CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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Neutrophil Homeostasis and Periodontal Health in Children and Adults

ABSTRACT

This review summarizes the current state of knowledge on neutrophil basic biology and discusses how the breakdown of neutrophil homeostasis affects periodontal health. The homeostasis of neutrophils is tightly regulated through coordinated bone marrow production, release into the circulation, transmigration to and activation in peripheral tissues, and clearance of senescent neutrophils. Dysregulation of any of these homeostatic mechanisms at any age can cause severe periodontitis in humans and animal models. Accordingly, both impaired and excessive neutrophil activity (in terms of numbers or immune function) can precipitate periodontitis. Neutrophil defects of congenital origin (*e.g.*, congenital neutropenia, leukocyte adhesion deficiency, and Chediak-Higashi syndrome) are associated with cutaneous and systemic infections and early-onset forms of periodontitis affecting both the primary and permanent dentitions of children. However, the strong association between congenital neutrophil disorders and early-onset periodontitis is not currently adequately explained mechanistically. This suggests the operation of as-yet-unknown molecular mechanisms, although the available body of evidence leaves no doubt that neutrophils are integral to periodontal tissue homeostasis and health.

KEY WORDS: bone marrow, congenital syndromes, periodontitis, leukocyte adhesion molecules, neutrophil infiltration, inflammation.

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INTRODUCTION

Neutrophils are terminally differentiated white blood cells, which are equipped with a plethora of microbicidal and pro-inflammatory mechanisms and form the first line of defense against pathogenic insults (Kobayashi and DeLeo, 2009; Amulic *et al.*, 2012). They are produced in huge numbers in the bone marrow (≈ 10^9 cells *per* kg bodyweight *per* day), from where they are released into the circulation. However, neutrophils are short-lived (6- to 8-hour circulating half-life) and undergo apoptosis (programmed cell death) (von Vietinghoff and Ley, 2008).

In response to infection and/or inflammation, circulating neutrophils can migrate to peripheral tissues, such as the skin, gut, lungs, or the periodontium (Amulic *et al.*, 2012; Hajishengallis and Chavakis, 2013). Defects in the function or numbers of neutrophils have been implicated in several forms of periodontal disease, ranging from early-onset forms in children to adult-type, chronic periodontitis (Deas *et al.*, 2003; Darveau, 2010; Nussbaum and Shapira, 2011). Intriguingly, both insufficient and excessive neutrophil function or numbers can contribute to the pathogenesis of periodontitis (Deas *et al.*, 2003; Ryder, 2010; Nussbaum and Shapira, 2011; Hasturk *et al.*, 2012; Hajishengallis and Chavakis, 2013). The major focus of this review is to discuss established and emerging concepts that neutrophil homeostasis is essential to periodontal health for reasons that do not necessarily relate to the traditional defense functions of neutrophils. Understanding the mechanisms of neutrophil involvement in the various forms of periodontal disease can lead to more effective therapeutic interventions.

NEUTROPHIL BIOLOGY AND HOMEOSTASIS

To better understand the role of neutrophils in periodontal disease, it is instructive to first discuss basic concepts of neutrophil biology and homeostasis. To maintain a balance between the protective and potentially destructive effects of neutrophils, several homeostatic mechanisms regulate their production, trafficking, and clearance (von Vietinghoff and Ley, 2008; Summers *et al.*, 2010). The granulocyte colony-stimulating factor (G-CSF) is the primary regulator of both granulopoiesis and neutrophil release from the bone marrow (Fig. 1). Mice or humans lacking a functional G-CSF receptor exhibit severe neutropenia (von Vietinghoff and Ley, 2008). The effects mediated by G-CSF include the commitment of progenitors to the myeloid lineage, proliferation of granulocytic precursors, and the release of mature neutrophils into the circulation (von Vietinghoff and Ley, 2008; Summers *et al.*, 2010). The latter effect is mediated, at least in part, by the ability of G-CSF to interfere

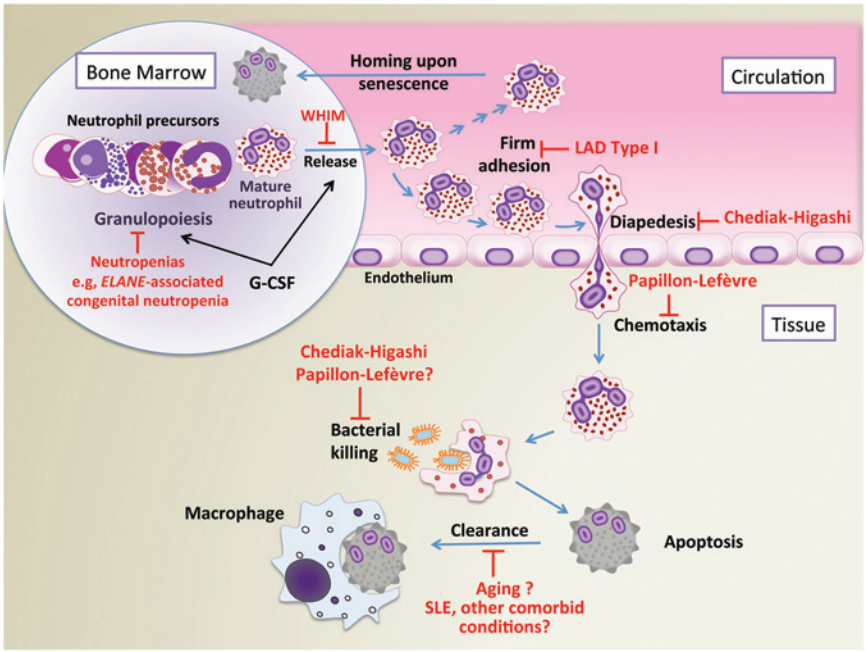

Figure 1. The life cycle and function of neutrophils: homeostatic perturbations in various syndromes or disorders. Key events in the life cycle of the neutrophil include granulopoiesis, release into the circulation, adhesion to the endothelium and chemotactic migration to tissues, antimicrobial function, and apoptotic cell clearance. Shown are congenital and other disorders that interfere with the indicated functions leading to development of severe forms of periodontitis (Deas *et al.*, 2003; Kollner *et al.*, 2006; Dotta *et al.*, 2011; Ye *et al.*, 2011; Hanna and Etzioni, 2012). LAD, leukocyte adhesion deficiency; SLE, systemic lupus erythematosus; WHIM, warts, hypogammaglobulinemia, infections, and myelokathexis (syndrome); G-CSF, granulocyte-colony stimulating factor.

with the interaction between the CXC chemokine receptor 4 (CXCR4) and the stromal-derived factor-1 (SDF-1), a chemokine (also known as CXCL12) produced constitutively by bone-marrow stromal cells. The CXCR4–SDF-1 interaction is a major mechanism of neutrophil retention in the bone marrow (von Vietinghoff and Ley, 2008). In mice, the circulating pool of neutrophils represents < 2% of the mature neutrophils in the bone marrow, although this percentage is increased to about 10% following G-CSF treatment (Summers *et al.*, 2010). Interleukin 17 (IL-17) promotes granulopoiesis and induces the recruitment, activation, and survival of neutrophils by acting mainly through up-regulation of G-CSF (Ye *et al.*, 2001; von Vietinghoff and Ley, 2008) (Fig. 2).

The process of neutrophil extravasation involves a cascade of low- and high-affinity adhesive interactions between the neutrophils and the vascular endothelium (Nourshargh *et al.*, 2010). The first step involves transient rolling interactions mediated by endothelial cell-surface molecules (P- or E-selectin) and their glycoprotein ligands on neutrophils. This rolling-dependent deceleration of neutrophils is followed by β_2 integrin-dependent firm adhesion and subsequent crawling on the endothelium, during which neutrophils seek an appropriate site for diapedesis through endothelial junctions (Fig. 1). Recently, a 52-kDa endothelial cell-secreted glycoprotein termed developmental endothelial locus-1 (Del-1; also known as EDIL3, for EGF-like repeats and discoidin I-like domains 3) was identified as a novel, perhaps the first, negative regulator of β_2 integrin-dependent neutrophil recruitment (Choi *et al.*, 2008; Hajishengallis and Chavakis, 2013). Specifically, Del-1 inhibits the interaction between the LFA-1 (CD11a/CD18) integrin on neutrophils and the intercellular adhesion molecule-1 (ICAM-1) on endothelial cells (Choi *et al.*, 2008), thereby suppressing firm adhesion and consequently the transendothelial migration of human neutrophils (Eskan *et al.*, 2012) (Fig. 3).

Neutrophil extravasation can be modulated by tissue-derived cytokines, which can up-regulate endothelial adhesion molecule expression, whereas tissue-derived chemokines decorating the apical endothelial cell surface can trigger conformational changes on leukocyte integrins, which thereby adopt their high-affinity state (Nourshargh *et al.*, 2010; Kolaczkowska and Kubes, 2013). Although transmigrating neutrophils initially follow the chemokine gradient deposited by the endothelium, they then have to move toward a gradient existing in the infected or inflamed tissue. Such gradients could also involve chemoattractants derived from infecting bacteria (*N*-formyl-methionyl-leucyl-phenylalanine; fMLP) or from local complement activation (C5a fragment) (Kolaczkowska and Kubes, 2013).

Extravasated senescent neutrophils are cleared locally by tissue phagocytes, such as macrophages or dendritic cells, whereas senescent neutrophils in the blood home back to the bone marrow for clearance upon up-regulation of CXCR4 expression (Martin *et al.*, 2003) (Fig. 1). Intriguingly, the clearance of transmigrated apoptotic neutrophils serves more than waste disposal, since it is crucial for the regulation of neutrophil production in the bone marrow (Stark *et al.*, 2005) (Fig. 2). Indeed, the engulfment of apoptotic neutrophils by tissue phagocytes triggers anti-inflammatory signals; which decrease their production of IL-23, a key cytokine for induction of IL-17 by both innate and adaptive immune cells (Stark *et al.*, 2005). The resulting inhibition of IL-17 production, in turn, leads to decreased production of G-CSF by cells such as fibroblasts, thereby limiting the stimulus for neutrophil production to maintain steady-state neutrophil counts (Stark *et al.*, 2005). In essence, this is a neutrophil rheostat ('neutrostat') feedback mechanism (Fig. 2). For instance, the phagocytosis of apoptotic neutrophils associated with resolution of inflammation will signal the down-regulation of neutrophil production, since neutrophils are no longer needed in great numbers.

NEUTROPHIL RECRUITMENT TO THE PERIODONTIUM

Neutrophils constitute the overwhelming majority of cells recruited to the gingival crevice (≥ 95% of total leukocytes) and

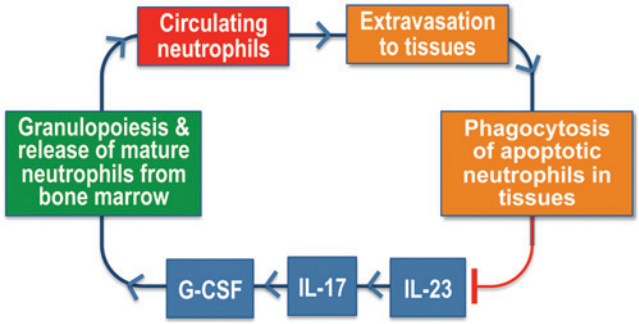

Figure 2. The neutrostat regulatory feedback loop. During infection or inflammation, IL-23-induced IL-17 promotes granulopoiesis and mobilization of mature neutrophils from the bone marrow by acting through up-regulation of G-CSF. Circulating neutrophils can normally extravasate into infected or inflamed tissues. Upon senescence, transmigrated neutrophils become apoptotic and undergo phagocytosis by tissue phagocytes, leading to suppression of IL-23 production, in turn, down-regulating the IL-17-G-CSF axis for maintaining steady-state neutrophil counts (Stark *et al.*, 2005).

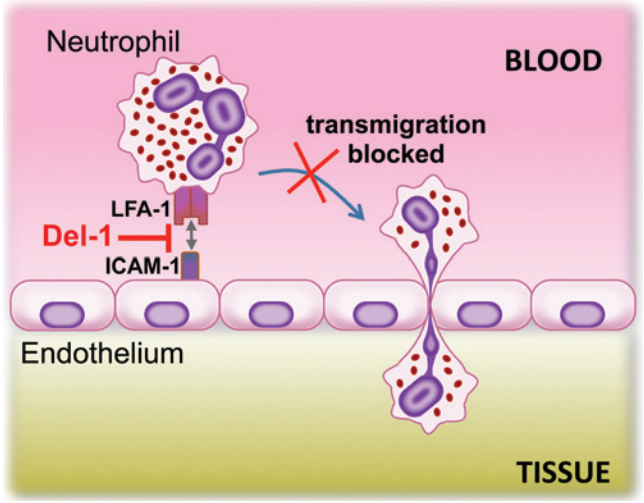

Figure 3. Del-1 acts as a local gatekeeper of normal neutrophil recruitment. Del-1 blocks the interaction between the LFA-1 integrin on neutrophils and ICAM-1 on endothelial cells. This interaction is required for firm neutrophil adhesion to the endothelium, which in turn is essential to subsequent transmigration (Choi *et al.*, 2008; Eskan *et al.*, 2012). As a consequence, Del-1 can suppress the migration of neutrophils from the circulation to peripheral tissues.

form a 'defense wall' against the tooth-associated subgingival biofilm (Delima and Van Dyke, 2003; Ryder, 2010). Extravasated neutrophils enter the crevice through the junctional epithelium, which, under inflammatory conditions, is largely occupied (≈ 60%) by trafficking neutrophils (Delima and Van Dyke, 2003).

Healthy human gingiva display coordinated gradients of chemokines and adhesion molecules that are thought to contribute to the directed migration of neutrophils to the gingival crevice. Specifically, gradients of interleukin (IL)-8 (CXCL8), ICAM-1, and E-selectin are topographically associated with the pathway of neutrophil migration, from the vasculature to the junctional epithelium and, ultimately, to the gingival crevice (Darveau, 2010). A recent study in mice demonstrated that neutrophil recruitment to the periodontium is entirely dependent upon the CXC chemokine receptor 2 (CXCR2), which responds to neutrophil-specific chemoattractants such as CXCL1 and CXCL2 (murine analogues of IL-8) (Zenobia *et al.*, 2013). This finding probably reflects the essential role of CXC chemoattractants in the initial phase of the directed extravasation of neutrophils (Kolaczkowska and Kubes, 2013), and it should not necessarily be interpreted as evidence of lack of involvement of C5a or fMLP in neutrophil recruitment to the periodontium.

Intriguingly, the migration of neutrophils to the periodontium does not require commensal bacterial colonization, since recruited neutrophils were also observed in germ-free mice (Zenobia *et al.*, 2013). This finding suggests that neutrophil recruitment may serve homeostatic function(s) that may not necessarily be related to infection control. In this respect, it should be noted that mechanisms sensing neutrophil recruitment to peripheral tissues also contribute to the homeostatic regulation of neutrophil numbers (Stark *et al.*, 2005) (Fig. 2).

NEUTROPHIL HOMEOSTASIS BREAKDOWN AND PERIODONTAL DISEASE

The importance of neutrophil homeostasis for periodontal health is emphatically underscored by the development of periodontitis in conditions associated with defects in mechanisms that regulate the production and life cycle of neutrophils (Fig. 1). Many of these disorders are congenital and affect periodontal health early in life. These forms of early-onset periodontitis are generally unresponsive to antibiotics and/or mechanical removal of the tooth-associated biofilm (Deas *et al.*, 2003; Nualart Grollmus *et al.*, 2007), suggesting the involvement of mechanisms unrelated to or in addition to defective neutrophil control of the periodontal microbiota. Congenital or acquired conditions associated with neutrophil dysfunction and periodontitis are discussed below in sections organized according to the different stages of the neutrophil life cycle.

Defective Neutrophil Production

Defective granulopoiesis leads to neutropenia, which represents a persistent reduction in the absolute neutrophil count in the circulation (< 1,500 cells/μL) and is generally associated with susceptibility to infections. It is classified as congenital or acquired (*i.e.*, autoimmune neutropenia, HIV-associated neutropenia, and neutropenia in cancer patients under chemotherapy or radiation therapy). Congenital neutropenias are further subdivided into those existing as the only phenotypic manifestations or those associated with other immunological or extra-hematopoietic abnormalities (Donadieu *et al.*, 2011). Periodontal disease, often severe, involving both the primary and the permanent dentition, occurs in many of these neutropenic conditions (Hart and Atkinson, 2007). To restore the number of circulating neutrophils, G-CSF is frequently administered to neutropenic patients who typically show improvement in their periodontal condition, thus supporting the importance of neutrophils in periodontal health (Nussbaum and Shapira, 2011).

Mutations in the *ELANE* gene, which encodes neutrophil elastase, are involved in cyclic neutropenia and also in 40% to 55%

of the cases of severe congenital neutropenia (Fig. 1). Elastase is a serine protease that is synthesized during the myeloblast-to-promyelocyte transition and stored in azurophilic granules. *ELANE* mutations cause the production of misfolded elastase protein molecules, which are thought to activate the unfolded protein response leading to accelerated apoptosis of neutrophil precursors in the bone marrow (Kollner *et al.*, 2006). Mutations in the *ELANE* gene were recently associated with periodontitis in patients with severe congenital neutropenia (Ye *et al.*, 2011).

Defective Neutrophil Release from the Bone Marrow

The syndrome known as WHIM is characterized by **w**arts, **h**ypogammaglobulinemia, **i**nfections, and **m**yelokathexis (*i.e.*, retention of neutrophils in the bone marrow) (Dotta *et al.*, 2011). WHIM patients have mutations in the CXCR4, a chemokine receptor involved in the release of mature neutrophils from the bone marrow and also the homing of senescent neutrophils back to the bone marrow (Martin *et al.*, 2003; von Vietinghoff and Ley, 2008). The *CXCR4* mutations associated with the WHIM syndrome affect the desensitization of CXCR4 upon stimulation with its ligand (SDF-1), leading to altered responsiveness of WHIM neutrophils to SDF-1 (Lagane *et al.*, 2008). How this defect relates to the clinical manifestations of the syndrome is not clear, although impaired cellular homeostasis and trafficking appear to be a factor. In this regard, WHIM patients cannot release neutrophils into the circulation (Fig. 1), leading to severe reduction of neutrophil counts in the blood and increased numbers of neutrophils in the bone marrow (Dotta *et al.*, 2011). It should be noted that lymphocyte function and trafficking are also affected in WHIM patients. Periodontitis associated with premature tooth loss is common in children with WHIM syndrome, who generally suffer from recurrent superficial infections, such as cellulitis and cutaneous abscess, but also deeper tissue infections, including pneumonia, osteomyelitis, and meningitis (Gorlin *et al.*, 2000; Mc Guire *et al.*, 2010; Dotta *et al.*, 2011).

Impaired or Excessive Neutrophil Recruitment to Peripheral Tissues

Leukocyte adhesion deficiency (LAD) represents a group of inherited disorders, which inhibit the normal extravasation of circulating neutrophils to sites of infection or inflammation (Hanna and Etzioni, 2012). In LAD patients, leukocytes have defects in the expression or function of β_2 integrins or other adhesion molecules and consequently cannot adhere to vascular endothelial cells (Fig. 1). LAD type I (LAD-I) is caused by deficiency in β_2 integrins, LAD-II is due to defective glycosylation of selectin ligands, and LAD-III involves dysfunction of signaling intermediates affecting integrin activation. The most common type is LAD-I, an autosomal-recessive immunodeficiency caused by mutations in the CD18-encoding *ITGB2* gene (Hanna and Etzioni, 2012). Few, if any, neutrophils can be found in extravascular sites in these patients, who display neutrophilia (increased blood neutrophil counts) even in the absence of infection. The neutrophilia of LAD patients is explained by the disruption of the neutrostat mechanism, since the lack of neutrophils recruited to tissues – and hence the lack of apoptotic neutrophil phagocytosis – would lead to unrestrained expression of granulopoiesis factors (Fig. 2). LAD-I patients suffer from recurrent infections and develop severe periodontitis early in life, affecting both the primary and permanent dentitions (Waldrop *et al.*, 1987; Deas *et al.*, 2003; Hanna and Etzioni, 2012). Mice genetically deficient in CD18 or CD11a reproduce several aspects of the LAD-I phenotype, such as neutrophilia and defective neutrophil adhesion and extravasation (Stark *et al.*, 2005; von Vietinghoff and Ley, 2008). More recently, LFA-1 (CD11a)-deficient mice were shown to develop spontaneous dysbiosis and alveolar bone loss early in life, mimicking the periodontal phenotype of LAD-I patients (Hajishengallis *et al.*, 2011). A similar periodontal phenotype is seen in mice with combined P- and E-selectin deficiency, a model equivalent to LAD-II (Niederman *et al.*, 2001). In either study, however, it is uncertain whether the dysbiosis of the microbiota was the cause or the consequence of inflammation, and further research is warranted to determine whether dysbiosis is a direct outcome of the neutrophil defects.

Other conditions associated with defective neutrophil recruitment are attributed to impaired neutrophil chemotaxis, such as the Chediak-Higashi syndrome and the Papillon-Lefèvre syndrome. Similar to LAD patients, individuals with defective chemotaxis develop rapidly advancing periodontal bone loss at a very young age (Deas *et al.*, 2003; Hart and Atkinson, 2007; Hanna and Etzioni, 2012). These conditions are reproduced in CXCR2-deficient mice, which cannot recruit neutrophils to the periodontium and develop severe bone loss early in life (Hajishengallis *et al.*, 2011; Zenobia *et al.*, 2013).

The Chediak-Higashi syndrome is associated with mutations of the *LYST* gene, which encodes for a protein involved in the regulation of lysosomal trafficking (Kaplan *et al.*, 2008). Phenotypically, this disorder is characterized by fusion of cytoplasmic granules, which, in the case of myelocytes, occurs early in myelopoiesis. As a consequence, many myeloid precursors die in the marrow, causing moderate neutropenia. In surviving neutrophils, the giant granules appear to interfere mechanically with the process of diapedesis (Clawson *et al.*, 1978) (Fig. 1). Moreover, because the Chediak-Higashi syndrome neutrophils have reduced content of hydrolytic enzymes, they exhibit delayed intracellular killing of phagocytosed bacteria (Fig. 1). Affected individuals are susceptible to infections involving mucous membranes, the respiratory tract, and skin, whereas severe periodontitis and oral ulcerations are common oral manifestations of the syndrome (Delcourt-Debruyne *et al.*, 2000; Nualart Grollmus *et al.*, 2007).

The Papillon-Lefèvre syndrome is caused by deficiency in cathepsin C (dipeptidyl peptidase-I), a lysosomal exo-cysteine protease also involved in pro-enzyme activation (*e.g.*, activation of neutrophil-derived serine proteases such as cathepsin G, elastase, and proteinase 3) (Dickinson, 2002; Kobayashi *et al.*, 2013). Neutrophils from patients with Papillon-Lefèvre syndrome have defective chemotaxis (Van Dyke *et al.*, 1984; Liu *et al.*, 2000) but are not invariably affected in their bacterial killing capacity (Pham *et al.*, 2004; de Haar *et al.*, 2006) (Fig. 1). These defects could probably contribute to the clinical condition of Papillon-Lefèvre patients, who show dramatic susceptibility to periodontitis affecting both the primary and permanent dentitions, whereas a subset of patients is susceptible to cutaneous

and systemic infections (Pham *et al.*, 2004; Hart and Atkinson, 2007; Nualart Grollmus *et al.*, 2007). Additionally or alternatively, the periodontal tissue destruction associated with the Papillon-Lefèvre syndrome could be related in part to inflammation resulting from defective degradation and hence excessive accumulation of the macrophage inflammatory protein-1α, due to the patients' cathepsin C deficiency and thus failure to activate neutrophil-derived serine proteases (Ryu *et al.*, 2005).

Apart from host-related factors, periodontal bacteria, such as the keystone pathogen *Porphyromonas gingivalis* (Hajishengallis *et al.*, 2012; Hajishengallis and Lambris, 2011), can impair neutrophil recruitment by interfering with the coordinated expression of chemokines and cell adhesion molecules, such as IL-8 and E-selectin (Darveau *et al.*, 1998; Darveau, 2010). The inhibitory effect on IL-8 was termed 'local chemokine paralysis' and depends on the capacity of *P. gingivalis* to invade the epithelial cells (Darveau *et al.*, 1998) and secrete the serine phosphatase SerB (Takeuchi *et al.*, 2013). *P. gingivalis*-invaded epithelial cells are prevented from eliciting IL-8 responses, even when exposed to bacteria like *F. nucleatum* that are potent inducers of IL-8 on their own (Darveau *et al.*, 1998). *In vivo* mouse studies have shown that these subversive effects (inhibition of IL-8 and E-selectin expression) are transient (Hajishengallis *et al.*, 2011), but, at least in principle, could allow adequate time for *P. gingivalis* to initiate colonization while delaying the influx of neutrophils. In this regard, a SerB-deficient isogenic mutant (hence, incapable of causing local chemokine paralysis) induces enhanced neutrophil recruitment to the periodontium and causes reduced bone loss compared with wild-type *P. gingivalis* (Bainbridge *et al.*, 2010). Therefore, mechanisms by which bacteria delay neutrophil recruitment can contribute to periodontal pathogenesis.

Recent studies have suggested that the unrestrained recruitment of neutrophils to the periodontium is as problematic as the impaired neutrophil recruitment in LFA-1–deficient mice (Hajishengallis *et al.*, 2011; Eskan *et al.*, 2012). In line with the recent identification of Del-1 as an antagonist of LFA-1–dependent leukocyte adhesion (Hajishengallis and Chavakis, 2013) (Fig. 3), Del-1–deficient mice spontaneously exhibit excessive neutrophil recruitment to the gingiva, leading to destructive inflammation and alveolar bone loss (Eskan *et al.*, 2012). Therefore, Del-1 appears to act as a gatekeeper for homeostatic recruitment of neutrophils to the periodontium. Consistent with this notion, inflamed human gingiva express lower levels of Del-1 than do healthy gingiva (Eskan *et al.*, 2012). Whether loss-of-function or hypofunctional polymorphisms in the gene encoding Del-1 (*EDIL3*) exist and can account for increased susceptibility to periodontitis is yet to be investigated. Intriguingly, wild-type mice develop age-associated Del-1 deficiency, which correlates with heavy neutrophil infiltration and alveolar bone loss in old age, although both features are suppressed by local treatment with Del-1 (Eskan *et al.*, 2012). Following induction of experimental gingivitis, the elderly exhibit increased recruitment of inflammatory cells to the gingiva as compared with young individuals, despite comparable dental plaque accumulation in the 2 age groups (Fransson *et al.*, 1996). Although it is uncertain whether this heightened inflammatory state in old individuals is related to age-associated decline in periodontal Del-1 expression, as shown in mice (Eskan *et al.*, 2012), it is nevertheless a testable hypothesis.

Excessive Neutrophil Activation

Because of their rich and potentially harmful assortment of antimicrobial and pro-inflammatory mechanisms, the activation of neutrophils at inflammatory sites should be tightly regulated to prevent unwarranted tissue damage. However, neutrophils from individuals with a specific polymorphism (131H/H) in the Fcγ receptor IIa (which mediates neutrophil activation and phagocytosis) exhibit a hyper-responsive phenotype, as compared with neutrophils from individuals with the more common (131R/R) Fcγ receptor IIa genotype (Nicu *et al.*, 2007). Specifically, upon stimulation, the 131H/H neutrophils express higher levels of degranulation markers and release more elastase than do the 131R/R neutrophils, although no significant differences were observed regarding their oxidative burst. Importantly, periodontitis patients with the 131H/H genotype have deeper periodontal pockets and more bone loss than those with the 131H/R or 131R/R genotype (Wolf *et al.*, 2006; Nicu *et al.*, 2007). It should be noted, however, that longitudinal analysis failed to demonstrate an association between these polymorphisms and response to conventional periodontal therapy (Wolf *et al.*, 2006). In general, whereas gene polymorphisms can play a contributory role in susceptibility or resistance to periodontitis, their effects may not always be readily detectable, given the multifactorial etiology of periodontitis and the redundancy and compensatory mechanisms of the immune system.

Peripheral neutrophil hyper-responsiveness associated with excessive production of reactive oxygen species has been observed in chronic periodontitis. Neutrophils from such patients exhibit a hyper-responsive phenotype, even in the absence of exogenous stimulation (Matthews *et al.*, 2007). It is thought that the recruitment of hyper-responsive neutrophils to the periodontium could contribute to periodontitis by causing oxidative tissue damage (Chapple and Matthews, 2007).

Localized aggressive periodontitis (LAP), a rapidly advancing form of periodontitis that primarily affects young patients, has been traditionally associated with hypofunctional neutrophils (Kantarci *et al.*, 2003; Ryder, 2010). However, more recent evidence suggests a rather hyperfunctional phenotype for LAP neutrophils, which display elevated secretion of inflammatory mediators and oxidative stress, combined with chemotactic and phagocytic abnormalities (Kantarci *et al.*, 2003; Ryder, 2010). It is therefore thought that periodontal tissue destruction in LAP could result, at least in part, from an inherent incapacity of LAP neutrophils to restrain their immune and inflammatory responses, while failing to control the periodontal microbial challenge.

Defective Neutrophil Clearance

Although hyperactive neutrophils may contribute to the pathogenesis of periodontitis (Chapple and Matthews, 2007), even normally activated neutrophils could cause unwarranted collateral tissue damage if not cleared properly when they become apoptotic. In this context, the resolution of inflammation, which includes clearance of apoptotic cells and cellular debris, is essential to homeostasis and tissue repair (Serhan *et al.*, 2008; Ricklin *et al.*, 2010). The inappropriate persistence of neutrophils due to defective clearance mechanisms could lead to their necrosis and the release of toxic contents. The notion that periodontal tissue

damage could result from eventual necrosis of non-cleared apoptotic neutrophils is consistent with findings that neutrophil necrosis predominates over apoptosis in the diseased periodontium (Crawford *et al.*, 2000). In principle, therefore, this mechanism could contribute to the transition from gingivitis to periodontitis or the aggravation of existing periodontitis.

Inefficient removal and accumulation of apoptotic cells and cellular debris have been associated with autoimmune inflammatory disorders such as systemic lupus erythematosus, attributed to deficiencies in complement components involved in apoptotic cell removal (*e.g.*, C1q) (Ricklin *et al.*, 2010). The importance of defective apoptotic cell clearance in periodontal pathogenesis is currently uncertain, although such a mechanism may operate in periodontitis patients with co-morbid conditions associated with impaired apoptotic cell clearance (*e.g.*, lupus) (Fig. 1). Interestingly, periodontitis and systemic lupus erythematosus are thought to share common risk factors associated with phagocytic receptor polymorphisms (Kobayashi *et al.*, 2007). Independently of genetic deficiencies predisposing to ineffective apoptotic cell removal, aging could be associated with defective clearance of apoptotic neutrophils (Aprahamian *et al.*, 2008) (Fig. 1), perhaps due to reduced expression of apoptotic cell uptake receptors in macrophages (Devitt and Marshall, 2011). Defective clearance of apoptotic neutrophils is a plausible mechanism that could account, at least in part, for the association of old age with increased susceptibility to periodontitis (Hajishengallis, 2010).

CONCLUSIONS AND FUTURE DIRECTIONS

Neutrophil homeostasis is maintained by a fine balance among several functions, including granulopoiesis, retention *vs*. release of mature neutrophils from the bone marrow, trafficking and transmigration, and clearance of apoptotic neutrophils (Figs. 1-3). Neutrophil defects affecting these functions can all potentially lead to severe periodontitis in human patients and related animal models. Many of these defects are congenital and can initiate periodontitis early in life, often leading to premature loss of primary and permanent teeth, with adverse psychological and functional consequences. In contrast to the syndromes and disorders discussed (Fig. 1), patients with chronic granulomatous disease are not susceptible to periodontitis, although their neutrophils have defective bactericidal activity, and they suffer from recurrent or persistent infections (*e.g.*, pneumonia and abscesses of the skin) (Nussbaum and Shapira, 2011). Combined with the evidence discussed above, that neutrophils are associated with important regulatory mechanisms, this suggests that the increased susceptibility to periodontitis of individuals with congenital neutrophil dysfunctions (Fig. 1) may not be explained adequately by defective immune control of the periodontal bacteria, especially since these conditions are generally refractory to standard periodontal therapy and antibiotics. Moreover, systems-biology-level approaches investigating the transcriptome, proteome, and metabolome of neutrophils have started providing a more comprehensive understanding of the role of neutrophils beyond pathogen phagocytosis and killing, including host response regulation and resolution of inflammation (Kobayashi and DeLeo, 2009). We thus suggest that neutrophil-associated diseases may not necessarily or exclusively be related to defective killing function by neutrophils but could, alternatively or additionally, involve breakdown of neutrophil-associated homeostatic mechanisms. Future research may uncover hitherto-unknown local regulatory defects forming a causal link between congenital neutrophil disorders and early-onset forms of periodontitis. A better molecular understanding of periodontitis-associated neutrophil dysfunctions, owing to congenital or acquired causes, could be exploited for targeted therapeutic interventions in affected individuals.

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