (2005) Antimicrobial peptides in the oral environment: expression and function in health and disease. *Current Issues in Molecular Biology* **7**, 119–133.

Dale, B. A., Kimball, J. R., Krisanaprakornkit, S., Roberts, F., Robinovitch, M., O'Neal, R., Valore, E. V., Ganz, T., Anderson, G. M. & Weinberg, A. (2001) Localized antimicrobial peptide expression in human gingiva. *Journal of Periodontal Research* **36**, 285–294.

Daley, J. M., Thomay, A. A., Connolly, M. D., Reichner, J. S. & Albina, J. E. (2008) Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. *Journal of Leukocyte Biology* **83**, 64–70.

de Haar, S. F., Hiemstra, P. S., van Steenbergen, M. T., Everts, V. & Beertsen, W. (2006) Role of polymorphonuclear leukocyte-derived serine proteinases in defense against *Actinobacillus actinomycetemcomitans*. *Infection and Immunity* **74**, 5284–5291.

Deasy, M. J., Vogel, R. I., Macedo-Sobrinho, B., Gertzman, G. & Simon, B. (1980) Familial benign chronic neutropenia associated with periodontal disease. A case report. *Journal of Periodontology* **51**, 206–210.

Dias, I. H. K., Matthews, J. B., Chapple, I. L. C., Wright, H. J., Dunston, C. R. & Griffiths, H. R. (2011) Activation of the neutrophil respiratory burst by plasma from periodontitis patients is mediated by pro-inflammatory cytokines. *Journal of Clinical Periodontology* **38**, 1–7.

Eick, S., Pfister, W., Sigusch, B. & Straube, E. (2000) Phagocytosis of periodontopathogenic bacteria by crevicular granulocytes is depressed in progressive periodontitis. *Infection* **28**, 301–304.

Fadok, V. A., Bratton, D. L., Konowal, A., Freed, P. W., Westcott, J. Y. & Henson, P. M. (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *Journal of Clinical Investigation* **101**, 890–898.

Frohm Nilsson, M., Sandstedt, B., Sorensen, O., Weber, G., Borregaard, N. & Stahle-Backdahl, M. (1999) The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. *Infection and Immunity* **67**, 2561–2566.

Gaffen, S. L., Kramer, J. M., Yu, J. J. & Shen, F. (2006) The IL-17 cytokine family. *Vitamins and Hormones* **74**, 255–282.

Ganz, T. (2003) Defensins: antimicrobial peptides of innate immunity. *Nature Reviews Immunology* **3**, 710–720.

Giannopoulou, C., Krause, K. H. & Muller, F. (2008) The NADPH oxidase NOX2 plays a role in periodontal pathologies. *Seminars in Immunopathology* **30**, 273–278.

Gribar, S. C., Anand, R. J., Sodhi, C. P. & Hackam, D. J. (2008) The role of epithelial Toll-like receptor signaling in the pathogenesis of intestinal inflammation. *Journal of Leukocyte Biology* **83**, 493–498.

Guzik, K. & Potempa, J. (2008) Friendly fire against neutrophils: proteolytic enzymes confuse the recognition of apoptotic cells by macrophages. *Biochimie* **90**, 405–415.

Hajishengallis, G. & Harokopakis, E. (2007) *Porphyromonas gingivalis* interactions with complement receptor 3 (CR3): innate immunity or immune evasion? *Frontiers in Bioscience* **12**, 4547–4557.

Hajishengallis, G., Ratti, P. & Harokopakis, E. (2005) Peptide mapping of bacterial fimbrial epitopes interacting with pattern recognition receptors. *Journal of Biological Chemistry* **280**, 38902–38913.

Han, H., Roberts, J., Lou, O., Muller, W. A., Nathan, N. & Nathan, C. (2006) Chemical inhibitors of TNF signal transduction in human neutrophils point to distinct steps in cell activation. *Journal of Leukocyte Biology* **79**, 147–154.

Han, H., Stessin, A., Roberts, J., Hess, K., Gautam, N., Kamenetsky, M., Lou, O., Hyde, E., Nathan, N., Muller, W. A., Buck, J., Levin, L. R. & Nathan, C. (2005) Calcium-sensing soluble adenylyl cyclase mediates TNF signal transduction in human neutrophils. *Journal of Experimental Medicine* **202**, 353–361.

Harokopakis, E., Albzreh, M. H., Martin, M. H. & Hajishengallis, G. (2006) TLR2 transmodulates monocyte adhesion and transmigration via Rac1- and PI3K-mediated inside-out signaling in response to *Porphyromonas gingivalis* fimbriae. *Journal of Immunology* **176**, 7645–7656.

Hart, T. C., Hart, P. S., Bowden, D. W., Michalec, M. D., Callison, S. A., Walker, S. J., Zhang, Y. & Firatli, E. (1999) Mutations of the cathepsin C gene are responsible for Papillon-Lefevre syndrome. *Journal of Medical Genetics* **36**, 881–887.

Hasturk, H., Tezcan, I., Yel, L., Ersoy, F., Sanal, O., Yamalik, N. & Berker, E. (1998) A case of chronic severe neutropenia: oral findings and consequences of short-term granulocyte colony-stimulating factor treatment. *Australian Dental Journal* **43**, 9–13.

Hasui, M. (1999) Chronic granulomatous disease in Japan: incidence and natural history. The Study Group of Phagocyte Disorders of Japan. *Pediatrics International* **41**, 589–593.

Hayashi, F., Means, T. K. & Luster, A. D. (2003) Toll-like receptors stimulate human neutrophil function. *Blood* **102**, 2660–2669.

Heissig, B., Nishida, C., Tashiro, Y., Sato, Y., Ishihara, M., Ohki, M., Gritli, I., Rosenkvist, J. & Hattori, K. (2010) Role of neutrophil-derived matrix metalloproteinase-9 in tissue regeneration. *Histology and Histopathology* **25**, 765–770.

Heitz-Mayfield, L. J. & Lang, N. P. (2010) Comparative biology of chronic and aggressive periodontitis vs. peri-implantitis. *Periodontology 2000* **53**, 167–181.

Hemmi, H., Takeuchi, O., Kawai, T., Kaisho, T., Sato, S., Sanjo, H., Matsumoto, M., Hoshino, K., Wagner, H., Takeda, K. & Akira, S. (2000) A Toll-like receptor recognizes bacterial DNA. *Nature* **408**, 740–745.

Heryanto, B., Girling, J. E. & Rogers, P. A. (2004) Intravascular neutrophils partially mediate the endometrial endothelial cell proliferative response to oestrogen in ovariectomised mice. *Reproduction* **127**, 613–620.

Holland, S. M. (2010) Chronic granulomatous disease. *Clinical Reviews in Allergy and Immunology* **38**, 3–10.

Holmstrup, P. & Glick, M. (2002) Treatment of periodontal disease in the immunodeficient patient. *Periodontology 2000* **28**, 190–205.

Hou, G. L. & Tsai, C. C. (1988) Oral manifestations of agranulocytosis associated with methimazole therapy. *Journal of Periodontology* **59**, 244–248.

Huynh, M. L., Fadok, V. A. & Henson, P. M. (2002) Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. *Journal of Clinical Investigation* **109**, 41–50.

Johansson, A., Sandstrom, G., Claesson, R., Hanstrom, L. & Kalfas, S. (2000) Anaerobic neutrophil-dependent killing of *Actinobacillus actinomycetemcomitans* in relation to the bacterial leukotoxicity. *European Journal of Oral Sciences* **108**, 136–146.

Kamma, J. J., Lygidakis, N. A. & Nakou, M. (1998) Subgingival microflora and treatment in prepubertal periodontitis associated with chronic idiopathic neutropenia. *Journal of Clinical Periodontology* **25**, 759–765.

Kantarci, A., Oyaizu, K. & Van Dyke, T. E. (2003) Neutrophil-mediated tissue injury in periodontal disease pathogenesis: findings from localized aggressive periodontitis. *Journal of Periodontology* **74**, 66–75.

Kinane, D. F., Cullen, C. F., Johnston, F. A. & Evans, C. W. (1989a) Neutrophil chemotactic behaviour in patients with early-onset forms of periodontitis (I). Leading front analysis in Boyden chambers. *Journal of Clinical Periodontology* **16**, 242–246.

Kinane, D. F., Cullen, C. F., Johnston, F. A. & Evans, C. W. (1989b) Neutrophil chemotactic behaviour in patients with early-onset forms of periodontitis (II). Assessment using the under agarose technique. *Journal of Clinical Periodontology* **16**, 247–251.

Kinane, D. F., Demuth, D. R., Gorr, S. U., Hajishengallis, G. N. & Martin, M. H. (2007) Human variability in innate immunity. *Periodontology 2000* **45**, 14–34.

Kinane, D. F., Berglundh, T & Lindhe, J. (2008) Pathogeneis of periodontitis. In: Lindhe, J, Lang, NP & Karring, T (eds). *Clinical Periodontology and Implant Dentistry*, 5th edition, pp. 285–299, Oxford: Blackwell Publishing.

Kirstila, V., Sewon, L. & Laine, J. (1993) Periodontal disease in three siblings with familial neutropenia. *Journal of Periodontology* **64**, 566–570.

Kitami, S., Tanaka, H., Kawato, T., Tanabe, N., Katono-Tani, T., Zhang, F., Suzuki, N., Yonehara, Y. & Maeno, M. (2010) IL-17A suppresses the expression of bone resorption-related proteinases and osteoclast differentiation via IL-17RA or IL-17RC receptors in RAW264.7 cells. *Biochimie* **92**, 398–404.

Kobayashi, S. D., Braughton, K. R., Whitney, A. R., Voyich, J. M., Schwan, T. G., Musser, J. M. & DeLeo, F. R. (2003) Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 10948–10953.

Kobayashi, S. D., Voyich, J. M., Buhl, C. L., Stahl, R. M. & DeLeo, F. R. (2002) Global changes in gene expression by human polymorphonuclear leukocytes during receptor-mediated phagocytosis: cell fate is regulated at the level of gene expression. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 6901–6906.

Korostoff, J., Wang, J. F., Kieba, I., Miller, M., Shenker, B. J. & Lally, E. T. (1998) *Actinobacillus actinomycetemcomitans* leukotoxin induces apoptosis in HL-60 cells. *Infection and Immunity* **66**, 4474–4483.

Kostmann, R. (1956) Infantile genetic agranulocytosis; agranulocytosis infantilis hereditaria. *Acta Paediatrica* **45** (Suppl.), 1–78.

Lamster, I. B., Oshrain, R. L. & Harper, D. S. (1987) Infantile agranulocytosis with survival into adolescence: periodontal manifestations and laboratory findings. A case report. *Journal of Periodontology* **58**, 34–39.

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

Martire, B., Rondelli, R., Soresina, A., Pignata, C., Broccoletti, T., Finocchi, A., Rossi, P., Gattorno, M., Rabusin, M., Azzari, C., Dellepiane, R. M., Pietrogrande, M. C., Trizzino, A., Di Bartolomeo, P., Martino, S., Carpino, L., Cossu, F., Locatelli, F., Maccario, R., Pierani, P., Putti, M. C., Stabile, A., Notarangelo, L. D., Ugazio, A. G., Plebani, A. & De Mattia, D. (2008) Clinical features, long-term follow-up and outcome of a large cohort of patients with Chronic Granulomatous Disease: an Italian multicenter study. *Clinical Immunology* **126**, 155–164.

McKay, M. S., Olson, E., Hesla, M. A., Panyutich, A., Ganz, T., Perkins, S. & Rossomando, E. F. (1999) Immunomagnetic recovery of human neutrophil defensins from the human gingival crevice. *Oral Microbiology and Immunology* **14**, 190–193.

Meng, H., Xu, L., Li, Q., Han, J. & Zhao, Y. (2007) Determinants of host susceptibility in aggressive periodontitis. *Periodontology 2000* **43**, 133–159.

Meyle, J. (1994) Leukocyte adhesion deficiency and prepubertal periodontitis. *Periodontology 2000* **6**, 26–36.

Moser, M., Bauer, M., Schmid, S., Ruppert, R., Schmidt, S., Sixt, M., Wang, H. V., Sperandio, M. & Fassler, R. (2009) Kindlin-3 is required for beta2 integrin-mediated leukocyte adhesion to endothelial cells. *Nature Medicine* **15**, 300–305.

Mueller, M. D., Lebovic, D. I., Garrett, E. & Taylor, R. N. (2000) Neutrophils infiltrating the endometrium express vascular endothelial growth factor: potential role in endometrial angiogenesis. *Fertility and Sterility* **74**, 107–112.

Murakami, M., Ohtake, T., Dorschner, R. A. & Gallo, R. L. (2002) Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. *Journal of Dental Research* **81**, 845–850.

Murray, D. A. & Wilton, J. M. (2003) Lipopolysaccharide from the periodontal pathogen *Porphyromonas gingivalis* prevents apoptosis of HL60-derived neutrophils in vitro. *Infection and Immunity* **71**, 7232–7235.

Mydel, P., Takahashi, Y., Yumoto, H., Sztukowska, M., Kubica, M., Gibson, F. C. III, Kurtz, D. M. Jr., Travis, J., Collins, L. V., Nguyen, K. A., Genco, C. A. & Potempa, J. (2006) Roles of the host oxidative immune response and bacterial antioxidant rubrerythrin during *Porphyromonas gingivalis* infection. *PLoS Pathogens* **2**, e76.

Nakayama, K. (1994) Rapid viability loss on exposure to air in a superoxide dismutase-deficient mutant of *Porphyromonas gingivalis*. *Journal of Bacteriology* **176**, 1939–1943.

Nathan, C. (2006) Neutrophils and immunity: challenges and opportunities. *Nature Reviews Immunology* **6**, 173–182.

Nibali, L., O'Dea, M., Bouma, G., Parkar, M., Thrasher, A. J., Burns, S. & Donos, N. (2010) Genetic variants associated with neutrophil function in aggressive periodontitis and Healthy controls. *Journal of Periodontology* **81**, 527–534.

Nicu, E. A., Van der Velden, U., Everts, A., Van Winkelhoff, A. J., Roos, D. & Loos, B. G. (2007) Hyper-reactive PMNs in Fc γRIIa 131 H/H genotype periodontitis patients. *Journal of Clinical Periodontology* **34**, 938–945.

Nussbaum, C., Moser, M. & Sperandio, M. (2010) Leukocyte adhesion deficiency-III: when leukocytes cannot stop. *Pediatric Research* **67**, 339.

Ocana, M. G., Asensi, V., Montes, A. H., Meana, A., Celada, A. & Valle-Garay, E. (2008) Autoregulation mechanism of human neutrophil apoptosis during bacterial infection. *Molecular Immunology* **45**, 2087–2096.

Odell, E. W. & Wu, P. J. (1992) Susceptibility of *Porphyromonas gingivalis* and *P. asaccharolytica* to the non-oxidative killing mechanisms of human neutrophils. *Archives of Oral Biology* **37**, 597–601.

Ozer, H., Armitage, J. O., Bennett, C. L., Crawford, J., Demetri, G. D., Pizzo, P. A., Schiffer, C. A., Smith, T. J., Somlo, G., Wade, J. C., Wade, J. L. III, Winn, R. J., Wozniak, A. J. & Somerfield, M. R. (2000) 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. American Society of Clinical Oncology Growth Factors Expert Panel. *Journal of Clinical Oncology* **18**, 3558–3585.

Page, R. C., Beatty, P. & Waldrop, T. C. (1987) Molecular basis for the functional abnormality in neutrophils from patients with generalized prepubertal periodontitis. *Journal of Periodontal Research* **22**, 182–183.

Parker, L. C., Whyte, M. K., Dower, S. K. & Sabroe, I. (2005) The expression and roles of Toll-like receptors in the biology of the human neutrophil. *Journal of Leukocyte Biology* **77**, 886–892.

Permpanich, P., Kowolik, M. J. & Galli, D. M. (2006) Resistance of fluorescent-labelled *Actinobacillus actinomycetemcomitans* strains to phagocytosis and killing by human neutrophils. *Cell Microbiology* **8**, 72–84.

Pernu, H. E., Pajari, U. H. & Lanning, M. (1996) The importance of regular dental treatment in patients with cyclic neutropenia. Follow-up of 2 cases. *Journal of Periodontology* **67**, 454–459.

Pham, C. T., Ivanovich, J. L., Raptis, S. Z., Zehnbauer, B. & Ley, T. J. (2004) Papillon-Lefevre syndrome: correlating the molecular, cellular, and clinical consequences of cathepsin C/dipeptidyl peptidase I deficiency in humans. *Journal of Immunology* **173**, 7277–7281.

Porter, S. R., Luker, J., Scully, C. & Oakhill, A. (1994) Oral features of a family with benign familial neutropenia. *Journal of the American Academy of Dermatology* **30**, 877–880.

Pouliot, M., Clish, C. B., Petasis, N. A., Van Dyke, T. E. & Serhan, C. N. (2000) Lipoxin A(4) analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry* **39**, 4761–4768.

Preshaw, P. M., Schifferle, R. E. & Walters, J. D. (1999) *Porphyromonas gingivalis* lipopolysaccharide delays human polymorphonuclear leukocyte apoptosis in vitro. *Journal Periodontal Research* **34**, 197–202.

Prichard, J. F., Ferguson, D. M., Windmiller, J. & Hurt, W. C. (1984) Prepubertal periodontitis affecting the deciduous and permanent dentition in a patient with cyclic neutropenia. A case report and discussion. *Journal of Periodontology* **55**, 114–122.

Putsep, K., Carlsson, G., Boman, H. G. & Andersson, M. (2002) Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. *Lancet* **360**, 1144–1149.

Reichart, P. A. & Dornow, H. (1978) Gingivo-periodontal manifestations in chronic benign neutropenia. *Journal of Clinical Periodontology* **5**, 74–80.

Ryder, M. I. (2010) Comparison of neutrophil functions in aggressive and chronic periodontitis. *Periodontology 2000* **53**, 124–137.

Rylander, H. & Ericsson, I. (1981) Manifestations and treatment of periodontal disease in a patient suffering from cyclic neutropenia. *Journal of Clinical Periodontology* **8**, 77–87.

Saftig, P., Beertsen, W. & Eskelinen, E. L. (2008) LAMP-2: a control step for phagosome and autophagosome maturation. *Autophagy* **4**, 510–512.

Saglam, F., Atamer, T., Onan, U., Soydinc, M. & Kirac, K. (1995) Infantile genetic agranulocytosis (Kostmann type). A case report. *Journal of Periodontology* **66**, 808–810.

Scully, C., MacFadyen, E. & Campbell, A. (1982) Oral manifestations in cyclic neutropenia. *British Journal of Oral Surgery* **20**, 96–101.

Soler-Palacin, P., Margareto, C., Llobet, P., Asensio, O., Hernandez, M., Caragol, I. & Espanol, T. (2007) Chronic granulomatous disease in pediatric patients: 25 years of experience. *Allergology and Immunopathology (Madrid)* **35**, 83–89.

Stabholz, A., Soskolne, V., Machtei, E., Or, R. & Soskolne, W. A. (1990) Effect of benign familial neutropenia on the periodontium of Yemenite Jews. *Journal of Periodontology* **61**, 51–54.

Stabholz, A., Soskolne, W. A. & Shapira, L. (2010) Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontology 2000* **53**, 138–153.

Stephens-Romero, S. D., Mednick, A. J. & Feldmesser, M. (2005) The pathogenesis of fatal outcome in murine pulmonary aspergillosis depends on the neutrophil depletion strategy. *Infection and Immunity* **73**, 114–125.

Svensson, L., Howarth, K., McDowall, A., Patzak, I., Evans, R., Ussar, S., Moser, M., Metin, A., Fried, M., Tomlinson, I. & Hogg, N. (2009) Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nature Medicine* **15**, 306–312.

Sztukowska, M., Bugno, M., Potempa, J., Travis, J. & Kurtz, D. M. Jr. (2002) Role of rubrerythrin in the oxidative stress response of *Porphyromonas gingivalis*. *Molecular Microbiology* **44**, 479–488.

Takahashi, K., Ohyama, H., Kitanaka, M., Sawa, T., Mineshiba, J., Nishimura, F., Arai, H., Takashiba, S. & Murayama, Y. (2001) Heterogeneity of host immunological risk factors in patients with aggressive periodontitis. *Journal of Periodontology* **72**, 425–437.

Takeda, K., Kaisho, T. & Akira, S. (2003) Toll-like receptors. *Annual Review of Immunology* **21**, 335–376.

Theilgaard-Monch, K., Knudsen, S., Follin, P. & Borregaard, N. (2004) The transcriptional activation program of human neutrophils in skin lesions supports their important role in wound healing. *Journal of Immunology* **172**, 7684–7693.

Toomes, C., James, J., Wood, A. J., Wu, C. L., McCormick, D., Lench, N., Hewitt, C., Moynihan, L., Roberts, E., Woods, C. G., Markham, A., Wong, M., Widmer, R., Ghaffar, K. A., Pemberton, M., Hussein, I. R., Temtamy, S. A., Davies, R., Read, A. P., Sloan, P., Dixon, M. J. & Thakker, N. S. (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nature Genetics* **23**, 421–424.

Van Dyke, T. E. (2011) Proresolving lipid mediators: potential for prevention and treatment of periodontitis. *Journal of Clinical Periodontology* **38**, 119–125.

Verzeletti, G. N., Gaio, E. J. & Rosing, C. K. (2007) Effect of methotrexate on alveolar bone loss in experimental periodontitis in Wistar rats. *Acta Odontologica Scandinavica* **65**, 348–351.

Vitkov, L., Klappacher, M., Hannig, M. & Krautgartner, W. D. (2009) Extracellular neutrophil traps in periodontitis. *Journal of Periodontal Research* **44**, 664–672.

Waldrop, T. C., Anderson, D. C., Hallmon, W. W., Schmalstieg, F. C. & Jacobs, R. L. (1987) Periodontal manifestations of the heritable Mac-1, LFA-1, deficiency syndrome. Clinical, histopathologic and molecular characteristics. *Journal of Periodontology* **58**, 400–416.

Walsh, N. C., Crotti, T. N., Goldring, S. R. & Gravallese, E. M. (2005) Rheumatic diseases: the effects of inflammation on bone. *Immunological Reviews* **208**, 228–251.

Watanabe, K. (1990) Prepubertal periodontitis: a review of diagnostic criteria, pathogenesis, and differential diagnosis. *Journal of Periodontal Research* **25**, 31–48.

Weston, B., Todd, R. F. III, Axtell, R., Balazovich, K., Stewart, J., Locey, B. J., Mayo-Bond, L., Loos, P., Hutchinson, R. & Boxer, L. A. (1991) Severe congenital neutropenia: clinical effects and neutrophil function during treatment with granulocyte colony-stimulating factor. *of Laboratory and Clinical Medicine* **117**, 282–290.

Winkelstein, J. A., Marino, M. C., Johnston, R. B. Jr., Boyle, J., Curnutte, J., Gallin, J. I., Malech, H. L., Holland, S. M., Ochs, H., Quie, P., Buckley, R. H., Foster, C. B., Chanock, S. J. & Dickler, H. (2000) Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine (Baltimore)* **79**, 155–169.

Wright, H. J., Matthews, J. B., Chapple, I. L., Ling-Mountford, N. & Cooper, P. R. (2008) Periodontitis associates with a type 1 IFN signature in peripheral blood neutrophils. *Journal of Immunology* **181**, 5775–5784.

Yoshinari, N., Kameyama, Y., Aoyama, Y., Nishiyama, H. & Noguchi, T. (1994) Effect of long-term methotrexate-induced neutropenia on experimental periodontal lesion in rats. *Journal of Periodontal Research* **29**, 393–400.

Yu, J. J. & Gaffen, S. L. (2008) Interleukin-17: a novel inflammatory cytokine that bridges innate and adaptive immunity. *Frontiers in Bioscience* **13**, 170–177.

Yu, J. J., Ruddy, M. J., Wong, G. C., Sfintescu, C., Baker, P. J., Smith, J. B., Evans, R. T. & Gaffen, S. L. (2007) An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor-dependent signals. *Blood* **109**, 3794–3802.

Zitzmann, N. U., Berglundh, T. & Lindhe, J. (2005) Inflammatory lesions in the gingiva following resective/non-resective periodontal therapy. *Journal of Clinical Periodontology* **32**, 139–146.

Zubery, Y., Moses, O. & Kozlovsky, A. (1991) Agranulocytosis – periodontal manifestations and treatment of the acute phase: a case report. *Clinical Preventive Dentistry* **13**, 5–8.

Address:
Lior Shapira
Department of Periodontology
Faculty of Dental Medicine
Hadassah-Hebrew University Medical Center
PO Box 12272 Jerusalem
Israel 91120
E-mail: shapiral@cc.huji.ac.il

Clinical Relevance

Scientific rational for the study: The aim of the present manuscript is to review the existing evidence regarding the role of the neutrophil in the protection of the periodontium against infection and in inflammation-induced destruction processes.
Principal findings: The evidence suggests that normal neutrophil number and function is essential to prevent penetration of bacteria into the host tissue, and neutrophils play a crucial role in tissue repair following periodontal treatment. Bacterial evasion of neutrophil clearance might be a major characteristic of periodontitis. Accumulation of neutrophils due to diminished neutrophil clearance and delayed resolution of inflammation may cause tissue damage.
Practical implications: Clinicians should consider their patients' neutrophils as ''partners'' in the defense against periodontitis; however, resolution of neutrophil mediated inflammation may also be critical for healing. Pharmacological or surgical elimination of the inflammatory infiltrate during periodontal therapy can therefore improve healing. Potential future therapies aimed at the neutrophil must account for how periodontal bacteria evade neutrophil clearance in order to block neutrophil-mediated tissue damage while preserving the neutrophil's beneficial antibacterial activity.