Epub ahead of print December 29, 2014 - doi:10.1189/jlb.3VMR1014-468R

JLB

Review

Neutrophil homeostasis and inflammation: novel paradigms from studying periodontitis

George Hajishengallis,[,1] Triantafyllos Chavakis,[†] Evlambia Hajishengallis,[‡] and John D. Lambris[§]

Departments of *Microbiology and [‡]Preventive and Restorative Sciences, Division of Pediatric Dentistry, Penn Dental Medicine, and [§]Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA; and [†]Technische Universität Dresden, Faculty of Medicine, Department of Clinical Pathobiochemistry and Institute for Clinical Chemistry and Laboratory Medicine, Dresden, Germany

RECEIVED OCTOBER 6, 2014; REVISED NOVEMBER 8, 2014; ACCEPTED NOVEMBER 28, 2014. DOI: 10.1189/jlb.3VMR1014-468R

ABSTRACT

Once viewed as simply antibacterial effector cells packed with antimicrobials, neutrophils are now increasingly appreciated for their regulatory roles in immunity and inflammation. The homeostatic regulation of neutrophils is thus crucial for optimal operation of the immune system. An attractive model to understand mechanistically the role of neutrophils is periodontitis, an oral inflammatory disease that is particularly sensitive to neutrophil alterations in numbers or function. The recruitment and proper activation of neutrophils are largely dependent on leukocyte integrins and complement. This review discusses how these processes are affected by host genetic or microbial factors leading to the development of periodontitis. For instance, both hypo- and hyper-recruitment of neutrophils as a result of deficiencies in the expression of β_2 integrins or their negative regulators, respectively, causes unwarranted IL-17-dependent inflammatory bone loss. Moreover, microbial hijacking of C5aR (CD88) signaling in neutrophils impairs their antimicrobial function while promoting destructive inflammatory responses. These studies not only support the concept that neutrophil homeostasis is key to periodontal health but also reveal promising, new therapeutic targets as discussed in the review. *J. Leukoc. Biol.* 98: 000–000; 2015.

Introduction

Historically, neutrophils have been viewed in the context of an acute inflammatory response and considered as short lived antibacterial effector cells that do not recirculate. However, recent and emerging evidence suggests that neutrophils are quite versatile and perform previously unsuspected functions, including reverse transmigration and the ability to cross-talk with and regulate other innate and adaptive immune leukocytes [1–4]. Moreover, the circulatory half-life of neutrophils is now thought to be longer than previously estimated (several days as opposed to hours) [5]. Besides their antimicrobial and cytotoxic mechanisms (release of reactive oxygen species; antimicrobial peptides, such as α-defensins and cathelicidin; proteases, such as elastase, cathepsin G, or matrix metalloproteinases; and neutrophil extracellular traps), neutrophils display a remarkable, de novo biosynthetic capacity for C-X-C and C-C chemokines, cytokines with proinflammatory, anti-inflammatory, or immunoregulatory properties as well as angiogenic and fibrogenic factors [1].

The triggering of complement via distinct cascade mechanisms (classic, lectin, or alternative, all of which converge at C3, the 3rd component of the system) leads to the generation of effector molecules that contribute to neutrophil activation. For instance, C3b is important for microbial opsonization and complement receptor 1-dependent phagocytosis by neutrophils, whereas C5a mediates neutrophil recruitment and activation by acting on C5aR (CD88) [6]. Complement synergizes with TLRs in the activation of neutrophils and innate immunity in general, although pathogens can subvert such cross-talk interactions to their advantage [7]. Interestingly, complement and leukocytes can reciprocally activate each other. In this regard, neutrophils and macrophages can generate C5a from C5, although macrophages exhibit more selective proteolytic activity (involving an inducible serine protease) and thus, generate higher levels of biologically active C5a for C5aR activation [8]. The source of C5 is not necessarily plasma derived (i.e., from inflammatory exudates), as leukocytes and especially macrophages constitute a source of local C5 production [9].

It is now well established that neutrophils are not solely involved in acute infections and inflammation but are also implicated in chronic inflammatory disorders or aging-related diseases, such as atherosclerosis, psoriasis, rheumatoid arthritis, inflammatory bowel disease, diabetes, and cancer [1, 10–13]. This review discusses recent mechanistic insights into new and emerging roles for neutrophils and particularly, those pertinent to their integrins and complement receptors in the pathogenesis of periodontal disease. This is a biofilm-induced inflammatory

Abbreviations: C5aR = complement 5aR, Del-1 = developmental endothelial locus 1, EAE = experimental autoimmune encephalomyelitis, LAD = leukocyte adhesion deficiency, Mac-1 = macrophage adhesion ligand 1, Mal = MyD88 adaptor-like, Rac2 = Ras-related C3 botulinum toxin substrate 2, RANKL = receptor activator of NF-κB ligand, Smurf1 = SMAD-specific E3 ubiquitin-protein ligase 1, WT = wild-type

1. Correspondence: University of Pennsylvania, Penn Dental Medicine, 240 South 40th St., Philadelphia, PA 19104-6030, USA. E-mail: geoh@upenn.edu

Copyright 2014 by The Society for Leukocyte Biology.

condition that leads to the destruction of the tissues that surround and support the teeth (periodontal ligament, gingiva, and alveolar bone, collectively known as the periodontium) [14]. The evidence discussed below shows that despite their potentially protective role, neutrophils are implicated in the initiation and progression of periodontitis when their function is subverted by periodontal bacteria or altered as a result of host immunodeficiencies or immunoregulatory defects. The literature discussed also indicates that periodontitis represents an attractive model to study the impact and mechanistic basis of disrupted neutrophil homeostasis on host health.

NEUTROPHIL HOMEOSTASIS

To understand better the role of neutrophils in periodontal disease, it is instructive to outline first basic concepts of neutrophil homeostasis. Neutrophils are produced in great numbers in the bone marrow ($\sim 10^9$ cells/kg bodyweight per day) from where they are released into the circulation [15, 16]. Central to their function is their ability to be recruited swiftly to sites of infection or tissue injury through the so-called leukocyte adhesion cascade, which is one of the major paradigms in immunity [17–19]. To prevent unwarranted neutrophil infiltration of peripheral tissues, the extravasation process is tightly regulated and entails a cascade of low- and high-affinity adhesive interactions between the neutrophils (or other leukocytes) and the endothelium lining the blood vessels of the infected or inflamed tissue [17, 18]. The initial step involves transient rolling interactions, mediated by the binding of endothelial receptors (P- or E-selectin) to their glycoprotein ligands on neutrophils. This selectin-dependent leukocyte rolling, is followed by chemokine-induced leukocyte activation and slow rolling, mediated by interactions of selectins and integrins with their respective ligands [17, 18]. This slow rolling-dependent deceleration of neutrophils leads to their firm adhesion and subsequent crawling on the endothelium, activities mediated primarily by β_2 integrins. These are heterodimeric molecules, each with a distinct CD11 subunit and a common CD18 subunit that interact with endothelial counter-receptors, such as the ICAM-1 and -2. The LFA-1 integrin (CD11a/CD18) plays a crucial role in firm adhesion, whereas the Mac-1 integrin (CD11b/CD18) mediates crawling, an activity during which neutrophils seek an appropriate site for transmigration [20]. Firm adhesion and intraluminal crawling are critical for subsequent transmigration of neutrophils to peripheral tissues [4, 18, 21]. Intriguingly, transmigrated neutrophils can potentially re-enter the circulation through reverse transmigration (movement in an abluminal-to-luminal direction), a process that is thought to contribute to the dissemination of systemic inflammation [2, 22].

The various steps of neutrophil extravasation can be modulated by cytokines or chemokines. Specifically, tissue-derived cytokines (e.g., TNF) can up-regulate endothelial adhesion molecule expression, whereas tissue-derived chemokines, decorating the apical endothelial cell surface (e.g., IL-8 and other CXCR2 ligands), can induce the high-affinity binding conformation of leukocyte integrins [10, 17]. Once in the tissue, neutrophils follow chemotactic gradients existing in the inflamed (infected or injured) tissue. Such gradients often involve chemoattractants derived from local complement activation (e.g., C5a fragments) or bacterial components (e.g., fMLP) [10].

In contrast to the plethora of described adhesion molecules promoting the different steps of neutrophil extravasation, very little was known until recently about locally produced, negative regulators of this inflammatory process. Newly identified, endogenous inhibitors of the leukocyte adhesion cascade include Del-1, pentraxin 3, and growth-differentiation factor 15 [20]. Del-1 (also known as epidermal growth factor-like repeats and discoidin I-like domain 3) is an endothelial, cell-secreted, 52-kDa glycoprotein that acts as an antagonistic ligand of the LFA-1 and Mac-1 integrins [23, 24]. Indeed, unlike ICAM-1, which interacts with LFA-1 to promote neutrophil extravasation, Del-1 suppresses LFA-1-dependent endothelial adhesion of neutrophils [23] (**Fig. 1**). Importantly, at equimolar amounts, Del-1 outcompetes ICAM-1 for binding to LFA-1 [23].

Because of their rich and potentially harmful assortment of antimicrobial and proinflammatory mechanisms, neutrophils are tightly regulated by several homeostatic mechanisms to prevent unwarranted tissue damage [26, 27]. Neutrophil homeostasis involves more than regulation of extravasation, also including tight control of granulopoiesis, release of mature neutrophils from the bone marrow into the circulation, and clearance of apoptotic or senescent neutrophils [26–28]. In this regard, a neutrophil rheostat ("neutrostat") mechanism was described, which, at least in mice, senses neutrophil recruitment and clearance in peripheral tissues and regulates granulopoiesis through a negative-feedback loop involving a cascade of cytokines, namely the IL-23–IL-17 G-CSF axis [29] (**Fig. 2**). Specifically, the phagocytosis of apoptotic neutrophils by tissue phagocytes triggers anti-inflammatory signals that limit their production of IL-23 [29], a key cytokine for induction of IL-17 by innate and adaptive immune cells [30]. The resulting inhibition of IL-17 production, in turn, leads to decreased production of G-CSF by cells, such as fibroblasts [29]. If neutrophils cannot transmigrate to peripheral tissues, as in leukocyte adhesion molecule-deficient mice, then the neutrostat regulatory circuit is disrupted (as a result of the lack of the regulatory signals associated with the presence of apoptotic neutrophils), thereby leading to unrestrained expression of IL-23 and downstream cytokines IL-17 and G-CSF. As G-CSF is a primary regulator of neutrophil production and release from the bone marrow, its overproduction in leukocyte adhesion molecule-deficient mice explains, at least in part, their increased granulopoiesis and blood neutrophilia [26, 27]. Stated in brief, the clearance of transmigrated apoptotic neutrophils is crucial for the regulation of neutrophil production in the bone marrow and thus, serves more than waste disposal.

NEUTROPHILS AND PERIODONTITIS

Neutrophils are an integral component of the periodontal host response and represent the overwhelming majority (≥95%) of the leukocytes recruited to the gingival crevice (space between the tooth surface and the free gingiva) in response to the tooth-associated biofilm [31–33]. Neutrophils exit the gingival plexus of blood vessels and enter the crevice via the gingival junctional

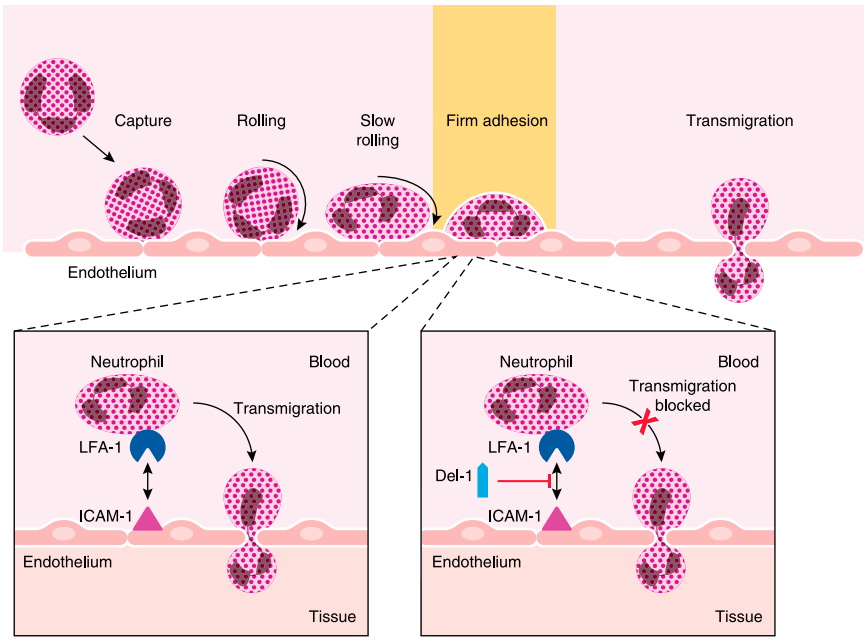

Figure 1. Del-1 antagonizes LFA-1 and regulates neutrophil extravasation. The neutrophil extravasation process is a cascade of low- and high-affinity adhesive interactions between the neutrophils and the vascular endothelium and involves distinct steps, including capturing, rolling, slow rolling, firm adhesion of activated neutrophils, and transmigration [10, 20]. Del-1 binds the LFA-1 integrin and antagonizes its interaction with ICAM-1, thereby blocking LFA-1-dependent leukocyte adhesion onto the vascular endothelium. As firm neutrophil adhesion to the endothelium is essential to subsequent transmigration, Del-1 restrains the migration of neutrophils from the circulation to peripheral tissues [23, 25].

epithelium, which under inflammatory conditions, is largely occupied by trafficking neutrophils [33]. In the gingival crevice or periodontal pocket (pathologically deepened crevice in the case of periodontitis), neutrophils form a "defense wall" against the tooth-associated biofilm, purportedly to prevent bacterial invasion into the underlying tissues [34].

Neutrophils are required for the maintenance of periodontal health, as revealed by the development of aggressive forms of periodontal disease in conditions associated with defects in the production and life cycle of neutrophils [32]. Many of these disorders are congenital and rare (defined as affecting ≤1 in 1250 individuals) and include the Chediak-Higashi syndrome; Papillon-Lefèvre syndrome; warts, hypogammaglubulinemia, infections, and myelokathexis syndrome; severe congenital neutropenia; and LAD (for reviews, see refs. [32, 35]). Being congenital, these conditions lead to periodontal bone loss early in life, thus affecting the primary and permanent dentition [32]. Rare diseases constitute an important medical and social issue that cumulatively affects 25 million patients in North America alone [36]. Moreover, rare diseases with a defined genetic defect constitute real-life models to understand human biology and (patho)physiologic mechanisms, thereby providing useful insights into common diseases [37]. In this context, the study of LAD, which is discussed in the next section, can offer insights

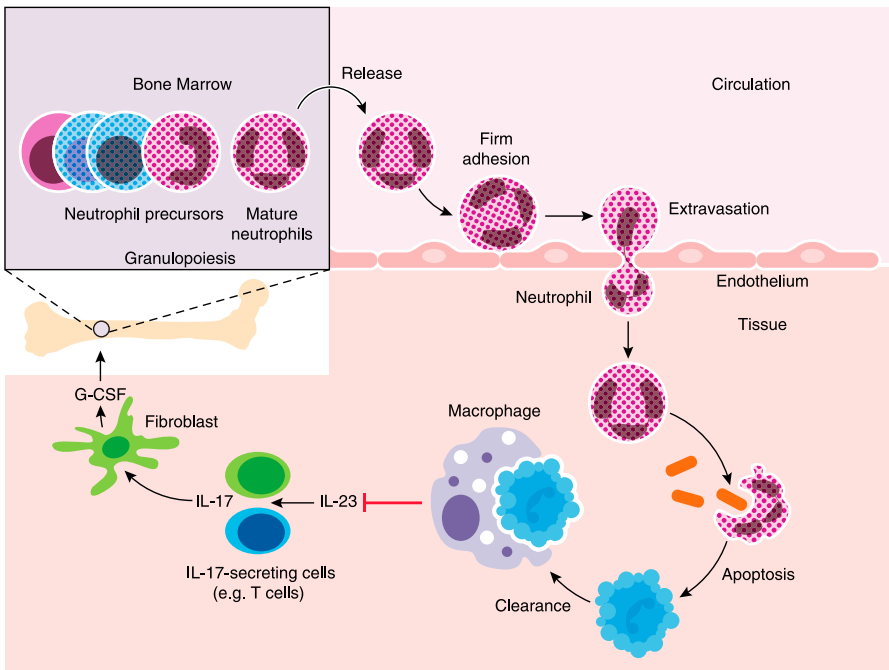

Figure 2. The neutrophil rheostat (neutrostat) mechanism. IL-17 promotes granulopoiesis and mobilization of mature neutrophils from the bone marrow by acting through up-regulation of G-CSF. The neutrostat senses neutrophil recruitment and clearance in peripheral tissues and regulates neutrophil production through a negative-feedback loop involving the IL-23–IL-17–G-CSF cytokine cascade. Specifically, after their release from the bone marrow, circulating neutrophils can normally extravasate to tissues in response to inflammation or infection. Upon senescence, neutrophils become apoptotic and are phagocytosed by tissue phagocytes, leading to suppression of their IL-23 production, in turn, down-regulating the IL-17–G-CSF axis for maintaining steady-state neutrophil counts [29].

into neutrophil biology and function, as well as into mechanisms that govern host-microbe homeostasis in the periodontal tissue.

The development of aggressive periodontitis associated with congenital deficiencies in neutrophil numbers or function directly supports the importance of neutrophils for maintaining a healthy periodontium; however, the presence of neutrophils is not necessarily protective. Indeed, there is adequate evidence that a significant portion of the inflammatory destruction of the periodontium occurs as a result of collateral damage by hyperactive neutrophils or neutrophils present in excessive numbers [25, 34, 38–44]. In a related context, the local counts of neutrophils in the periodontal tissues correlate positively with periodontal disease severity [45]. As the expanding roles of neutrophils also include regulatory activities [1, 46, 47], it is possible that neutrophils can contribute to the pathogenesis of periodontitis, not only by overexuberant activity but also by failing to perform regulatory functions.

Although neutrophils constitute the great majority of leukocytes recruited to the gingival crevice or periodontal pockets, the inflammatory infiltrate, in the underlying connective tissue of established periodontal lesions, is dominated by lymphocytes (T, B, and plasma cells occupying ~40% of the inflamed periodontal tissue volume) [33]. It is possible that the chronic recruitment of neutrophils exacerbates the formation of the lymphocyte-rich periodontal lesion that characterizes advanced periodontitis. In this regard, in other disease settings, neutrophils were shown to recruit pathogenic, IL-17-producing $CD4^+$ Th17 cells to sites of chronic inflammation, to enhance plasma cell survival via a proliferation-inducing ligand secretion, and to engage in cross-talk with other leukocytes (dendritic cells, NK cells, B cells, and both $CD4^+$ and $CD8^+$ T cells) for reciprocal activation and modulation of lifespan [1, 48]. A mechanism by which neutrophils can recruit Th17 cells that are implicated in autoimmune and inflammatory conditions, including periodontitis [49–51], involves neutrophil production of CCL2 and CCL20 chemokines. CCL2 and CCL20 are ligands for CCR2 and CCR6 chemokine receptors, respectively, that are characteristically expressed by Th17 cells, which are thereby attracted to sites of neutrophil accumulation. Th17 cells, in turn, reciprocally attract more neutrophils via IL-17 production [52] (**Fig. 3**).

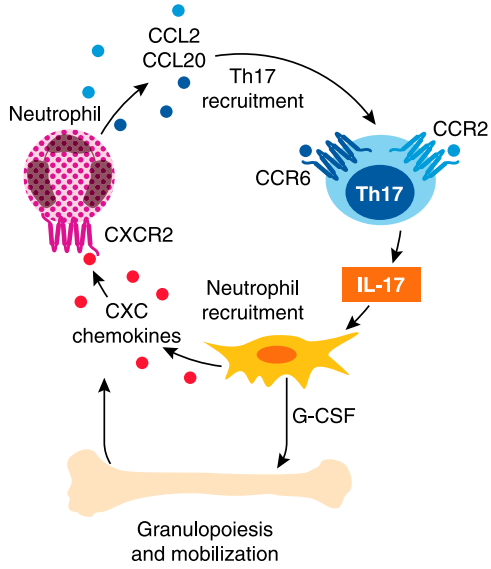

Figure 3. Cross-talk and reciprocal recruitment of neutrophils and Th17 cells. Neutrophils produce CCL2 and CCL20 chemokines, which can selectively recruit Th17 cells by acting on Th17-expressed CCR2 and CCR6. Th17 cells secrete IL-17, which by acting mainly through fibroblast up-regulation of G-CSF and CXC chemokines, can orchestrate bone marrow production and release of neutrophils and their chemotactic recruitment to inflammatory sites [**52**].

INFLAMMATION AS A RESULT OF IMPAIRED NEUTROPHIL RECRUITMENT

LAD represents a group of inherited disorders that all inhibit the normal extravasation of circulating neutrophils and their recruitment to sites of infection or inflammation [53, 54]. The genetic defects involve impaired expression or function of β_2 integrins or other adhesion molecules, thereby impeding neutrophil adherence to vascular endothelial cells. LAD-I involves β_2 integrin deficiency, LAD-II is caused by defective glycosylation of selectin ligands, and LAD-III entails dysfunction of signaling intermediates required for integrin activation. The most common LAD type is LAD-I, an autosomal, recessive immunodeficiency caused by mutations in the CD18-encoding the integrin β_2 gene [53, 54]. Only few, if any, neutrophils can be found in extravascular sites in LAD-I patients, who exhibit increased blood neutrophil counts (neutrophilia), suffer from frequent infections at mucosal or skin surfaces, and experience early-onset aggressive periodontitis [35, 47, 54, 55].

Periodontitis associated with LAD-I has been historically attributed to lack of neutrophil surveillance of the periodontal infection [35, 55–60]. However, a recent study in LAD-I patients and relevant animal models has shown that the fundamental cause of this form of periodontitis involves dysregulated overproduction of IL-23 and IL-17 [47]. IL-17 is a proinflammatory and pro-osteoclastogenic cytokine involved in the pathogenesis of inflammatory bone loss in humans and animal models of arthritis [61, 62]. In LAD-I patients and mice with defective neutrophil recruitment to the periodontal tissue (LFA-1-deficient mice), infiltrating T cells constitute the main source of IL-17 [47]. In mice, these cells represent $CD3^+$ $\gamma\delta$ T cells, which in the presence of IL-23, become a major cellular source of IL-17 in periodontal or other mucosal tissues [25, 63]. In LAD-I patients, the IL-17-producing T cells were identified as $CD3^+CD8^-CD56^-TCR\gamma\delta^-$ cells and most likely represent $CD4^+$ (Th17) cells [47].

The overexpression of IL-17 mRNA and protein in the periodontal tissue of LAD-I patients (as well as of IL-23 and G-CSF, which were also up-regulated in these patients) [47] is consistent with the breakdown of the neutrostat mechanism, discussed above (Fig. 2). According to the neutrostat model, as established in mice, the engulfment of apoptotic neutrophils by tissue phagocytes triggers anti-inflammatory signals that suppress the production of IL-23, a key cytokine for the induction of IL-17, in turn, a stimulus for G-CSF production [26, 29]. The IL-23–IL-17–G-CSF axis becomes dysregulated also in LAD-I patients [47] (**Fig. 4**). Whereas the elevated G-CSF could explain the increased granulopoiesis and blood neutrophilia in LAD-I patients, the

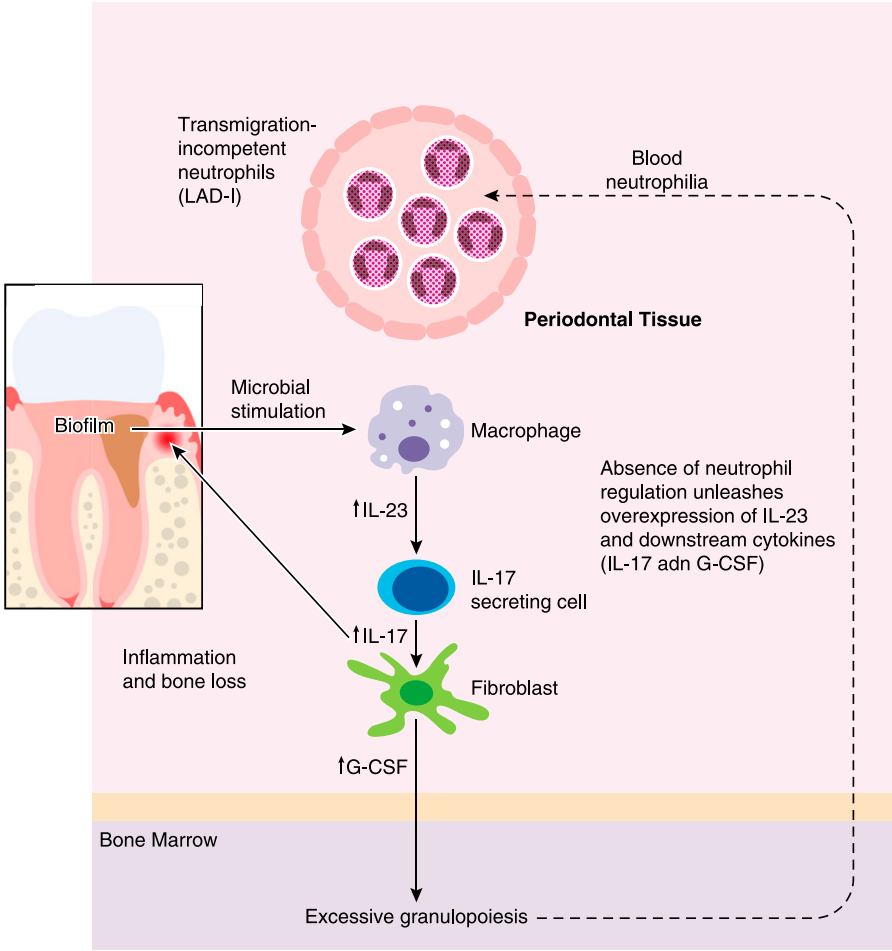

Figure 4. Disruption of the neutrostat in LAD-I causes IL-17-dependent inflammatory bone loss. In LAD-I, β_2 integrin-deficient neutrophils fail to extravasate, thereby disrupting the neutrostat circuit. The absence of recruited neutrophils to the periodontal tissue of LAD-I patients leads to unrestrained local production of IL-23 and hence, IL-17 and G-CSF. Whereas increased G-CSF leads to excessive granulopoiesis and blood neutrophilia, elevated IL-17 leads to inflammatory bone loss [47].

LAD-I-associated overproduction of IL-17 had not been associated previously with any of the disease manifestations of the LAD-I syndrome or even in related animal models. Not only were IL-23 and IL-17 associated recently with LAD-I periodontitis in humans and relevant mouse models, but furthermore, these cytokines were causally linked to inflammatory bone loss in LFA-1-deficient mice [47]. Specifically, LFA-1-deficient mice, treated locally in the gingiva with anti-IL-17A or anti-IL-23p19 antibody (from the age of 4 to 18 weeks, by which time dramatic bone loss occurs), were protected from bone loss, whereas untreated or control antibody-treated, LFA-1-deficient mice experienced progressive disease [47].

LAD-I patients and LFA-1-deficient mice display higher periodontal bacterial load than their respective healthy controls. Intriguingly, however, antibody-mediated neutralization of IL-17 or IL-23 in LFA-1-deficient mice diminishes their total periodontal bacterial counts to normal levels, i.e., similar to those of WT, healthy mice [47]. These findings suggest that the high bacterial burden is driven by IL-23/IL-17-dependent inflammation rather than by lack of neutrophil surveillance of the periodontal infection. In this regard, inflammation generates tissue-breakdown products, such as degraded collagen peptides and heme-containing compounds, which promote the growth of periodontitis-associated bacteria [64]. Therefore, control of inflammation appears to limit bacterial growth. The fact that LAD-I periodontitis is recalcitrant to antibiotic treatment and/or mechanical removal of the tooth-associated biofilm [35, 57, 65] is also consistent with the notion that the disease cannot be explained adequately as a failure of immune surveillance of the infection. Moreover, in contrast to LAD-I patients, individuals with chronic granulomatous disease do not have increased susceptibility to periodontitis (compared with the general population), even though the defective oxygen-dependent bactericidal activity of their neutrophils renders them susceptible to frequent infections, including pneumonia and abscesses of the skin [31]. Taken together, it could be argued that the extravasation competence of neutrophils is essential for periodontal tissue homeostasis. On the other hand, impaired control of the periodontal infection by neutrophils may not be a dominant factor in susceptibility to periodontitis, being at best a contributing factor. This concept does not imply that the tooth-associated microbiota is not involved in the pathogenic process. The ability of periodontal bacteria to stimulate IL-23 expression in macrophages [66] suggests that the bacteria might act as stimuli for IL-23 induction by macrophages in the periodontium, thereby unleashing the disinhibited IL-23–IL-17 axis. In this regard, the bacteria do not have to invade the periodontal tissue to stimulate inflammatory cells, as their released bacterial products (e.g., LPS) can readily penetrate through the highly porous gingival junctional epithelium [67]. In this context,

microbiological analysis of gingival tissue samples from LAD-I patients did not reveal any unusual tissue-invasive infection within the lesion driving tissue destruction [47].

Similar to LFA-1-deficient mice, mice with combined P- and E-selectin deficiency (a model resembling LAD-II) were shown to harbor increased periodontal bacterial load and to experience severe bone loss early in life [68]. Although the periodontal IL-17 response in P/E-deficient mice was not analyzed [68], these double-knockout mice do display elevated IL-17 mRNA expression in several other peripheral tissues examined, such as spleen, lung, and jejunum compared with WT mice [29]. Mice deficient in the chemokine receptor CXCR2, which also fails to recruit neutrophils to the periodontal tissue [69], similarly exhibit dysregulated IL-23 and IL-17 responses and susceptibility to periodontitis, similar to LFA-1-deficient mice [47]. A possible fourth type of LAD (LAD-IV) was proposed, which involves impaired neutrophil chemotaxis and margination, owing to mutations affecting the Rac2 GTPase [70]. Intriguingly, Rac2-deficient mice are significantly more susceptible to experimental periodontitis compared with WT controls, although the periodontal IL-17 response was not determined [71]. Nevertheless, consistent with being a LAD condition, Rac2-deficient mice have only a few neutrophils in the junctional epithelium and gingival connective tissue but display a rich mononuclear cellular infiltrate (monocytic cells and lymphocytes) in the same regions [71].

These recent advances suggest that neutralization of IL-17 (or IL-23) could effectively serve as an adjunctive therapy for aggressive forms of periodontitis associated with LAD-I and perhaps other types of LAD or conditions associated with poor or no accumulation of neutrophils in extravascular sites, owing to defective chemotaxis (e.g., Chediak-Higashi syndrome) or neutropenic states (e.g., congenital agranulocytosis) [72].

In summary, antibody-mediated neutralization of IL-17 in a mouse model of LAD-I inhibits inflammatory bone loss and reduces the bacterial load, despite the absence of the presumed, protective effects of neutrophils in the periodontium. Moreover, the inflammatory tissue destruction in LAD-I periodontitis is the consequence of the absence of neutrophils, as opposed to the usual bystander injury dogma that applies to many neutrophil-associated inflammatory diseases [1, 10–12], including the chronic form of periodontitis. These recent developments [47] also provide an explanation for the long-standing puzzle of why individuals with chronic granulomatous disease, who are particularly susceptible to infections elsewhere in the body, are not susceptible to periodontal disease. In these patients, the neutrophils are transmigration competent and thus, should normally regulate the activity of the IL-23–IL-17–G-CSF axis, despite their defective killing mechanisms.

INFLAMMATION AS A RESULT OF UNRESTRAINED NEUTROPHIL RECRUITMENT

As discussed above, Del-1 is an LFA-1 integrin antagonist that restrains neutrophil extravasation [23] (Fig. 1). Importantly, Del-1 was shown to serve as a mechanism by which the periodontium self-regulates local inflammation by preventing unwarranted neutrophil infiltration [25]. In this regard, Del-1-deficient mice display excessive LFA-1-dependent neutrophil infiltration and spontaneously develop periodontitis [25]. The associated inflammatory response is characterized by elevated expression of IL-17, which was shown to be responsible for periodontitis in this model. Indeed, treatment with anti-IL-17 antibody blocks inflammation and bone loss, and moreover, mice with combined deficiency in Del-1 and IL-17R are protected from the disease [25]. Although $\gamma\delta$ T cells are the major cellular source of IL-17 in the periodontium of Del-1-deficient mice, important secondary sources involve Th17 cells as well as recruited neutrophils [25]. The latter finding is consistent with a subsequent study that identified a population of mouse and human neutrophils that expresses the transcription factor retinoic acid-related orphan receptor γt and produces IL-17 [73].

Detailed analysis of in vitro and in vivo experiments, including bone marrow chimeras, has shown that Del-1 and IL-17 are reciprocally cross-regulated in the periodontium. Whereas Del-1 suppresses LFA-1-dependent neutrophil recruitment and IL-17 production, IL-17 inhibits Del-1 expression in endothelial cells and thereby, promotes neutrophil recruitment [25]. The down-regulation of this endogenous inhibitor of neutrophil transmigration may be a novel mechanism by which IL-17 facilitates neutrophil recruitment to inflamed or infected tissues. Although IL-17-mediated down-regulation of Del-1 may be beneficial in the acute response against pathogen infection, chronic IL-17 signaling can lead to persistent recruitment of neutrophils, thereby contributing to chronic inflammatory conditions. An inverse expression of Del-1 and IL-17 was also established in human gingival biopsy samples: Del-1 mRNA expression dominates in healthy gingiva, and IL-17 mRNA expression dominates in inflamed gingiva [25]. Intriguingly, the reciprocal regulatory loop between IL-17 and Del-1 also operates in the CNS, where Del-1 acts as a major homeostatic factor by preventing excessive neutrophil infiltration and IL-17-dependent neuroinflammation [74]. Specifically, whereas diminished expression of Del-1 is associated with human multiple sclerosis, and genetic deficiency of Del-1 exacerbates EAE (the rodent counterpart of multiple sclerosis), systemic administration of Del-1 inhibits clinical relapse in relapsing-remitting EAE [74].

Interestingly, at least in mice, the expression of Del-1 significantly declines as a function of age, in contrast to the expression of adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin, which are not affected significantly [25, 75]. Age-associated Del-1 deficiency in WT mice correlates with increased neutrophil infiltration, IL-17 expression, and bone loss, although these pathologic features are suppressed by local treatment with Del-1 [25]. The enhanced bone loss seen in Del-1 deficiency could be explained by several mechanisms: the elevated levels of IL-17, as a result of Del-1 deficiency, are likely to contribute to inflammatory periodontal bone loss by stimulating osteoblast expression of RANKL, a major osteoclastogenesis factor [76]. IL-17 is also thought to have direct stimulatory effects on osteoclastogenesis [77]. Moreover, neutrophils (the numbers of which are elevated in Del-1 deficiency) release tissue-degrading enzymes, such as collagenase, which is involved in the initiation of bone resorption [42–44, 78]. Moreover, TLR-activated

neutrophils express membrane-bound RANKL and can stimulate osteoclastogenesis [79].

Taken together, the studies in Del-1-deficient mice indicate that the unrestrained recruitment of neutrophils to the periodontium is as problematic as the impaired neutrophil recruitment in LFA-1-deficient mice [25, 80]. Interestingly, unrestrained and conversely, diminished neutrophil recruitment to the periodontium causes IL-17-dependent inflammatory bone loss, although the mechanisms of IL-17 production are completely different.

COMPLEMENT-DEPENDENT MICROBIAL SUBVERSION OF NEUTROPHILS

As alluded to above, patients with chronic or adult-type periodontitis have a greater number and longer-lived neutrophils in the oral tissues compared with healthy individuals [40]. The chronic recruitment and sustained presence of neutrophils in the periodontal pockets of patients are probably results of their inability to control the subgingival biofilm, which thus remains a constant stimulus for their recruitment. As crevicular neutrophils retain their viability and capacity to elicit immune responses [14, 34], the persistence of uncontrolled biofilms could be attributed, at least in part, to the capacity of the bacteria to subvert neutrophil functions in ways that inhibit immune-mediated killing, while promoting the nutritionally favorable inflammatory response (see above) [81].

A recent study has dissected a molecular strategy by which *Porphyromonas gingivalis*, a keystone pathogen in periodontitis [21, 80], can disengage bacterial clearance from inflammation and thereby, contribute to the persistence of microbial communities that drive periodontitis [82]. This subversive strategy depends on the capacity of *P. gingivalis* to instigate a cross-talk between TLR2 and C5aR in neutrophils (**Fig. 5**). TLR2 is critical for *P. gingivalis* recognition by neutrophils in vivo [83], whereas C5aR is under the control of *P. gingivalis* Arg-specific gingipains—C5 convertase-like enzymes that can locally generate high concentrations of the C5a ligand independently of complement activation [84, 85]. Although the TLR2 signaling adaptor MyD88 can potentially mediate the clearance of *P. gingivalis* [86], this activity is counteracted upon the activation of the C5aR–TLR2 cross-talk that induces proteasomal degradation of MyD88 [82]. Analyses in mouse and human neutrophils revealed that the MyD88 proteasomal degradation is mediated through ubiquitination by the E3 ligase Smurf1; conversely, antagonistic inhibition of C5aR signaling abrogates MyD88 ubiquitination and degradation and promotes bacterial killing.

As the MyD88 pathway is not only an antimicrobial but also a proinflammatory pathway, this begged the question as to how *P. gingivalis* could rescue the favorable (for the bacteria) inflammatory response in neutrophils. In this context, *P. gingivalis* was shown to activate an alternative pathway involving the TLR2 adaptor Mal and PI3K; Fig. 5). The Mal-PI3K signaling pathway mediates robust inflammatory responses, as genetic or pharmacological ablation of Mal or PI3K blocks the production of proinflammatory cytokines, such as TNF, IL-1β, and IL-6. Moreover, the same pathway suppresses actin polymerization through the small GTPase RhoA and thereby, inhibits

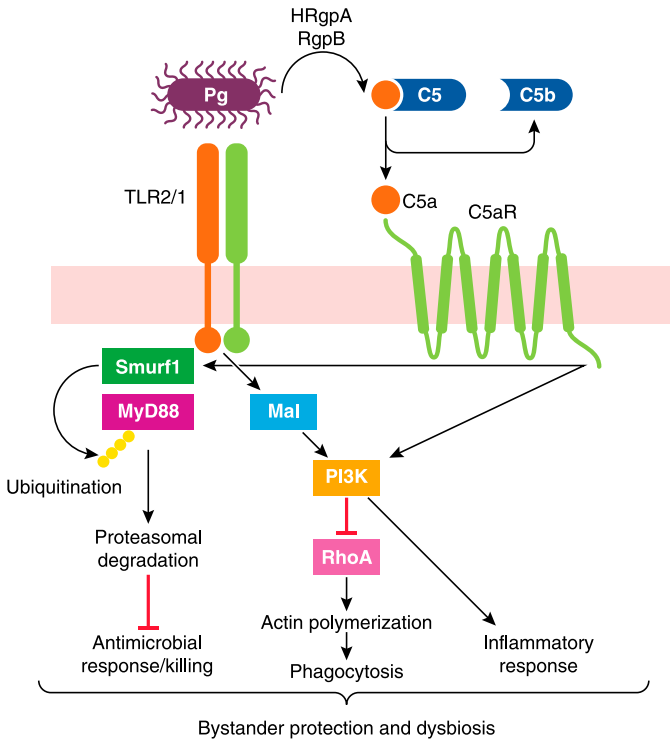

Figure 5. Microbial subversion of neutrophil function leads to periodontal inflammation. *P. gingivalis* can manipulate C5aR signaling in neutrophils by means of enzymes (HRgpA and RgpB gingipains) that have C5 convertase-like activity and generate high local concentrations of the C5a ligand. Therefore, the bacterium can coactivate C5aR and TLR2 in neutrophils, and the resulting cross-talk leads to E3 ubiquitin ligase Smurf1-dependent ubiquitination and proteasomal degradation of MyD88, thereby inhibiting a host-protective antimicrobial pathway. Moreover, the C5aR–TLR2 cross-talk activates PI3K, which prevents phagocytosis through inhibition of RhoA and actin polymerization, while promoting an inflammatory response. In contrast to MyD88, Mal is a component of the subversive pathway, acting upstream of PI3K. The integrated, subversive strategy provides "bystander" protection to otherwise susceptible bacterial species and promotes dysbiotic inflammation in vivo; from reference [82] with permission. HRgpA, high molecular mass arginine-specific gingipain A; Pg, *P. gingivalis*; P.RgpB, arginine-specific gingipain B.

phagocytosis. This subversive strategy of *P. gingivalis* moreover protects bystander bacteria that are otherwise susceptible to neutrophil killing [82]. Consistently, when the subversive C5aR–TLR2 cross-talk was inhibited in vivo in the context of *P. gingivalis*-induced periodontitis in mice, this led not only to the elimination of *P. gingivalis* from the periodontal tissue but also reversed the uncontrolled bacterial growth brought about earlier by *P. gingivalis* colonization, as well as inhibited periodontal inflammation. These subversive effects were abrogated also when a gingipain-deficient mutant of *P. gingivalis*, which fails to generate C5a and hence, to manipulate C5aR signaling, was used in lieu of the WT organism. Therefore, *P. gingivalis* manipulates neutrophils through 2 distinct mechanisms that together, ensure bacterial survival and the perpetuation of periodontal inflammation (Fig. 5). It should be noted, however, that the presence of *P. gingivalis* does not necessarily cause periodontitis. Indeed, periodontally healthy individuals may also harbor *P. gingivalis*, although with decreased frequency compared with periodontitis

patients [87, 88]. In this regard, there is considerable strain and virulence diversity within the population structure of *P. gingivalis*, and key virulence factors thereof (such as gingipains and lipid A phosphatases) are regulated by local environmental conditions that probably differ among individuals [89]. From a broader perspective, a susceptible host is required for the development of periodontal disease, as suggested by clinical cases of individuals who do not develop periodontitis, despite massive biofilm accumulation at dentogingival sites [90, 91].

CONCLUSIONS AND THERAPEUTIC OUTLOOK

The literature discussed above indicates that distinct forms of periodontitis are associated with defects in the recruitment or proper activation of neutrophils, in turn, highlighting the notion that neutrophil homeostasis is a sine qua non for periodontal health. Moreover, it should be noted that neutrophils can contribute to periodontitis also when affected by systemic conditions, such as diabetes mellitus. Diabetes increases the risk and severity of human periodontitis [92–94], and diabetic mice display higher inflammation than healthy mice to the same challenge with *P. gingivalis* [95, 96]. Although these findings could be attributed to a variety of diabetes-associated factors (e.g., increased formation of proinflammatory advanced glycation end products and defects in the resolution of inflammation), neutrophils are likely also involved, as diabetes causes defects in the chemotactic migration and immune responses of neutrophils [93, 94, 97–99].

Although representing diametrically opposed conditions, excessive and impaired neutrophil recruitment can lead to periodontal inflammation. Del-1 can be used therapeutically to block unwarranted neutrophil recruitment and destructive periodontal inflammation [25], and locally administered inhibitors of IL-17 or of IL-17R may be promising for the treatment of LAD-I periodontitis [47]. As IL-17 is also implicated in the pathogenesis of common, chronic forms of human periodontitis, these may also benefit from IL-17-based therapies [100–102]. Moreover, in chronic periodontitis, the pharmacological blockade of signaling pathways involved in neutrophil subversion by periodontal bacteria is likely to confer protection against the disease. This notion is supported by promising results in preclinical models of periodontitis by use of appropriate complement inhibitors [82, 103, 104]. Although Del-1 is still at the preclinical level, complement- and IL-17-targeted therapies were shown to be safe in human clinical trials and are currently considered for the treatment of other inflammatory diseases [105, 106].

ACKNOWLEDGMENTS

The authors are supported by grants from the U.S. National Institutes of Health DE015254, DE017138, and DE021685 (to G.H.) and AI068730, AI030040, EY020633, and GM094474 (to J.D.L.); from the European Commission (FP7-DIREKT 602699; to J.D.L. and T.C.); from the European Research Council (ENDHOMRET; to T.C.); and from the Deutsche Forschungsgemeinschaft (SFB-TRR 127 Project A3 and CH279/5-1 and CH279/6-2; to T.C.).

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

1. Scapini, P., Cassatella, M. A. (2014) Social networking of human neutrophils within the immune system. *Blood* **124,** 710–719.
2. Beyrau, M., Bodkin, J. V., Nourshargh, S. (2012) Neutrophil heterogeneity in health and disease: a revitalized avenue in inflammation and immunity. *Open Biol.* **2,** 120134.
3. Mócsai, A. (2013) Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J. Exp. Med.* **210,** 1283–1299.
4. Phillipson, M., Kubes, P. (2011) The neutrophil in vascular inflammation. *Nat. Med.* **17,** 1381–1390.
5. Tak, T., Tesselaar, K., Pillay, J., Borghans, J. A., Koenderman, L. (2013) What's your age again? Determination of human neutrophil half-lives revisited. *J. Leukoc. Biol.* **94,** 595–601.
6. Ricklin, D., Hajishengallis, G., Yang, K., Lambris, J. D. (2010) Complement: a key system for immune surveillance and homeostasis. *Nat. Immunol.* **11,** 785–797.
7. Hajishengallis, G., Lambris, J. D. (2011) Microbial manipulation of receptor crosstalk in innate immunity. *Nat. Rev. Immunol.* **11,** 187–200.
8. Huber-Lang, M., Younkin, E. M., Sarma, J. V., Riedemann, N., McGuire, S. R., Lu, K. T., Kunkel, R., Younger, J. G., Zetoune, F. S., Ward, P. A. (2002) Generation of C5a by phagocytic cells. *Am. J. Pathol.* **161,** 1849–1859.
9. Hetland, G., Johnson, E., Røyset, P., Eskeland, T. (1987) Human alveolar macrophages and monocytes generate the functional classical pathway of complement in vitro. *Acta Pathol. Microbiol. Immunol. Scand. [C]* **95,** 117–122.
10. Kolaczkowska, E., Kubes, P. (2013) Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* **13,** 159–175.
11. Mayadas, T. N., Cullere, X., Lowell, C. A. (2014) The multifaceted functions of neutrophils. *Annu. Rev. Pathol.* **9,** 181–218.
12. Fournier, B. M., Parkos, C. A. (2012) The role of neutrophils during intestinal inflammation. *Mucosal Immunol.* **5,** 354–366.
13. Tseng, C. W., Liu, G. Y. (2014) Expanding roles of neutrophils in aging hosts. *Curr. Opin. Immunol.* **29,** 43–48.
14. Hajishengallis, G. (2014) Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends Immunol.* **35,** 3–11.
15. Borregaard, N. (2010) Neutrophils, from marrow to microbes. *Immunity* **33,** 657–670.
16. Amulic, B., Cazalet, C., Hayes, G. L., Metzler, K. D., Zychlinsky, A. (2012) Neutrophil function: from mechanisms to disease. *Annu. Rev. Immunol.* **30,** 459–489.
17. Nourshargh, S., Hordijk, P. L., Sixt, M. (2010) Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat. Rev. Mol. Cell Biol.* **11,** 366–378.
18. Ley, K., Laudanna, C., Cybulsky, M. I., Nourshargh, S. (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat. Rev. Immunol.* **7,** 678–689.
19. Chavakis, E., Choi, E. Y., Chavakis, T. (2009) Novel aspects in the regulation of the leukocyte adhesion cascade. *Thromb. Haemost.* **102,** 191–197.
20. Hajishengallis, G., Chavakis, T. (2013) Endogenous modulators of inflammatory cell recruitment. *Trends Immunol.* **34,** 1–6.
21. Hajishengallis, G., Darveau, R. P., Curtis, M. A. (2012) The keystone-pathogen hypothesis. *Nat. Rev. Microbiol.* **10,** 717–725.
22. Woodfin, A., Voisin, M. B., Beyrau, M., Colom, B., Caille, D., Diapouli, F. M., Nash, G. B., Chavakis, T., Albelda, S. M., Rainger, G. E., Meda, P., Imhof, B. A., Nourshargh, S. (2011) The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. *Nat. Immunol.* **12,** 761–769.
23. Choi, E. Y., Chavakis, E., Czabanka, M. A., Langer, H. F., Fraemohs, L., Economopoulou, M., Kundu, R. K., Orlandi, A., Zheng, Y. Y., Prieto, D. A., Ballantyne, C. M., Constant, S. L., Aird, W. C., Papayannopoulou, T., Gahmberg, C. G., Udey, M. C., Vajkoczy, P., Quertermous, T., Dimmeler, S., Weber, C., Chavakis, T. (2008) Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. *Science* **322,** 1101–1104.
24. Mitroulis, I., Kang, Y. Y., Gahmberg, C. G., Siegert, G., Hajishengallis, G., Chavakis, T., Choi, E. Y. (2014) Developmental endothelial locus-1 attenuates complement-dependent phagocytosis through inhibition of Mac-1-integrin. *Thromb. Haemost.* **111,** 1004–1006.
25. Eskan, M. A., Jotwani, R., Abe, T., Chmelar, J., Lim, J. H., Liang, S., Ciero, P. A., Krauss, J. L., Li, F., Rauner, M., Hofbauer, L. C., Choi, E. Y., Chung, K. J., Hashim, A., Curtis, M. A., Chavakis, T., Hajishengallis, G. (2012) The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nat. Immunol.* **13,** 465–473.
26. Von Vietinghoff, S., Ley, K. (2008) Homeostatic regulation of blood neutrophil counts. *J. Immunol.* **181,** 5183–5188.

27. Summers, C., Rankin, S. M., Condliffe, A. M., Singh, N., Peters, A. M., Chilvers, E. R. (2010) Neutrophil kinetics in health and disease. *Trends Immunol.* **31,** 318–324.
28. Bugl, S., Wirths, S., Müller, M. R., Radsak, M. P., Kopp, H. G. (2012) Current insights into neutrophil homeostasis. *Ann. N. Y. Acad. Sci.* **1266,** 171–178.
29. Stark, M. A., Huo, Y., Burcin, T. L., Morris, M. A., Olson, T. S., Ley, K. (2005) Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity* **22,** 285–294.
30. Cua, D. J., Tato, C. M. (2010) Innate IL-17-producing cells: the sentinels of the immune system. *Nat. Rev. Immunol.* **10,** 479–489.
31. Sima, C., Glogauer, M. (2014) Neutrophil dysfunction and host susceptibility to periodontal inflammation: current state of knowledge. *Curr. Oral Health Rep.* **1,** 95–103.
32. Hajishengallis, E., Hajishengallis, G. (2014) Neutrophil homeostasis and periodontal health in children and adults. *J. Dent. Res.* **93,** 231–237.
33. Delima, A. J., Van Dyke, T. E. (2003) Origin and function of the cellular components in gingival crevice fluid. *Periodontol. 2000* **31,** 55–76.
34. Ryder, M. I. (2010) Comparison of neutrophil functions in aggressive and chronic periodontitis. *Periodontol. 2000* **53,** 124–137.
35. Deas, D. E., Mackey, S. A., McDonnell, H. T. (2003) Systemic disease and periodontitis: manifestations of neutrophil dysfunction. *Periodontol. 2000* **32,** 82–104.
36. Schieppati, A., Henter, J. I., Daina, E., Aperia, A. (2008) Why rare diseases are an important medical and social issue. *Lancet* **371,** 2039–2041.
37. Dodge, J. A., Chigladze, T., Donadieu, J., Grossman, Z., Ramos, F., Serlicorni, A., Siderius, L., Stefanidis, C. J., Tasic, V., Valiulis, A., Wierzba, J. (2011) The importance of rare diseases: from the gene to society. *Arch. Dis. Child.* **96,** 791–792.
38. Chapple, I. L., Matthews, J. B. (2007) The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol. 2000* **43,** 160–232.
39. Aboodi, G. M., Goldberg, M. B., Glogauer, M. (2011) Refractory periodontitis population characterized by a hyperactive oral neutrophil phenotype. *J. Periodontol.* **82,** 726–733.
40. Lakschevitz, F. S., Aboodi, G. M., Glogauer, M. (2013) Oral neutrophil transcriptome changes result in a pro-survival phenotype in periodontal diseases. *PLoS ONE* **8,** e68983.
41. Gangbar, S., Overall, C. M., McCulloch, C. A., Sodek, J. (1990) Identification of polymorphonuclear leukocyte collagenase and gelatinase activities in mouthrinse samples: correlation with periodontal disease activity in adult and juvenile periodontitis. *J. Periodontal Res.* **25,** 257–267.
42. Lee, W., Aitken, S., Sodek, J., McCulloch, C. A. (1995) Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. *J. Periodontal Res.* **30,** 23–33.
43. Hernández, M., Gamonal, J., Tervahartiala, T., Mäntylä, P., Rivera, O., Dezerega, A., Dutzan, N., Sorsa, T. (2010) Associations between matrix metalloproteinase-8 and -14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis: a longitudinal study. *J. Periodontol.* **81,** 1644–1652.
44. Ingman, T., Sorsa, T., Suomalainen, K., Halinen, S., Lindy, O., Lauhio, A., Saari, H., Konttinen, Y. T., Golub, L. M. (1993) Tetracycline inhibition and the cellular source of collagenase in gingival crevicular fluid in different periodontal diseases. A review article. *J. Periodontol.* **64,** 82–88.
45. Landzberg, M., Doering, H., Aboodi, G. M., Tenenbaum, H. C., Glogauer, M. (2014) Quantifying oral inflammatory load: oral neutrophil counts in periodontal health and disease. J. *Periodont. Res.* doi: 10.1111/jre.12211 [Epub ahead of print].
46. Wang, J., Arase, H. (2014) Regulation of immune responses by neutrophils. *Ann. N. Y. Acad. Sci.* **1319,** 66–81.
47. Moutsopoulos, N. M., Konkel, J., Sarmadi, M., Eskan, M. A., Wild, T., Dutzan, N., Abusleme, L., Zenobia, C., Hosur, K. B., Abe, T., Uzel, G., Chen, W., Chavakis, T., Holland, S. M., Hajishengallis, G. (2014) Defective neutrophil recruitment in leukocyte adhesion deficiency type I disease causes local IL-17-driven inflammatory bone loss. *Sci. Transl. Med.* **6,** 229ra240.
48. Kalyan, S., Kabelitz, D. (2014) When neutrophils meet T cells: beginnings of a tumultuous relationship with underappreciated potential. *Eur. J. Immunol.* **44,** 627–633.
49. Miossec, P., Kolls, J. K. (2012) Targeting IL-17 and TH17 cells in chronic inflammation. *Nat. Rev. Drug Discov.* **11,** 763–776.
50. Weaver, C. T., Elson, C. O., Fouser, L. A., Kolls, J. K. (2013) The Th17 pathway and inflammatory diseases of the intestines, lungs, and skin. *Annu. Rev. Pathol.* **8,** 477–512.
51. Cheng, W. C., Hughes, F. J., Taams, L. S. (2014) The presence, function and regulation of IL-17 and Th17 cells in periodontitis. *J. Clin. Periodontol.* **41,** 541–549.
52. Pelletier, M., Maggi, L., Micheletti, A., Lazzeri, E., Tamassia, N., Costantini, C., Cosmi, L., Lunardi, C., Annunziato, F., Romagnani, S., Cassatella, M. A. (2010) Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* **115,** 335–343.
53. Schmidt, S., Moser, M., Sperandio, M. (2013) The molecular basis of leukocyte recruitment and its deficiencies. *Mol. Immunol.* **55,** 49–58.
54. Hanna, S., Etzioni, A. (2012) Leukocyte adhesion deficiencies. *Ann. N. Y. Acad. Sci.* **1250,** 50–55.
55. 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. *J. Periodontol.* **58,** 400–416.
56. Kinane, D. F., Hart, T. C. (2003) Genes and gene polymorphisms associated with periodontal disease. *Crit. Rev. Oral Biol. Med.* **14,** 430–449.
57. Dababneh, R., Al-Wahadneh, A. M., Hamadneh, S., Khouri, A., Bissada, N. F. (2008) Periodontal manifestation of leukocyte adhesion deficiency type I. *J. Periodontol.* **79,** 764–768.
58. Nussbaum, G., Shapira, L. (2011) How has neutrophil research improved our understanding of periodontal pathogenesis? *J. Clin. Periodontol.* **38** (Suppl 11), 49–59.
59. Etzioni, A., Tonetti, M. (2000) Leukocyte adhesion deficiency II—from A to almost Z. *Immunol. Rev.* **178,** 138–147.
60. Sollecito, T. P., Sullivan, K. E., Pinto, A., Stewart, J., Korostoff, J. (2005) Systemic conditions associated with periodontitis in childhood and adolescence. A review of diagnostic possibilities. *Med. Oral Patol. Oral Cir. Bucal* **10,** 142–150.
61. Sato, K., Suematsu, A., Okamoto, K., Yamaguchi, A., Morishita, Y., Kadono, Y., Tanaka, S., Kodama, T., Akira, S., Iwakura, Y., Cua, D. J., Takayanagi, H. (2006) Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J. Exp. Med.* **203,** 2673–2682.
62. Van den Berg, W. B., Miossec, P. (2009) IL-17 as a future therapeutic target for rheumatoid arthritis. *Nat. Rev. Rheumatol.* **5,** 549–553.
63. Sutton, C. E., Lalor, S. J., Sweeney, C. M., Brereton, C. F., Lavelle, E. C., Mills, K. H. (2009) Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* **31,** 331–341.
64. Hajishengallis, G. (2014) The inflammophilic character of the periodontitis-associated microbiota. *Mol. Oral Microbiol.* **29,** 48–57.
65. Nualart Grollmus, Z. C., Morales Chávez, M. C., Silvestre Donat, F. J. (2007) Periodontal disease associated to systemic genetic disorders. *Med. Oral Patol. Oral Cir. Bucal* **12,** E211–E215.
66. Allam, J. P., Duan, Y., Heinemann, F., Winter, J., Götz, W., Deschner, J., Wenghoefer, M., Bieber, T., Jepsen, S., Novak, N. (2011) IL-23-producing CD68(+) macrophage-like cells predominate within an IL-17-polarized infiltrate in chronic periodontitis lesions. *J. Clin. Periodontol.* **38,** 879–886.
67. Bosshardt, D. D., Lang, N. P. (2005) The junctional epithelium: from health to disease. *J. Dent. Res.* **84,** 9–20.
68. Niederman, R., Westernoff, T., Lee, C., Mark, L. L., Kawashima, N., Ullman-Culler, M., Dewhirst, F. E., Paster, B. J., Wagner, D. D., Mayadas, T., Hynes, R. O., Stashenko, P. (2001) Infection-mediated early-onset periodontal disease in P/E-selectin-deficient mice. *J. Clin. Periodontol.* **28,** 569–575.
69. Zenobia, C., Luo, X. L., Hashim, A., Abe, T., Jin, L., Chang, Y., Jin, Z. C., Sun, J. X., Hajishengallis, G., Curtis, M. A., Darveau, R. P. (2013) Commensal bacteria-dependent select expression of CXCL2 contributes to periodontal tissue homeostasis. *Cell. Microbiol.* **15,** 1419–1426.
70. Pai, S. Y., Kim, C., Williams, D. A. (2010) Rac GTPases in human diseases. *Dis. Markers* **29,** 177–187.
71. Sima, C., Gastfreund, S., Sun, C., Glogauer, M. (2014) Rac-null leukocytes are associated with increased inflammation-mediated alveolar bone loss. *Am. J. Pathol.* **184,** 472–482.
72. Hajishengallis, G., Moutsopoulos, N. M. (2014) Etiology of leukocyte adhesion deficiency-associated periodontitis revisited: not a raging infection but a raging inflammatory response. *Expert Rev. Clin. Immunol.* **10,** 973–975.
73. Taylor, P. R., Roy, S., Leal, Jr., S. M., Sun, Y., Howell, S. J., Cobb, B. A., Li, X., Pearlman, E. (2014) Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, RORγt and dectin-2. *Nat. Immunol.* **15,** 143–151.
74. Choi, E.-Y., Lim, J.-H., Neuwirth, A., Economopoulou, M., Chatzigeorgiou, A., Chung, K.-J., Bittner, S., Lee, S.-H., Langer, H., Samus, M., Kim, H., Cho, G.-S., Ziemssen, T., Bdeir, K., Chavakis, E., Koh, J.-Y., Boon, L., Hosur, K., Bornstein, S. R., Meuth, S. G., Hajishengallis, G., Chavakis, T. (2014) Developmental endothelial locus-1 is a homeostatic factor in the central nervous system limiting neuroinflammation and demyelination. *Mol. Psychiatry* doi: 10.1038/mp.2014.146 [Epub ahead of print].
75. Shin, J., Hosur, K. B., Pyaram, K., Jotwani, R., Liang, S., Chavakis, T., Hajishengallis, G. (2013) Expression and function of the homeostatic molecule Del-1 in endothelial cells and the periodontal tissue. *Clin. Dev. Immunol.* **2013,** 617809.

76. Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., Saito, S., Inoue, K., Kamatani, N., Gillespie, M. T., Martin, T. J., Suda, T. (1999) IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest.* **103,** 1345–1352.
77. Yago, T., Nanke, Y., Ichikawa, N., Kobashigawa, T., Mogi, M., Kamatani, N., Kotake, S. (2009) IL-17 induces osteoclastogenesis from human monocytes alone in the absence of osteoblasts, which is potently inhibited by anti-TNF-alpha antibody: a novel mechanism of osteoclastogenesis by IL-17. *J. Cell. Biochem.* **108,** 947–955.
78. Holliday, L. S., Welgus, H. G., Fliszar, C. J., Veith, G. M., Jeffrey, J. J., Gluck, S. L. (1997) Initiation of osteoclast bone resorption by interstitial collagenase. *J. Biol. Chem.* **272,** 22053–22058.
79. 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.
80. Hajishengallis, G., Liang, S., Payne, M. A., Hashim, A., Jotwani, R., Eskan, M. A., McIntosh, M. L., Alsam, A., Kirkwood, K. L., Lambris, J. D., Darveau, R. P., Curtis, M. A. (2011) Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* **10,** 497–506.
81. Hajishengallis, G., Lamont, R. J. (2014) Breaking bad: manipulation of the host response by *Porphyromonas gingivalis*. *Eur. J. Immunol.* **44,** 328–338.
82. Maekawa, T., Krauss, J. L., Abe, T., Jotwani, R., Triantafilou, M., Triantafilou, K., Hashim, A., Hoch, S., Curtis, M. A., Nussbaum, G., Lambris, J. D., Hajishengallis, G. (2014) *Porphyromonas gingivalis* manipulates complement and TLR signaling to uncouple bacterial clearance from inflammation and promote dysbiosis. *Cell Host Microbe* **15,** 768–778.
83. 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. *J. Immunol.* **177,** 8296–8300.
84. Liang, S., Krauss, J. L., Domon, H., McIntosh, M. L., Hosur, K. B., Qu, H., Li, F., Tzekou, A., Lambris, J. D., Hajishengallis, G. (2011) The C5a receptor impairs IL-12-dependent clearance of *Porphyromonas gingivalis* and is required for induction of periodontal bone loss. *J. Immunol.* **186,** 869–877.
85. Popadiak, K., Potempa, J., Riesbeck, K., Blom, A. M. (2007) Biphasic effect of gingipains from *Porphyromonas gingivalis* on the human complement system. *J. Immunol.* **178,** 7242–7250.
86. 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. *J. Immunol.* **184,** 1455–1462.
87. Haffajee, A. D., Cugini, M. A., Tanner, A., Pollack, R. P., Smith, C., Kent, Jr., R. L., Socransky, S. S. (1998) Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. *J. Clin. Periodontol.* **25,** 346–353.
88. Abusleme, L., Dupuy, A. K., Dutzan, N., Silva, N., Burleson, J. A., Strausbaugh, L. D., Gamonal, J., Diaz, P. I. (2013) The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *ISME J.* **7,** 1016–1025.
89. Darveau, R. P., Hajishengallis, G., Curtis, M. A. (2012) *Porphyromonas gingivalis* as a potential community activist for disease. *J. Dent. Res.* **91,** 816–820.
90. Laine, M. L., Crielaard, W., Loos, B. G. (2012) Genetic susceptibility to periodontitis. *Periodontol. 2000* **58,** 37–68.
91. Socransky, S. S., Haffajee, A. D. (1994) Evidence of bacterial etiology: a historical perspective. *Periodontol. 2000* **5,** 7–25.
92. Chapple, I. L., Genco, R.; Working Group 2 of the Joint EFP/AAP Workshop. (2013) Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J. Periodontol.* **84**, (4 Suppl), S106–S112.
93. Lalla, E., Papapanou, P. N. (2011) Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. *Nat. Rev. Endocrinol.* **7,** 738–748.
94. Karima, M., Kantarci, A., Ohira, T., Hasturk, H., Jones, V. L., Nam, B. H., Malabanan, A., Trackman, P. C., Badwey, J. A., Van Dyke, T. E. (2005) Enhanced superoxide release and elevated protein kinase C activity in neutrophils from diabetic patients: association with periodontitis. *J. Leukoc. Biol.* **78,** 862–870.
95. Naguib, G., Al-Mashat, H., Desta, T., Graves, D. T. (2004) Diabetes prolongs the inflammatory response to a bacterial stimulus through cytokine dysregulation. *J. Invest. Dermatol.* **123,** 87–92.
96. Graves, D. T., Liu, R., Alikhani, M., Al-Mashat, H., Trackman, P. C. (2006) Diabetes-enhanced inflammation and apoptosis—impact on periodontal pathology. *J. Dent. Res.* **85,** 15–21.
97. Cohen, G., Ilic, D., Raupachova, J., Hörl, W. H. (2008) Resistin inhibits essential functions of polymorphonuclear leukocytes. *J. Immunol.* **181,** 3761–3768.
98. Sima, C., Rhourida, K., Van Dyke, T. E., Gyurko, R. (2010) Type 1 diabetes predisposes to enhanced gingival leukocyte margination and macromolecule extravasation in vivo. *J. Periodontal Res.* **45,** 748–756.
99. Pacios, S., Kang, J., Galicia, J., Gluck, K., Patel, H., Ovaydi-Mandel, A., Petrov, S., Alawi, F., Graves, D. T. (2012) Diabetes aggravates periodontitis by limiting repair through enhanced inflammation. *FASEB J.* **26,** 1423–1430.
100. Moutsopoulos, N. M., Kling, H. M., Angelov, N., Jin, W., Palmer, R. J., Nares, S., Osorio, M., Wahl, S. M. (2012) *Porphyromonas gingivalis* promotes Th17 inducing pathways in chronic periodontitis. *J. Autoimmun.* **39,** 294–303.
101. Ohyama, H., Kato-Kogoe, N., Kuhara, A., Nishimura, F., Nakasho, K., Yamanegi, K., Yamada, N., Hata, M., Yamane, J., Terada, N. (2009) The involvement of IL-23 and the Th17 pathway in periodontitis. *J. Dent. Res.* **88,** 633–638.
102. Gaffen, S. L., Hajishengallis, G. (2008) A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. *J. Dent. Res.* **87,** 817–828.
103. Maekawa, T., Abe, T., Hajishengallis, E., Hosur, K. B., DeAngelis, R. A., Ricklin, D., Lambris, J. D., Hajishengallis, G. (2014) Genetic and intervention studies implicating complement C3 as a major target for the treatment of periodontitis. *J. Immunol.* **192,** 6020–6027.
104. Abe, T., Hosur, K. B., Hajishengallis, E., Reis, E. S., Ricklin, D., Lambris, J. D., Hajishengallis, G. (2012) Local complement-targeted intervention in periodontitis: proof-of-concept using a C5a receptor (CD88) antagonist. *J. Immunol.* **189,** 5442–5448.
105. Jones, S. A., Sutton, C. E., Cua, D., Mills, K. H. (2012) Therapeutic potential of targeting IL-17. *Nat. Immunol.* **13,** 1022–1025.
106. Ricklin, D., Lambris, J. D. (2013) Complement in immune and inflammatory disorders: therapeutic interventions. *J. Immunol.* **190,** 3839–3847.

KEY WORDS:
leukocyte adhesion deficiency · integrins · Del-1 · complement · IL-17