



Dual role of neutrophils in periodontal disease: A Review

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Abstract

Neutrophils play an important role in the acute immune response. The neutrophils within the gingival sulcus provides the first cellular host defense mechanism to control periodontal diseases. They are considered the key protective cell type in the periodontal tissues. They act as a barrier between the junctional epithelium and dental plaque, which is the reservoir of periodontal pathogens. They are the most abundant leukocytes in the blood and their hyper and hypoactivity severely influences the immune response, causing tissue destruction either directly or indirectly.

Keywords: Neutrophils, Inflammation, Periodontal health, Periodontitis

INTRODUCTION

Neutrophils are the first line of defense of the body's immune system. Neutrophils are white blood cells that are short lived, non-mitotic, generated in large numbers from pluripotent stem cells residing in the bone marrow.[1] During the process of myelopoiesis, the neutrophil acquires the necessary capabilities to detect infection, migrate to the site of infection, and ingest and kill microorganisms.[2]

Approximately 10^{11} neutrophils are produced daily and this number may increase several folds in the face of a systemic infection.[3] The neutrophils are considered the key protective cell type in the periodontal tissues and their role in periodontal health and in the disease of periodontium is extensive. The role of the polymorphonuclear neutrophil in periodontitis is evident in severe forms of periodontitis when neutrophils are absent or have severely impaired function. Impaired neutrophil function disrupts the homeostasis of periodontal

tissues and hypofunction of neutrophils increases the susceptibility of individual to periodontal disease. On the other hand, the hyperactivity of neutrophils leads to neutrophil mediated tissue destruction. Many periodontal diseases are associated with neutrophil dysfunction, which implies the importance of neutrophils in periodontal health and disease.[4]

II. NEUTROPHIL ACTIVATION

The recruitment of neutrophils from blood vessels into the site of injury takes place in a coordinated series of steps. The initial step of the neutrophil response to infection is the detection of an appropriate signal and are tightly regulated.

Neutrophils migrate to site of tissue injury or bacterial ingress through the process of chemotaxis. Neutrophils migrate towards the source of chemoattractants that include bacterial products such as formylated peptides, C5a, a product of the complement cascade, products of phospholipid

metabolism, and chemokines such as IL-8. Binding of the chemoattractant to its cell-surface receptor triggers the cascade for activation. The steps in neutrophil recruitment consist of rolling mediated by selectins, adhesion mediated by integrins and transmigration, which occur by CD31 acting as a zipper to transverse the endothelium.[5]

III. NEUTROPHIL FUNCTION

The neutrophils kill the invading pathogens by an oxygen dependent or independent mechanism. The mechanism by which it acts is phagocytosis, generation of reactive oxygen species and degranulation. When these classical pathway are not sufficient to kill the pathogen, they form the neutrophil extracellular trap (NET).

IV. NEUTROPHIL IN PERIODONTAL HEALTH

Neutrophils are the main leukocytes recruited to the gingival sulcus. ($\geq 95\%$ of total leukocytes). They exit the gingival blood vessels and travel through the gingival junctional epithelium until they reach the sulcus. In the sulcus, neutrophils create a barrier against the growing bacteria biofilm to prevent bacteria from invading the underlying tissues. Apart from their role in acute inflammation, neutrophils also have the capacity to modify the overall immune response by interaction with the cells of adaptive immunity. Recent evidence shows that neutrophil-derived proteases also modulate chemokine activity.[6] For example, limited cleavage of IL-8 (CXCL8) by proteinase 3 increases the chemoattractive potency of this key proinflammatory mediator, while cathepsin-G-mediated cleavage lowers the chemotactic potency of CCL5 (RANTES).[7] Similarly, neutrophil-derived proteases can activate some cytokines, most notably TNF and IL-1 β and inactivate others, such as IL-6.[8] Azurocidin and proteinase-3 upregulate adhesion molecules on the vascular endothelium, while cathelicidin is a potent recruiter of monocytes to sites of bacterial infection.[9] Thus, these granule proteins combine to promote the intensity and longevity of the inflammatory response. Neutrophil proteases may also amplify and link the innate and adaptive arms of the immune system. Chimerin (tazarotene-induced gene 2 protein, TIG-2; retinoic acid receptor responder protein 2) is an important chemoattractant to professional antigen-presenting

cells (dendritic cells and macrophages) required to engage lymphocytes. Both cathepsin G and neutrophil elastase can convert prochemerin into the active form, chemerin.[10] Indeed, it seems that neutrophil serine proteases and MMPs can, between them, modulate multiple molecules that play important roles in inflammation and tissue remodeling, including: anti-bacterial peptides; growth factors (e.g., VEGF and TGF β release); adhesion molecules (e.g., ICAM-1 and VCAM-1 processing); and a host of cell surface signal transducers (e.g., TLR4 activation, TNFR inactivation).[11]

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.[12]

An animal 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). The author concluded that the migration of neutrophils to the periodontium does not require commensal bacterial colonization, since recruited neutrophils were also observed in germ-free mice.[13] This finding suggests that neutrophil recruitment may have homeostatic functions that may not necessarily be related to infection control. It should also be noted that mechanisms sensing neutrophil recruitment to peripheral tissues also contribute to the homeostatic regulation of neutrophil count.[14]

V. NEUTROPHIL DYSFUNCTION

Neutrophil dysfunctions causing tissue destruction are its hypofunction and hyper-responsiveness.

HYPOREACTIVE NEUTROPHIL FUNCTION HOMEOSTASIS

Numerous human mutations, though rare have been defined that give rise to defects in neutrophil maturation. These syndromes are collectively known as severe congenital neutropenia (SCN). Patients

with SCN typically present with recurrent life threatening infections in the first few months of life and have neutrophil counts of less than 500 cells/ μ l of blood for at least 3 months. The neutrophils of patients with SCN often show a characteristic maturation arrest at the promyelocyte stage of differentiation. Approximately 60–75% of SCN cases are due to mutations in the gene encoding neutrophil elastase. These mutations often act in a dominant manner and are thought to lead to accumulation of misfolded elastase molecules, causing an endoplasmic reticulum stress (unfolded protein) response in maturing neutrophils that induces apoptosis. Recessive mutations in the genes encoding the mitochondrial antiapoptotic protein HAX-1 [hematopoietic cell-specific Lyn substrate 1 (HCLS1)-associated protein X-1] and the glycosylation enzyme G6PC3 (glucose 6-phosphatase 3) also lead to a range of neutropenias. Nearly one-third of SCN patients have acquired mutations in the G-CSF receptor, which commonly lead to expression of a truncated protein that lacks signaling capacity. Studies done in cell lines and mouse models demonstrate that these mutations cause increased signaling responses to G-CSF, potentially leading to apoptosis. Patients with this form of SCN often shown to develop myeloid leukemia.[15]

RECRUITMENT

Inherited defects in neutrophil recruitment in patients with leukocyte adhesion deficiency (LAD) highlight the importance of selectins (LADII); β 2 integrins (LADI); and integrin activation, specifically Kindlin-3 (LADIII) in getting cells to the site of inflammation. Patients with LADI, LADII, or LADIII present with infections without pus formation, indicating affected neutrophil accumulation. Studies show mice with deficiencies in the β 2 integrins, selectins, or integrin activation exhibit profound defects in neutrophil recruitment. Patients have been found with mutations in the Rac2 GTPase, which lead to impairment in chemokine signaling and actin remodeling that result in recruitment defects.[16] Del-1 exhibit excessive neutrophil recruitment to the gingival, leading to destructive inflammation and alveolar bone loss.[17]

Apart from host-related factors, periodontal bacteria, such as the *Porphyromonas gingivalis* can impair

neutrophil recruitment by interfering with the coordinated expression of chemokines and cell adhesion molecules, such as IL-8 and E-selectin. The inhibitory effect on IL-8 was termed 'local chemokine paralysis' and depends on the capacity of *P. gingivalis* to invade the epithelial cells and secrete the serine phosphatase SerB. *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.[18]

ACTIVATION

Numerous disorders linked to alterations either in pathogen sensing or in the molecules involved in intracellular signaling downstream of pathogen-sensing receptors have now been defined in patients. Defects in TLRs or TLR signaling pathways present almost exclusively with fungal (often *Aspergillus*) infections. Although studies in neutrophils of these patients are not comprehensive, mice deficient in these molecules have defects in neutrophil cytotoxic responses. These observations highlight the point that the type of pathogen infection is often a significant clue to the molecular defect involved. Indeed, increased susceptibility to herpes virus encephalitis has been mapped to mutations in TLR3, the primary virus-sensing TLR, though these mutations affect antigen presenting cells more than neutrophils.[19]

PATHOGEN KILLING

The most common type of neutrophil functional defect is caused by genetic deficiency of any one of the subunits of NADPH oxidase, which results in chronic granulomatous disease (CGD). The most common form of CGD is due to loss of gp91phox, which is encoded on the X chromosome. Patients with this disease present early in life with chronic infections that often lead to formation of tissue granulomas. Autosomal-recessive forms of CGD result from deficiencies of the other NADPH oxidase subunits; these disorders tend to have a better prognosis than the X-linked form. Importantly, many patients with CGD develop chronic intestinal inflammation, mimicking Crohn's disease or ulcerative colitis, due to bacterial overgrowth and inability to maintain barrier function in the gut. This observation illuminates the frequent overlap between

immunodeficiency diseases and autoimmunity—often the former causes the latter.[20]

Other disorders of pathogen killing can arise from defects in granule formation and/or loss of granule enzymes. Chediak-Higashi syndrome (CHS) manifests in many granule containing cells because of mutations in the *LYST* (lysosomal trafficking regulator) gene, which encodes a protein essential for lysosomal trafficking. CHS patients also have defects in cytolytic T cell killing. Patients with loss of the granule enzyme myeloperoxidase are only modestly affected, suggesting that other neutrophil killing mechanisms are adequate to provide host defense in these patients. Another extremely rare disorder of neutrophil killing is neutrophil-specific granule deficiency (SGD). Neutrophils from SGD patients lack secondary and tertiary granules due to mutations in genes encoding transcription factors *C/EBP ϵ* or *Gfi-1* (growth factor independent 1). Numerous other disorders that affect many cell types can also result in neutrophil functional defects and impaired pathogen killing. A prime example is Wiskott-Aldrich syndrome (WAS), which is due to loss of the WAS protein, which is involved in actin remodeling. Neutrophils from WAS patients show migratory and bacterial killing defects.[21]

HYPERACTIVITY OF NEUTROPHILS

Neutrophils are potent regulators of inflammation. They can promote and control inflammation, so the activation of neutrophils at inflammatory sites should be tightly regulated to prevent unwarranted tissue damage. Hyperactivity of neutrophils, has been described in the periodontal literature to mean 'elevated function', e.g. increased enzymatic activity, particularly an increased respiratory burst.[22] Neutrophils from individuals with a specific polymorphism (131H/H) in the *Fc γ 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) *Fc γ receptor IIa* genotype. 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.[23] It should be noted, however, that longitudinal analysis failed to demonstrate an association between these polymorphisms and response to conventional periodontal therapy.[24] Though 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.. It is thought that the recruitment of hyper-responsive neutrophils to the periodontium could contribute to periodontitis by causing oxidative tissue damage.[25]

Neutrophils are thought to play key roles in promoting edema as a consequence of interactions with the vascular endothelia during diapedesis through the secretion of arachidonic acid derivatives, chemokines (such as CXCL1, 2, 3 and 8) and heparin binding protein (CAP37 or neutrophil azurocidin 1), with neutrophil elastase and cathepsin G also reported to promote vascular permeability in vitro studies. Of these mediators, the arachidonic acid metabolites may be of particular importance due to their ability to enhance the permeability-inducing capacities of other endogenous (e.g. C5a, histamine) and exogenous (e.g. fMLP) proinflammatory mediators. Several factors generated de novo by neutrophils during the immune response program help to promote edema, most notably TNF, CXCL2 and CXCL8. Finally, in addition to edema induced by the permeability-increasing (and proangiogenic) factors, neutrophils also contribute to edema by damaging the integrity of the vascular endothelium through the actions of ROS and serine proteases.[26]

Dias et al have shown that plasma from periodontitis subjects is more efficient than plasma from healthy subjects in priming neutrophils to fMLP responsiveness and in directly inducing the oxidative burst. Antibodies against IL-8, GM-CSF and IFN- α abrogated this superoxide-inducing potential of plasma.[27] *F. nucleatum* exposure results in the upregulation of multiple ROS-related genes in neutrophils and that pro-inflammatory transcripts,

including several ROS response-related genes, are differentially regulated in cells from periodontitis subjects and healthy controls.[28]

Neutrophil subset

It has been reported that neutrophil subset differ in health and periodontitis. Hyper-reactive neutrophil phenotype has been reported particularly with regard to ROS release.[29] Although this phenotype has been attributed to higher levels of circulating bacterial products and pro-inflammatory cytokines originating from the periodontium, there is a possibility that these neutrophils may belong to a distinct subset. The pro-inflammatory phenotype of periodontitis oral neutrophils was confirmed by elevated degranulation, phagocytosis, ROS production, and NET formation. In oral health, two different populations of oral neutrophils was observed. The other, more activated population showed higher expression of CD55 and CD63, having decreased levels of inhibitory receptor CD170 and CD16.[30]

Kobayashi et al. reported impaired phagocytosis and ROS release in response to IgG-opsonized *Porphyromonas gingivalis* and that these peripheral blood neutrophils from patients with periodontitis displayed a specific Fc γ RIIIb allotype (NA2).[31] They further reported that GCF-derived neutrophils from these patients had increased Fc α RI and Fc γ RI levels and lower Fc γ RIIa and Fc γ RIIIb levels than blood neutrophils, leading to the same impairment of phagocytosis.[32] A neutrophil subset with defective chemotaxis in aggressive was described by Van Dyke et al.[33]

DEFECTIVE NEUTROPHIL CLEARANCE

Although hyperactive neutrophils may contribute to the pathogenesis of periodontitis, even normally activated neutrophils could cause unwarranted collateral tissue damage if not cleared properly when they become apoptotic. The resolution of inflammation, includes clearance of apoptotic cells and cellular debris, is essential for homeostasis and tissue repair.[34] The prolonged persistence of neutrophils due to defective clearance mechanisms could lead to necrosis and the release of toxic contents. The defect in neutrophil clearance causing periodontal tissue damage could result from necrosis of non-cleared apoptotic neutrophils is consistent with findings that neutrophil necrosis predominates over

apoptosis in the diseased periodontium. 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.[35] This mechanism could contribute to the transition from gingivitis to periodontitis or the aggravation of existing periodontitis. The importance of defective apoptotic cell clearance in periodontal pathogenesis is may operate in periodontitis patients with co-morbid conditions associated with impaired apoptotic cell clearance (e.g., lupus). Genetic deficiencies predisposing to ineffective apoptotic cell removal, aging could be associated with defective clearance of apoptotic neutrophils, perhaps due to reduced expression of apoptotic cell uptake receptors in macrophage.[36]

VI. CONCLUSION

Because of the constant irritation of the periodontal tissues, these tissues are always active and there is a balance between destruction and repair. Regeneration of the periodontal tissues is part of this balance, and also an important part of the desired outcome of periodontal therapy. Neutrophils were once ignored as cells unimportant in the process of tissue regeneration, but there is now an increasing body of evidence that neutrophils can affect tissue regeneration by the release of neutrophil derived factors that promote angiogenesis. With newer concepts about the role of neutrophils, host modulation to prevent neutrophil mediated tissue damage, while preserving beneficial antibacterial activity. can be considered as a new approach. However, a greater understanding of the pathways controlling neutrophil apoptosis and neutrophil subsets in the periodontal disease is required in order to design new therapeutic interventions.

VII. REFERENCE

1. Segal BH, Holland SM. Primary phagocytic disorders of childhood. *Pediatr Clin N Am* 2000; 47: 1311–1338.
2. Holland SM, Gallin J. Disorders of granulocytes and monocytes. In: *Harrison's Internal medicine*. New York: McGraw-Hill, 2001; 366–373.
3. Trowbridge H, Emling R. The blood

- leukocytes. In: *Inflammation: a review of the process*. Chicago: Quintessence, 1997; 41–58.
4. Hart TC, Shapira L, Van Dyke TE. Neutrophil defects as risk factors for periodontal diseases. *J Periodontol* 1994; 65:521–529.
 5. Nordenfelt P, Tapper H. Phagosome dynamics during phagocytosis by neutrophils. *J. Leukoc. Biol.* 2011; 90:271–284.
 6. Pham CT: Neutrophil serine proteases fine-tune the inflammatory response. *Int J Biochem Cell Biol* 2008; 40:1317–1333.
 7. Lim JK, Lu W, Hartley O, DeVico AL: N-terminal proteolytic processing by cathepsin G converts RANTES/CCL5 and related analogs into a truncated 4–68 variant. *J Leukoc Biol* 2006; 80:1395–1404.
 8. Coeshott C, Ohnemus C, Pilyavskaya A, Ross S, Wiczorek M, Kroona H, Leimer AH, Cheronis J: Converting enzyme-independent release of tumor necrosis factor alpha and IL-1beta from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. *Proc Natl Acad Sci USA* 1999; 96:6261–6266.
 9. de Haar SF, Hiemstra PS, van Steenberg MT, Everts V, Beertsen W: Role of polymorphonuclear leukocyte-derived serine proteinases in defense against *Actinobacillus actinomycetemcomitans*. *Infect Immun* 2006; 74:5284–5291.
 10. Wittamer V, Bondue B, Guillabert A, Vassart G, Parmentier M, Communi D: Neutrophil-mediated maturation of chemerin: a link between innate and adaptive immunity. *J Immunol* 2005; 175:487–493.
 11. Meyer-Hoffert U: Neutrophil-derived serine proteases modulate innate immune responses. *Front Biosci* 2009; 14:3409–3418.
 12. Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. *Nat Rev Microbiol.* 2010; 8:481–490.
 13. Zenobia C, Luo XL, Hashim A, Abe T, Jin L, Chang Y, et al. Commensal bacteria-dependent select expression of CXCL2 contributes to periodontal tissue homeostasis. *Cell Microbiol.* 2003; 15:1419–1426.
 14. Stark MA, Huo Y, Burcin TL, Morris MA, Olson TS, Ley K. Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity.* 2005; 22:285–294.
 15. Curtis MA, Zenobia C, Darveau RP: The relationship of the oral microbiota to periodontal health and disease. *Cell Host Microbe* 2011 10:302–306.
 16. Chakravarti, A., Raquil, M. A., Tessier, P. & Poubelle, P. E. Surface RANKL of Toll-like receptor 4-stimulated human neutrophils activates osteoclastic bone resorption. 2009; *Blood* 114, 1633–1644.
 17. Eskan MA, Jotwani R, Abe T, et al. The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nat Immunol.* 2012; 13(5):465–473.
 18. Gustafsson A, Ito H, Asman B, Bergstrom K. Hyper-reactive mononuclear cells and neutrophils in chronic periodontitis. *J Clin Periodontol* 2006; 33: 126–129.
 19. Germeshausen M, Ballmaier M, Welte K. 2007. Incidence of CSF3R mutations in severe congenital neutropenia and relevance for leukemogenesis: results of a long-term survey. *Blood* 109:93–99.
 20. Ambruso DR, Knall C, Abell AN, Panepinto J, Kurkchubasche A, et al. 2000. Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. *Proc. Natl. Acad. Sci. USA* 97:4654–4659.
 21. Vieira OV, Botelho RJ, Grinstein S. Phagosome maturation: aging gracefully. *Biochem. J.* 2002; 366:689–704.
 22. Ryder MI: Comparison of neutrophil functions in aggressive and chronic periodontitis. *Periodontol* 2000 2010; 53:124–137.

23. Nicu EA, Van der Velden U, Everts V, Van Winkelhoff AJ, Roos D, Loos BG. Hyper-reactive PMNs in FcγRIIa 131 H/H genotype periodontitis patients. *J Clin Periodontol.*2007; 34:938-945.
24. Wolf DL, Neiderud AM, Hinckley K, Dahlén G, van de Winkel JG, Papananou PN. Fcγ receptor polymorphisms and periodontal status: a prospective follow-up study. *J Clin Periodontol.*2006; 33:691-698.
25. Matthews JB, Wright HJ, Roberts A, Ling-Mountford N, Cooper PR, Chapple IL. Neutrophil hyper-responsiveness in periodontitis. *J Dent Res.*2007. 86:718-722.
26. DiStasi MR, Ley K: Opening the flood-gates: how neutrophil-endothelial interactions regulate permeability. *Trends Immunol* 2009; 30:547–556.
27. Dias IH, Matthews JB, Chapple IL, Wright HJ, Dunston CR, Griffiths HR: Activation of the neutrophil respiratory burst by plasma from periodontitis patients is mediated by pro-inflammatory cytokines. *J Clin Periodontol* 2011; 38:1–7.
28. Wright HJ, Chapple IL, Matthews JB, Cooper PR: *Fusobacterium nucleatum* regulation of neutrophil transcription. *J Periodontal Res* 2011; 46:1–12.
29. Johnstone AM, Koh A, Goldberg MB, Glogauer M. A hyperactive neutrophil phenotype in patients with refractory periodontitis. *J Periodontol.*2007;78:1788–94.
30. Matthews JB, Wright HJ, Roberts A, Cooper PR, Chapple ILC. Hyperactivity and reactivity of peripheral blood neutrophils in chronic periodontitis. *Clin Exp Immunol.* 2007; 147:255–64.
31. Kobayashi T, Van Der Pol WL, Van De Winkel JG, Hara K, Sugita N, Westerdal NA, et al. Relevance of IgG receptor IIIb (CD16) polymorphism to handling of *Porphyromonas gingivalis*: implications for the pathogenesis of adult periodontitis. *J Periodontal Res.*2000; 35:65–73.
32. Kobayashi T, Yamamoto K, Sugita N, Van Spruiel AB, Kaneko S, Van De Winkel JG, et al. Effective in vitro clearance of *Porphyromonas gingivalis* by Fc alpha receptor I (CD89) on gingival crevicular neutrophils. *Infect Immun.*2001; 69:2935–42.
33. Van Dyke TE, Warbington M, Gardner M, Offenbacher S. Neutrophil surface protein markers as indicators of defective chemotaxis in LJP. *J Periodontol.*1990;61:180–4.
34. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol.*2010; 11:785-797.
35. Crawford JM, Wilton JM, Richardson P. Neutrophils die in the gingival crevice, periodontal pocket, and oral cavity by necrosis and not apoptosis. *J Periodontol.*2010;71:1121-1129.
36. Devitt A, Marshall LJ. The innate immune system and the clearance of apoptotic cells. *J Leukoc Biol.*2011; 90:447-457.