



UvA-DARE (Digital Academic Repository)

Reactivity of neutrophils, monocytes and platelets in periodontitis

Nicu, E.A.

Publication date
2008

[Link to publication](#)

Citation for published version (APA):

Nicu, E. A. (2008). *Reactivity of neutrophils, monocytes and platelets in periodontitis*. [Thesis, fully internal, Universiteit van Amsterdam].

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Chapter 1

General Introduction

Introduction

The periodontium is the tooth-supporting organ. It comprises the periodontal ligament, the alveolar bone, the radicular cementum and the gingiva. The periodontal ligament is the soft connective tissue interposed between the root of each tooth and the inner wall of the alveolar socket. At the soft-hard tissue borders of the periodontal ligament, the principal periodontal ligament fibers are embedded in alveolar bone on one side and radicular cementum on the other side. Covering and protecting these structures, the gingiva forms the fourth component of the periodontium.

The periodontium can become inflamed; the mildest and most frequent inflammatory condition of the periodontium is gingivitis. Gingivitis is highly prevalent and readily reversible by effective oral hygiene. Gingivitis affects 50-90% of adults worldwide, depending on its precise definition (1). Inflammation that extends deep into the tissues and causes loss of supportive connective tissue and alveolar bone is known as periodontitis. Periodontitis results in the formation of periodontal pockets; these are deepened crevices between gingiva and tooth roots eventually leading to tooth loss. After the age of fifty, virtually all individuals may present with some mild periodontal destruction, one or two pockets deeper than 4 mm, or small gingival recessions. But the severe forms of periodontitis will only occur in approximately 10% of the adult population (2). Treatment of periodontitis includes mechanical removal of subgingival bacterial plaque with scalers, curettes or ultra-sonic devices and intensive oral hygiene instructions for the patient. A close to ideal oral hygiene is the only way to prevent formation of new dental plaque deposits and re-infection of the subgingival tissues.

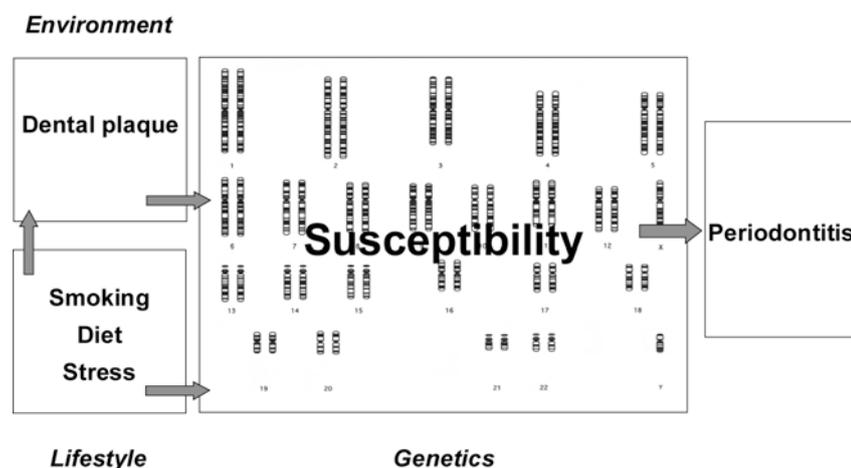


Fig. 1.1 Periodontitis is a multifactorial disease.

Periodontitis is a multifactorial disease. In the light of current knowledge, genetic factors represent the basis of susceptibility for periodontitis, and environment and lifestyle are major modifying factors (Fig. 1.1). Dental plaque is the major environmental factor. As dental plaque matures to a state that is associated with periodontitis, the number of Gram-negative and anaerobe micro-organisms increases. Certain bacterial species have been often associated with disease, and intensively studied, including *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* (3). Several hundred different bacterial species colonize the subgingival tissues, covering the root surfaces and the epithelial lining of the pocket with a complex biofilm. A biofilm is a structured community of micro-organisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface. Evidence is accumulating that the aggregated organisms in biofilms are not merely passive neighbors, but rather are involved in a wide range of physical, metabolic and molecular interactions (4). Dental biofilm pathogenicity in the oral cavity is magnified by two biofilm characteristics: increased antibiotic resistance and the inability of the community to be phagocytosed by host inflammatory cells.

Smoking and emotional stress are lifestyle-associated factors that have been often incriminated as major disease-modifiers. Smokers are much more likely to develop periodontitis than non-smokers and smoking has a strong negative effect on the response to periodontal treatment and other oral surgical interventions (1). Traumatic life events that lead to depression or individual inability to cope with stress could increase the person's risk for periodontitis, most likely due to adverse immune responses (5).

The periodontal immune response has been compared to a double-edged sword, on the one hand fighting microorganisms with which it comes into contact and, on the other hand, mediating injury to the host. Maintaining a balance between these two conflicting properties and the preservation of health is assured by proper immunoregulation. Therefore, knowledge of the immune response to periodontal pathogens is ultimately relevant for understanding pathogenesis of periodontitis.

The immune response in periodontitis

The local defense against pathogens from dental plaque is based on the integrity and activity of the epithelial lining, secretion of gingival crevicular fluid and saliva, and local inflammatory reactions. Subgingival accumulation of oral bacteria triggers inflammation in the periodontium. Inflammation is the response of the host to infection or other insults

and comprises a series of vascular reactions at the site of injury. The results of these vascular reactions are exudation of fluid and plasma proteins and recruitment of leukocytes to the site of injury. The goal of inflammation is confining the injury and initiating the immune response, through which the infection is eliminated and the injury repaired (6).

Professional phagocytes, comprising polymorphonuclear neutrophilic leukocytes (PMNs) and monocyte/macrophage cells, play an important role in the host defense against bacterial infections, and, as such, play an important role in periodontal disease. The functional responses of the phagocytes to bacterial infections include chemotaxis, migration, phagocytosis, degranulation and reactive oxygen species generation.

PMNs form the first line of defense, are the most abundant leukocytes in peripheral blood (50-65%) and are characterized by a segmented nucleus and a rich granular cytoplasm. The PMN granules contain large amounts of anti-microbial substances and enzymes. The primary (or azurophilic) granules contain myeloperoxidase (MPO), serine proteases (elastase, cathepsin G, and others), defensins and lysozyme. The secondary (or specific) granules contain collagenase, lysozyme, vitamin B-binding protein and lactoferrin (7).

PMNs have the ability of sensing and moving towards chemical signals, chemotaxins, generated at infectious sites. Among the chemotaxins recognized by PMNs are N-formyl-methionyl peptides from bacteria, C5a from complement and IL-8 from epithelial and other cells (8). Activated PMNs adhere to the vessel wall via selectins and integrins, leave the blood circulation, and migrate to the site of infection with the ultimate goal to phagocytose and kill pathogens. The bacterial killing by PMN takes place in a membrane-confined vacuole, the phagolysosome, formed after fusion of PMN granules with the bacterium-containing phagosome. The bacterial killing and digestion are accomplished via oxygen-dependent and oxygen-independent pathways. In the oxygen-dependent the NADPH-oxidase system (residing in the cytosol) is activated and generates toxic oxygen species; in the oxygen-independent pathway, proteolytic enzymes stored in the granules are activated and released within the phagolysosome. In periodontitis, a hyper-production of proteolytic enzymes (9,10) and reactive oxygen species (11) has been documented and these may play a role in the host-derived destruction of periodontal tissues.

In inflammation, PMNs arrive at the site of injury first followed by monocytes (6). Tissue macrophages differentiate from peripheral-blood monocytes. The monocytes, and

their progeny, the macrophages and the dendritic cells are important players in the pathogenesis of periodontitis. Various bacterial species in dental plaque are gram-negative and their LPS interacts with CD14 and Toll-like receptors inducing the production of cytokines and other mediators by macrophages or dendritic cells. A key cytokine in the monocyte-associated periodontal destruction is interleukin-1 (IL-1), which is capable of stimulating the collagenolytic and bone-destructive processes (12).

Not only PMNs and monocytes/macrophages can interact with bacteria, platelets also have the ability to respond to infection. Activated platelets release anti-microbial peptides and chemokines such as platelet factor 4, RANTES (13), and expose pro-inflammatory receptors, facilitating their binding to leukocytes and endothelial cells (14). Due to this interaction, leukocytes and endothelial cells increase the expression of adhesion molecules and various cytokines (15,16). Bacteria-platelet interactions are made possible either directly through a bacterial surface protein or indirectly by a bridging molecule from plasma that links bacteria with platelet surface receptors. Gingipains, a family of cysteine proteases produced by the periodontal pathogen *P. gingivalis*, can directly activate platelets. *Streptococcus sanguis* was found to require immunoglobulin G (IgG) interacting with an IgG receptor (FcγRIIIa) to mediate platelet activation (17).

Recognition of bacteria by phagocytes: the receptors

In general, phagocytes lack the ability to recognize bacteria, and instead, depend on opsonization. Opsonization is the process of coating of bacteria with plasma proteins in order to signal and facilitate phagocytosis. There are three identified mechanisms: I) the recognition of complement C3b by CR3 and CR4 (complement receptor 3 and 4); II) the recognition of antibodies by Fc receptors; and III) the recognition of lipopolysaccharide-binding protein by CD14 (18).

The PMN and monocyte complement receptors - CR3 (Mac-1; CD11b/CD18) and CR4 (p150,95; CD11c/CD18) recognize and bind particles that have been coated by the complement-derived opsonin, C3b.

Phagocytes bind and recognize targets also via antibodies (immunoglobulins). Immunoglobulin G (IgG) is the predominant serum isotype during bacterial infections. IgG₁ and IgG₃ are produced in response to proteinaceous antigens (such as *P. gingivalis* hemagglutinin) and viruses; IgG₂ is formed in response to the bacterial polysaccharides and outer membrane proteins of gram-negative periodontal pathogens (19). IgG molecules

contain two regions: one region (the Fab-domain) recognizes the pathogen, whereas the other region (the Fc-domain) activates the immune system. Depending on their expression on effector cells, Fc γ R exert different effects. Based on their affinity for monomeric IgG, three types of Fc γ R on phagocytes have been identified: Fc γ RI, Fc γ RII, and Fc γ RIII (20).

Fc γ RI (CD64) is constitutionally highly expressed by monocytes and macrophages. Under resting condition, PMNs do not express Fc γ RI (21). Expression of Fc γ RI on PMNs can be induced by stimulation with interferon- γ (IFN- γ) or granulocyte colony-stimulating factor (G-CSF) (22,23,24). Fc γ RII (CD32) is the most widely distributed Fc γ R, expressed on PMNs, platelets, eosinophils, basophils, lymphocytes and monocytes. There are several isoforms of Fc γ RII, with highly similar extracellular and transmembrane domains, but with different intracellular signaling motifs (25,26). Fc γ RIIa and Fc γ RIIc contain an immunoreceptor tyrosine-based *activation* motif (ITAM), thus they function as activating receptors. Fc γ RIIb, on the other hand, contains an immunoreceptor tyrosine-based *inhibitory* motif (ITIM), thus it is an inhibitory receptor (27,28). PMNs and monocytes express both Fc γ RIIa and Fc γ RIIb (29,21). The balance between activating and inhibitory Fc γ RII can shift in inflammatory states. IFN- γ induces upregulation of Fc γ RIIa and concomitant downregulation of Fc γ RIIb, whereas interleukin-4 (IL-4) can have the opposite effect (29). Two genes, *FCG3A* and *FCG3B* encode for Fc γ RIII. Fc γ RIIIa is expressed by monocytes and NK cells (30,31,32), whereas Fc γ RIIIb is solely expressed by PMNs and eosinophils (33).

Genetic polymorphisms with consequences for the receptor affinities for IgG-subclasses have been identified in *FCG2A*, *FCG3A* and *FCG3B* (34,35,36,21). At aminoacid position 131, Fc γ RIIa expresses either an arginine (R) or a histidine (H). The genetic variation in the Fc γ RIIa has functional consequences: the Fc γ RIIa-H131 variant binds human IgG₂, whereas Fc γ RIIa-R131 does not (35). It has been speculated that this polymorphism may have important consequences for the pathogenesis of periodontitis (37). The polymorphism in Fc γ RIIIa yields either a valine (V) or a phenylalanine (F) at aminoacid position 158. This substitution results in an increased affinity for IgG1 and IgG3 of the Fc γ RIIIa-V158 variant (36,34). In the case of Fc γ RIIIb, the polymorphism involves 4 aminoacids, combined in the NA1 and NA2 variants (38). Fc γ RIIIb-NA1 binds IgG1 and IgG3 more efficiently than Fc γ RIIIb-NA2 (21).

Plasma-derived lipopolysaccharide-binding protein (LBP) and septin can directly opsonize Gram-negative bacteria by interaction with the lipopolysaccharide (LPS) that

forms part of the Gram-negative bacterial outer membrane (18). CD14 is an LPS co-receptor and is mainly expressed on mature monocytes, macrophages and activated PMNs (39). Soluble CD14 (sCD14) can be released from blood monocytes or produced in the liver. Acting as a soluble LPS-receptor, sCD14 helps inducing responses in cells that naturally lack CD14, such as endothelial cells (40). Another role is the neutralization and clearance of LPS in Gram-negative infectious states (endotoxemic states). Acting as a decoy receptor for serum LPS, sCD14 prevents extreme pro-inflammatory responses from monocytes/macrophages (41).

Periodontitis in relation to atherosclerosis and cardiovascular diseases

Periodontitis shares its multifactorial character with other frequent diseases of the modern world, such as diabetes and cardiovascular diseases. Furthermore, it has been postulated that periodontitis and cardiovascular diseases have more in common than unfavorable lifestyle (such as smoking and stress), as epidemiologic studies identified periodontitis as a risk factor for future cardiovascular events such as stroke and myocardial infarction. In a meta-analysis cumulating the results from several studies investigating the relationship between periodontitis and coronary artery disease, presence of periodontitis associated with an increase of 19% in the risk for myocardial infarction (Fig. 1.2), compared to subjects without periodontitis (42); the risk increase was larger (44%) when only subjects <65 years old were considered. However, the underlying mechanisms of the link between periodontitis and cardiovascular diseases are still unclear.

Atherosclerosis, which lies at the base of myocardial infarction and stroke, is now

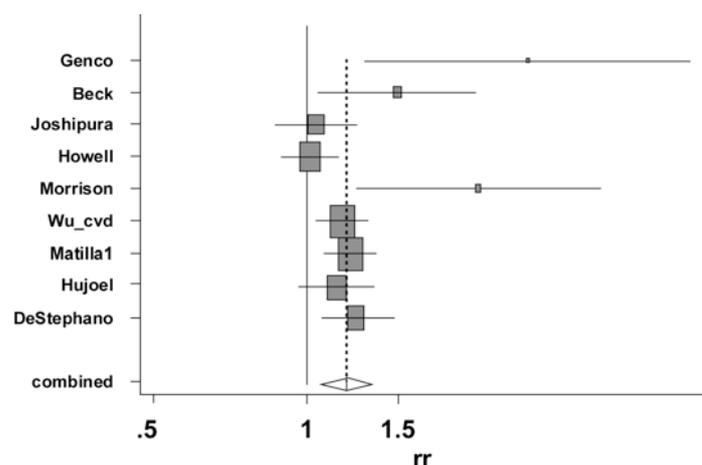


Fig. 1.2 Meta-analysis of nine studies on the impact of periodontitis for risk of future cardiovascular events (adopted from Janket et al., 2003).

also considered as an inflammatory disease (43). The atherosclerotic process is initiated when cholesterol-containing low-density lipoproteins accumulate in the intima and activate the endothelium. Leukocyte adhesion molecules and chemokines promote recruitment of monocytes and T cells. Monocytes differentiate into macrophages that accumulate intracellular lipoprotein, which leads to foam-cell formation. Intensified inflammatory activation may lead to local proteolysis, plaque rupture, and thrombus formation, which causes ischemia and infarction. Chronic infections with *Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus (CMV) have been implicated in atherosclerosis by stimulating disease progression and possibly plaque activation (43). This activation could be due either to direct action in plaques or to remote signaling via inflammatory mediators. Similar mechanisms of action are conceivable in periodontitis, where chronic subgingival infection with periodontal pathogens is accompanied by transient, low-grade bacteremias during dental procedures or daily activities like tooth brushing or chewing (44,45). Moreover, a systemic inflammatory reaction is documented in periodontitis, with increased production of IL-1 β , IL-6, C-reactive protein and tumor-necrosis factor (TNF)- α (46,47). There is evidence of the presence of periodontal pathogens in atherosclerotic plaques (48). Therefore, direct or indirect priming of PMNs, monocytes and platelets by periodontal pathogens, leading to increased inflammation at sites of atherosclerotic activity, might be an important mechanism underlying the increased risk for CVD in periodontitis patients.

General aim and outline of this thesis

The general scope of this thesis was to characterize the reactivity of PMNs, monocytes and platelets against periodontal pathogens. The added knowledge will increase our understanding of some patho-physiological processes in periodontitis, and may explain inter-individual variation in clinical responses to pathogens. Moreover, this interaction is important to dissect possible pathways of the association between periodontitis and cardiovascular diseases, as endotoxemia, systemic exposure to periodontal pathogens and the induced systemic inflammation seem to be important (49,50).

There is a need to incorporate host genetic diversity in functional studies in the pathogenesis of periodontitis. One aspect of the host-derived breakdown of periodontal tissues seems related to a hyper-reactive trait of PMNs (51,11). The nature of this hyper-reactivity might be genetically-determined or derived from increased expression levels of

phagocytic receptors (e.g Fc γ R, complement receptor, CD14) in response to the pathologic processes accompanying periodontitis. The hypothesis that there are inter individual differences in phagocyte reactivity due to variations in phagocytic receptors led to three research questions.

1. Does the PMN activation via Fc γ RIIa among periodontitis patients with different Fc γ RIIa genotypes (R/R and H/H) after stimulation with IgG-opsonized *A. actinomycetemcomitans* differ? (Chapter 2)
2. Is the PMN and monocyte reactivity in periodontitis attributable to modified expression of Fc γ RI, Fc γ RIIa, Fc γ RIII, CR3 or CD14? (Chapter 3)
3. Is periodontitis leading to increased sCD14 levels? (Chapter 4)

Furthermore, we hypothesized that in response to transient bacteremic episodes of periodontal origin, PMNs, monocytes and platelets are primed in periodontitis patients. This priming would than be a part of the pathogenic processes linking periodontitis and cardiovascular disease. We analyzed the cellular response of PMNs, monocytes and platelets to the periodontal pathogens *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia*. This interest materialized in two research questions.

4. Are circulating platelets from patients with periodontitis more activated than control platelets? (Chapter 5)
5. Do platelets, PMNs and monocytes from patients with periodontitis respond in a hyper-reactive fashion to periodontal pathogens compared to cells from periodontally healthy controls? (Chapter 6)

Finally, Chapter 7 summarizes and interprets the main findings of the studies in the context of the research questions, and suggests new directions for future research.

The chapters 2-6 of this thesis have been published as individual studies, thus some repetition, especially in the introductory sections, is inherent.

REFERENCES

- 1 **Pihlstrom BL, Michalowicz BS, Johnson NW.** 2005. Periodontal diseases. *Lancet* **366**:1809-1820.
- 2 **Brown LJ, Oliver RC, Loe H.** 1990. Evaluating periodontal status of US employed adults. *J Am Dent Assoc* **121**:226-232.
- 3 **van Winkelhoff AJ, Loos BG, van der Reijden WA, van der Velden U.** 2002. *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *J Clin Periodontol* **29**:1023-1028.
- 4 **Thomas JG and Nakaishi LA.** 2006. Managing the complexity of a dynamic biofilm. *J Am Dent Assoc* **137**:10S-115.
- 5 **Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA.** 1999. Relationship of stress, distress and inadequate coping behaviors to periodontal disease. *J Periodontol* **70**:711-723.
- 6 **Majno G.** 1982. Inflammation and infection: historic highlights. *Monogr Pathol* 1-17.
- 7 **Kuijpers TW, Tool AT, van der Schoot CE, Ginsel LA, Onderwater JJ, Roos D, Verhoeven AJ.** 1991. Membrane surface antigen expression on neutrophils: a reappraisal of the use of surface markers for neutrophil activation. *Blood* **78**:1105-1111.
- 8 **Zadeh HH, Nichols FC, Miyasaki KT.** 1999. The role of the cell-mediated immune response to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontitis. *Periodontology 2000* **20**:239-288.
- 9 **Figueredo CM, Gustafsson A, Asman B, Bergstrom K.** 1999. Increased release of elastase from in vitro activated peripheral neutrophils in patients with adult periodontitis. *J Clin Periodontol* **26**:206-211.

-
- 10 **Gustafsson A and Asman B.** 1996. Increased release of free oxygen radicals from peripheral neutrophils in adult periodontitis after Fc delta-receptor stimulation. *J Clin Periodontol* **23**:38-44.
 - 11 **Matthews JB, Wright HJ, Roberts A, Cooper PR, Chapple IL.** 2007. Hyperactivity and reactivity of peripheral blood neutrophils in chronic periodontitis. *Clin Exp Immunol* **147**:255-264.
 - 12 **Tatakis DN.** 1993. Interleukin-1 and bone metabolism: a review. *J Periodontol* **64**:416-431.
 - 13 **Fitzgerald JR, Foster TJ, Cox D.** 2006. The interaction of bacterial pathogens with platelets. *Nat Rev Microbiol* **4**:445-457.
 - 14 **Klinger MH and Jelkmann W.** 2002. Role of blood platelets in infection and inflammation. *J Interferon Cytokine Res* **22**:913-922.
 - 15 **Celi A, Pellegrini G, Lorenzet R, De Blasi A, Ready N, Furie BC, Furie B.** 1994. P-selectin induces the expression of tissue factor on monocytes. *PNAS* **91**:8767-8771.
 - 16 **Slupsky JR, Kalbas M, Willuweit A, Henn V, Kroczeck RA, Muller-Berghaus G.** 1998. Activated platelets induce tissue factor expression on human umbilical vein endothelial cells by ligation of CD40. *Thromb Haemost* **80**:1008-1014.
 - 17 **McNicol A, Zhu R, Pesun R, Pampolina C, Jackson EC, Bowden GH, Zelinski T.** 2006. A role for immunoglobulin G in donor-specific *Streptococcus sanguis*-induced platelet aggregation. *Thromb Haemost* **95**:288-293.
 - 18 **Wright SD, Ramos RA, Patel M, Miller DS.** 1992. Septin: a factor in plasma that opsonizes lipopolysaccharide-bearing particles for recognition by CD14 on phagocytes. *J Exp Med* **176**:719-727.
 - 19 **Ebersole JL and Taubman MA.** 1994. The protective nature of host responses in periodontal diseases. *Periodontol 2000* **5**:112-141.
 - 20 **Deo YM, Graziano RF, Repp R, Van de Winkel JG.** 1997. Clinical significance of IgG Fc receptors and Fc gamma R-directed immunotherapies. *Immunol Today* **18**:127-135.

- 21 **Huizinga TW, Kerst M, Nuyens JH, Vlug A, dem Borne AE, Roos D, Tetteroo PA.** 1989. Binding characteristics of dimeric IgG subclass complexes to human neutrophils. *J Immunol* **142**:2359-2364.

- 22 **Repp R, Valerius T, Sandler A, Gramatzki M, Iro H, Kalden JR, Platzer E.** 1991. Neutrophils express the high affinity receptor for IgG (Fc gamma RI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. *Blood* **78**:885-889.

- 23 **Guyre PM, Morganelli PM, Miller R.** 1983. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. *J Clin Invest* **72**:393-397.

- 24 **Perussia B, Kobayashi M, Rossi ME, Anegon I, Trinchieri G.** 1987. Immune interferon enhances functional properties of human granulocytes: role of Fc receptors and effect of lymphotoxin, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor. *J Immunol* **138**:765-774.

- 25 **Brooks DG, Qiu WQ, Luster AD, Ravetch JV.** 1989. Structure and expression of human IgG FcRII(CD32). Functional heterogeneity is encoded by the alternatively spliced products of multiple genes. *J Exp Med* **170**:1369-1385.

- 26 **Warmerdam PA, Van de Winkel JG, Gosselin EJ, Capel PJ.** 1990. Molecular basis for a polymorphism of human Fc gamma receptor II (CD32). *J Exp Med* **172**:19-25.

- 27 **van den Herik-Oudijk IE, Capel PJ, van der BT, van de Winkel JG.** 1995. Identification of signaling motifs within human Fc gamma RIIIa and Fc gamma RIIb isoforms. *Blood* **85**:2202-2211.

- 28 **van den Herik-Oudijk IE, Westerdal NA, Henriquez NV, Capel PJ, van de Winkel JG.** 1994. Functional analysis of human Fc gamma RII (CD32) isoforms expressed in B lymphocytes. *J Immunol* **152**:574-585.

- 29 **Pricop L, Redecha P, Teillaud J-L, Frey J, Fridman WH, Sautes-Fridman C, Salmon JE.** 2001. Differential Modulation of Stimulatory and Inhibitory Fc{{gamma}} Receptors on Human Monocytes by Th1 and Th2 Cytokines. *J Immunol* **166**:531-537.

-
- 30 **Ravetch JV and Kinet JP.** 1991. Fc receptors. *Annu Rev Immunol* **9**:457-492.
- 31 **Van de Winkel JG and Anderson CL.** 1991. Biology of human immunoglobulin G Fc receptors. *J Leukoc Biol* **49**:511-524.
- 32 **Hulett MD and Hogarth PM.** 1994. Molecular basis of Fc receptor function. *Adv Immunol* **57**:1-127.
- 33 **Huizinga TW, van der Schoot CE, Jost C, Klaassen R, Kleijer M, dem Borne AE, Roos D, Tetteroo PA.** 1988. The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature* **333**:667-669.
- 34 **De Haas M, Koene HR, Kleijer M, de Vries E, Simsek S, van Tol MJ, Roos D, dem Borne AE.** 1996. A triallelic Fc gamma receptor type IIIA polymorphism influences the binding of human IgG by NK cell Fc gamma RIIIa. *J Immunol* **156**:2948-2955.
- 35 **Warmerdam PA, Van de Winkel JG, Vlug A, Westerdal NA, Capel PJ.** 1991. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. *J Immunol* **147**:1338-1343.
- 36 **Koene HR, Kleijer M, Algra J, Roos D, dem Borne AE, De Haas M.** 1997. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. *Blood* **90**:1109-1114.
- 37 **Wilson ME and Bronson PM.** 1997. Opsonization of *Actinobacillus actinomycetemcomitans* by immunoglobulin G antibodies to the O polysaccharide of lipopolysaccharide. *Infect Immun* **65**:4690-5.
- 38 **Huizinga TW, Kleijer M, Tetteroo PA, Roos D, von dem Borne AE.** 1990. Biallelic neutrophil Na-antigen system is associated with a polymorphism on the phosphoinositol-linked Fc gamma receptor III (CD16). *Blood* **75**:213-7.
- 39 **Ziegler-Heitbrock HW and Ulevitch RJ.** 1993. CD14: cell surface receptor and differentiation marker. *Immunol Today* **14**:121-125.

- 40 **Ulevitch RJ and Tobias PS.** 1995. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* **13**:437-457.
- 41 **Maliszewski CR.** 1991. CD14 and immune response to lipopolysaccharide. *Science* **252**:1321-1322.
- 42 **Janket SJ, Baird AE, Chuang SK, Jones JA.** 2003. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **95**:559-569.
- 43 **Hansson GK, Robertson AK, Soderberg-Naucler C.** 2006. Inflammation and atherosclerosis. *Annu Rev Pathol* **1**:297-329.
- 44 **Forner L, Larsen T, Kilian M, Holmstrup P.** 2006. Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *J Clin Periodontol* **33**:401-407.
- 45 **Geerts SO, Nys M, De MP, Charpentier J, Albert A, Legrand V, Rompen EH.** 2002. Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. *J Periodontol* **73**:73-78.
- 46 **Loos BG.** 2005. Systemic markers of inflammation in periodontitis. *J Periodontol* **76**:2106-2115.
- 47 **Paraskevas S, Huizinga JD, Loos BG.** 2008. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* **35**:277-290.
- 48 **Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ.** 2000. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* **71**:1554-1560.
- 49 **Pussinen PJ, Alfthan G, Tuomilehto J, Asikainen S, Jousilahti P.** 2004. High serum antibody levels to *Porphyromonas gingivalis* predict myocardial infarction. *Eur J Cardiovasc Prev Rehabil* **11**:408-411.
- 50 **Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V.** 2007. Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events. *Arterioscler Thromb Vasc Biol* **27**:1433-1439.

-
- 51 **Fredriksson M, Gustafsson A, Asman B, Bergstrom K.** 1998. Hyper-reactive peripheral neutrophils in adult periodontitis: generation of chemiluminescence and intracellular hydrogen peroxide after in vitro priming and Fcγ₃R-stimulation. *J Clin Periodontol* **25**:394-8.