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# Aging, inflammation, immunity and periodontal disease

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Poor periodontal health, along with its increased prevalence and severity, have long been associated with more advanced age, whereby this oral condition affects the majority of the adult population, with an estimated 10–15% developing severe periodontitis (149, 184). The etiology of periodontal disease is associated with tissue damage, which is primarily initiated by an excessive immune response by the host to subgingival pathogens. The aging process is indirectly controlled by a variety of defense and/or anti-stress responses globally acting as anti-aging mechanisms (56, 57, 96). The immune system is a major player in this global anti-aging response (56, 96). To this end, aging can be characterized by quantitative and qualitative modifications of the immune system. A phenomenon known as 'immunosenescence' is a progressive modification of the immune system that leads to greater susceptibility to infections, neoplasia and autoimmune manifestations, mainly as a result of prolonged antigenic stimulation and/or stress responses across the lifespan (121, 123, 188). This global reduction in the capability to cope with antigenic stimuli and or stressors is usually coupled with a progressive increase in proinflammatory status termed 'inflammaging' (57, 166). Interestingly, this increased inflammatory status is shared by the elderly who age successfully (i.e. no comorbidities) and those who age unsuccessfully (11, 57, 117). Thus, it has been hypothesized that a threshold exists beyond which the adverse effects of immunosenescence and inflammaging drive an individual toward unsuccessful aging (57). Indeed, recent studies are showing that the coincident loss of normal innate and adaptive immune response capacity with aging, combined with low-grade chronic inflammation, coalesce to alter immunocompetence and promote the pathogenesis of a diverse number of diseases (58). In addition, it has been suggested that genetics and epigenetics can confer 'healthy' aging, and studies have provided some ranking of genes with the greatest association toward increased lifespan, some of which are immune system genes (106). To this end, elderly individuals exhibit increased susceptibilities to a number of autoimmune, infectious and inflammatory diseases, including periodontitis (73, 77, 85, 124). Indeed, aging alone leads to physiological loss of periodontal attachment and alveolar bone, but these changes are not pathological and thus have marginal clinical significance (77, 85, 98). It is only in the presence of periodontal inflammation that changes in these clinical parameters are exacerbated and lead to loss of function (1, 77, 98). This concomitant change in periodontal health and periodontal inflammation is actually seen in a significant portion of the aged population (85, 180).

The immune system is essential for survival and has evolved to defend against pathogens, as well as to maintain homeostasis within an organism. From the perspective of the characteristics of the host response to noxious challenge, the immune system can be separated into the inflammatory response, innate immunity and adaptive immunity. Inflammation is a complex biological response to harmful stimuli; this is generally a protective response involving immune cells, blood vessels and an array of soluble mediators designed to eliminate the noxious challenge and initiate tissue repair. Both acute and chronic inflammation are nonspecific responses, the actions of which are considered to be one mechanism of innate immunity. Insufficient levels of protective inflammation

leads to continued tissue destruction by the harmful stimulus and chronic inflammation contributes to an array of diseases, including atherosclerosis, rheumatoid arthritis, and periodontitis. As with the inflammatory response, innate immunity is based on nonspecific responses to the noxious challenge, for example, microbial pathogens, to which both nonimmune and immune cells respond to provide immediate defense against the infection but do not confer long-lasting protective immunity. Thus, whilst this system engages a range of protective cells and biomolecules (e.g. complement and cytokines) to provide a physical and chemical barrier to infectious agents, it is also critical for activating the adaptive immune system by presenting antigens from the pathogens to cells of acquired immunity. Adaptive, or acquired, immunity specifically recognizes the infecting agent and incorporates cells and molecules to prevent colonization, replication and tissue dysfunctions caused by the infection. A critical component of adaptive immunity is the function of 'immunological memory' with enhanced protective responses upon re-exposure to the infecting agent, and is the fundamental principle of vaccine-based immunity. Although the innate and adaptive systems are frequently described as separate entities, in reality, components of the innate and adaptive responses overlap and both systems are required to work in conjunction to maintain homeostasis and defend against insult. Thus, systems biology approaches are necessary to define the multivariate changes that occur with healthy and diseased aging (133). Franceschi et al. (60) put forth the notion of a 'network theory of aging'. This concept emphasizes a global reduction in the ability of the host-response systems to cope with an array of stressors, coupled with a progressive increase in an overall intrinsic proinflammatory environment during the aging process. The likelihood is that this 'inflammaging' status is generated through continuous antigenic load and stress changes affecting the immune system (Fig. 1). In line with the definition of immunosenescence, it has been suggested that the cumulative effect of prolonged exposure of the periodontium to microbial challenge is, at least in part, a contributor to the effects of aging on these tissues (68, 77). With that said, no significant difference in the accumulation of biofilm between young and aged subjects was observed when using an experimental gingivitis model, although the aged cohort did develop more severe gingivitis (61). Thus, it has also been hypothesized that alterations in the function of resident immune and nonimmune cells of the periodontium contribute to the phenomenon of inflammaging in periodontal disease (77, 85). Whilst the majority of aging research has focused on the adaptive immune response, it is becoming increasingly clear that the innate immune compartment is also highly affected by aging and that the reorganization of both innate and adaptive immune systems, which occurs with aging, are responsible for the phenomenon of immunosenescence and inflammaging and thus age-associated changes within the periodontium (Fig. 2).

## Innate immune cell compartment

Overall, studies in aged mice, nonhuman primates and humans have shown that activation of the aged innate immune system results in dysregulated inflammation, with dysregulation involving alterations in basal inflammation, impairment in mounting efficient innate cell effector function and induction of adaptive immunity (56) (Fig. 2). The diverse cell lineages that mediate innate immunity show heterogonous aging phenotypes in which the observed aging-related mechanisms depend on the cell and tissue context (i.e. the microenvironment) (14, 56). The term 'inflammaging' was coined to describe the phenomenon of elevated levels of pro-inflammatory cytokines, clotting factors and acute-phase proteins in the steady state (56, 57). The mechanisms that underlie inflammaging are incompletely understood

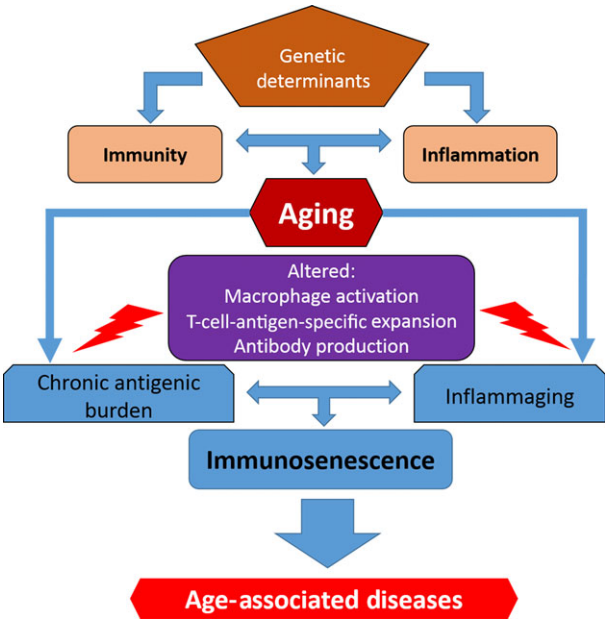


**Fig. 1. Aspects of genetic contribution to alterations in immunity and inflammation that play a role in the dysregulation of host responses in aging. The chronic antigenic burden and uncontrolled inflammatory responses (inflammaging) can then combine to enhance immunosenescence and aging-associated diseases.**

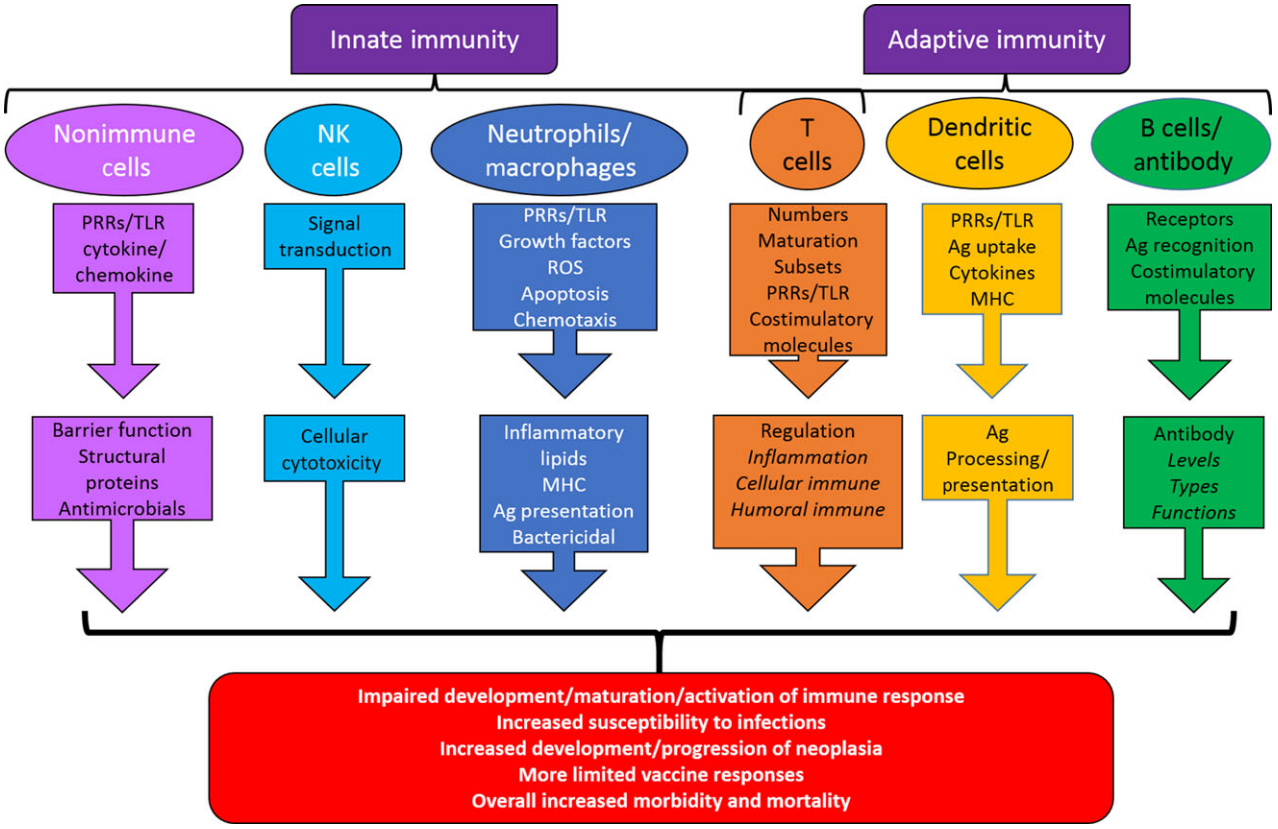


**Fig. 2. Effects of aging on cellular functions critical for innate and adaptive immunity. Changes in cellular functions, and in some cases cell numbers and cell subsets, result in the array of health-related alterations noted with aging, including increases in infections, autoimmunity, chronic inflammatory diseases and cancer. Ag, antigen; MHC, major histocompatibility complex; NK, natural killer; PRRs, pattern recognition receptors; ROS, reactive oxygen species; TLR, toll-like receptor.**

but have been suggested to involve either changes in the numbers and frequencies of innate immune cells or alterations in their functions, most likely through altered expression of, activation of, or signaling through, pattern recognition receptors (11). Importantly, age-related alterations in innate immune function are not associated with immunodeficiency but rather with dysregulation of the immune response (77).

Although the main task of the epithelium overlying mucosal surfaces is to provide an effective barrier to the vast majority of macromolecules and microorganisms, it has become clear that the epithelial layer is much more than a simple physical barrier. With that said, a defective mucosal barrier has substantive consequences beyond the mucosal surfaces and the local environment, whereby a lack of barrier integrity is linked to systemic disorders (116). As such, these declines in function at mucosal surfaces affect the ability to maintain homeostasis with the resident microbiota, as well as to respond to pathogenic challenge, compromising the overall health of the host. To this end, bacterial and viral infections of mucosal surfaces become more prevalent with aging and represent a major cause of morbidity and mortality, potentially through alterations in the molecular components of tight junctions, thus decreasing the innate barrier functions of the intestinal epithelium with aging (114). In addition, the importance of epithelial cell responses to the environment, and how they communicate warning signals to the underlying immune apparatus, may also be compromised with aging. Thus, it is plausible that similar phenomena occur with the oral cavity contributing to the development and maintenance of periodontal disease progression.

All immune cells are differentiated from a common hematopoietic precursor into either a common lymphoid progenitor or a common myeloid progenitor. Most innate immune cells, including neutrophils, macrophages and some dendritic cells, differentiate from the common myeloid progenitor. Studies have demonstrated that aged hematopoietic stem cells have reduced regenerative potential and fail to reconstitute myeloablated recipients following transplantation (11). In addition, hematopoietic stem cells from

older donors are biased toward myeloid differentiation at the expense of lymphopoiesis (13, 30, 41, 141). As such, the data suggest that most innate immune cell populations either remain stable in size or slightly decrease with age (166). Specifically, neutrophil and monocyte numbers have been reported to be unchanged in older adults (28, 36, 157, 165), whereas decreased percentages and numbers of both plasmacytoid and myeloid dendritic cells have been reported in some cases (70, 88, 138). Thus, despite the skewing of aged hematopoietic stem cells to the myeloid cell lineage, aging does not seem to be associated with global increases in the numbers of myeloid cells (41, 166).

Conversely, many aspects of the effector functions of innate immune cells appear to be affected by aging. Great emphasis has been placed on the age-related alterations in neutrophils, macrophages and natural killer cells, as these are the main innate immune cells recruited to the periodontal tissues during disease. Neutrophils are the first cells to migrate to a site of insult, including the gingival crevice, whereby they have three major methods for directly attacking microorganisms: phagocytosis; release of soluble antimicrobial peptides; and generation of neutrophil extracellular traps, which are scaffolds of extruded chromatin containing antimicrobial peptides and protease that facilitate the capture and killing of microorganisms. Here, the speed of neutrophil movement to the site of infection, known as chemokinesis, is unaltered in older individuals (189), although in contrast, their chemotaxis (i.e. response to a gradient of stimulus) is significantly impaired (130, 189). Importantly, this function affects both the migration to and egress from inflammatory sites (166). Thus, such alteration in this cell trafficking could affect not only the initiation but also the resolution of inflammation in aging. To this end, the expression of a negative regulator of neutrophil endothelial transmigration, named Del-1 (EDIL3), was significantly diminished with age in mice. Del-1 is an endogenous inhibitor of lymphocyte function-associated antigen 1 (an integrin that is key for neutrophil endothelial adhesion and further transmigration), whereby Del-1-deficient mice developed spontaneous periodontitis characterized by excessive gingival neutrophil infiltration. (47). Neutrophils from older individuals also demonstrate impaired phagocytosis as well as diminished capacity to kill phagocytosed microorganisms (23, 54, 67, 167, 181). Similarly, neutrophil extracellular trap formation has been demonstrated to be decreased in aged mice, but it remains to be determined whether neutrophil extracellular traps are altered in aged humans (23, 181). An additional factor that may contribute to the reduced antimicrobial activity is the increased susceptibility of neutrophils from older individuals to apoptotic signals (24). Finally, age-specific changes appear in the antimicrobial arsenal of neutrophils, rendering them less capable of killing pathogens as well as altering the necessary cross-talk with other immune cells necessary for the resolution of inflammation (182). Although mechanisms associated with the age-associated defects in neutrophil effector function cannot be linked to changes in expression of receptors (77), because of the age-associated decrease in cell-membrane fluidity as a result of increased cholesterol content of the lipid rafts, alterations in receptor engagement have been proposed to contribute to

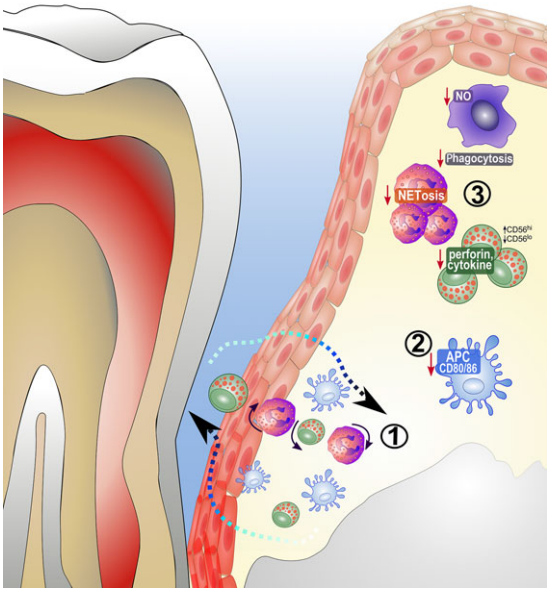


**Fig. 3. Age-associated alterations in innate immune function within the periodontium. 1. The chemotaxis of innate immune cells, such as neutrophils, macrophages, dendritic cells and natural killer cells, is significantly impaired in aged individuals. Importantly, this function affects both the migration to and egress from inflammatory sites. Thus, such alteration in this cell trafficking could affect not only the initiation, but also the resolution, of inflammation. 2. In older individuals, the antigen-presenting function of innate immune cells, such as dendritic cells, is dysfunctional because of decreased expression of costimulatory proteins. APC, antigen-presenting cell. 3. Innate immune cells from older individuals demonstrate impaired phagocytosis as well as diminished capacity to kill phagocytosed microorganisms through decreased neutrophil extracellular traposis (NETosis) in the case of neutrophils, decreased nitric oxide production in the case of macrophages and decreased perforin and cytokine production in the case of natural killer cells. This ineffective innate immunity leads to chronic persistence of pathogens and unresolved destructive inflammation.**

these altered functions (67, 100). In addition, impaired signal-transduction functions mostly centered on the Janus kinase–signal transducer and activator of transcription (JAK–STAT) signaling pathway have been reported (52, 53, 180). Under conditions of periodontal health, neutrophils are also present within the gingival crevice, albeit at low numbers, to maintain homeostasis between the biofilm and the periodontal tissues. Thus, it could be envisioned that these age-associated alterations result in an influx (and retention) of ineffective neutrophils that fail to contain an expanding biofilm through decreased effector functions and increased apoptosis. Through this increased neutrophil cell death, there is an increase in the release of toxic substances, which have the potential to initiate and perpetuate damage to the periodontal tissues. While these products may not be produced at high quantities on a per-cell basis in older age, in the setting of chronic inflammation, such as periodontal disease, tissue damage could result from repeated and chronic exposures to these neutrophil products (77).

Shortly after the arrival of neutrophils, macrophages and dendritic cells appear within the periodontal tissues, whereby they too can directly attach microorganisms through phagocytosis and release of soluble antimicrobial peptides. Similarly to neutrophils, defects in the migration of these phagocytes have been reported in older individuals (1, 166, 197). In addition, age-associated impairment in phagocytosis of apoptotic cells and microorganisms, as well as declines in nitric oxide production, have been reported in humans (1, 8, 82, 166). Specifically, autophagy, which allows for the delivery of endogenous or exogenous biomolecules to specific intracellular compartments and thus is an important mechanism of delivery of antigens from pathogens to major histocompatibility complex-class I and II compartments for processing and presentation, can malfunction during aging. This process regulates phagocytosis, modulates inflammasome function and controls cytokine/chemokine production (35), whereby, during aging, phagocytosis and nitrite burst as microbiocidal capabilities are reduced, whereas proinflammatory cytokine responses are significantly increased. Thus, this age-related modulation of autophagy would be predicted to allow excess inflammation and undermine macrophage function, contributing to decreased protective immune responses and supporting an increase in chronic inflammation (173). Variable results regarding the impact of aging on phagocytosis have been obtained using aged and young mouse or rat macrophages (73, 150). In the context of periodontal disease, macrophages from young and old mice exhibit comparable capacities for the uptake of *Porphyromonas gingivalis*, a major periodontal pathogen (103).

A unifying model for aging impact on dendritic cell antigen processing and presenting capabilities has been put forth, with aging impairing the antigen-presenting functions of dendritic cells under some biological conditions, but preserving this dendritic cell function under other conditions associated with aging (192). In addition, dendritic cells from aged humans show impaired capacity to ingest apoptotic cells, whilst demonstrating enhanced proinflammatory responses and increased reactivity to self-antigens (76). Therefore, a defective maturation of dendritic cells in aging may alter the balance between their tolerigenic and immunogenic functions (2). An important function of both macrophages and dendritic cells is the induction of adaptive immunity through the processing and presentation of antigen to lymphocytes. Studies of the antigen-presenting function in aged mice have generally shown impairment in this function (102, 127, 147, 151, 192). Specifically, studies have demonstrated an age-dependent decrease in the expression of costimulatory proteins and the ability to prime T-cells (102, 127). Owing to the relatively small numbers of subjects evaluated, whether these processes are altered in human cells remains unclear, although these limited studies have demonstrated a preservation of the antigen-presentation function in human macrophages and dendritic cells (32, 166, 171).

A final key function of these innate immune cells is to set the stage for the 'flavor' of the adaptive immune response, invoked through a coordinated expression of adhesion molecules, chemokines and cytokines following the engagement of pattern recognition receptor. As an example, high levels of interleukin-6, interleukin-1, tumor necrosis factor-alpha and C-reactive protein are detected in older individuals and are generally correlated with frailty and an increased risk of morbidity and mortality (123). Aging is also associated with aberrant pattern recognition receptor expression on, and impaired pattern recognition receptor signaling within, innate immune cells (166). To this end, Dunston & Griffiths (40) hypothesized that despite a poor response via pattern recognition receptor activation, the ineffective clearance of pathogens by macrophages increases the duration of their activation and contributes to perpetuation of deleterious inflammatory responses with aging. Although there are multiple classes of pattern recognition receptor, with even more members within in

each class, the majority of aging research has focused on toll-like receptor biology. Decreased surface expression of toll-like receptor-1, toll-like receptor-3 and toll-like receptor-8 by human monocytes and myeloid dendritic cells, along with decreased expression of toll-like receptor-7 and toll-like receptor-9 in human plasmacytoid dendritic cells, have been associated with aging (88, 140). These decreases in expression correlate with diminished ligand-induced cytokine production (166). Specifically, multiple subsets of monocytes from older adults produce less tumor necrosis factor-alpha and interleukin-6 in response to toll-like receptor-1/2 stimulation, with an associated decrease in mitogen-activated protein kinase signaling (132, 166, 39). Like monocytes, primary myeloid dendritic cells and plasmacytoid dendritic cells have a generalized age-associated decreased in toll-like receptor-induced cytokine production, including interleukin-12, interferon-gamma and type 1 interferons induced by toll-like receptor-4, toll-like receptor-7 and toll-like receptor-9, respectively (26, 154, 168, 185). Again, in the context of periodontal disease, *P. gingivalis* induction of interleukin-6 in macrophages was shown to decline with age (103, 159). In addition, toll-like-receptor-dependent expression of the costimulatory molecules CD80 and CD86 are also blunted in monocytes, myeloid dendritic cells and plasmacytoid dendritic cells from older individuals (154, 168). Conversely, lipopolysaccharide-induced production of tumor necrosis factor-alpha by $CD14^+$ $CD16^+$ human monocytes is increased with age (82). In addition, toll-like-receptor-5-induced production of interleukin-8 and interleukin-6 in adherent human macrophages also exhibits an age-associated increase (153). Similarly, tumor necrosis factor-alpha and interleukin-6 production following toll-like receptor-4 or toll-like receptor-8 stimulation, and tumor necrosis factor-alpha and interferon-alpha production following exposure to self-DNA were elevated in human myeloid dendritic cells from aged subjects (1, 3). Finally, basal levels of intracellular cytokines in primary myeloid dendritic cells and plasmacytoid dendritic cells are elevated in older individuals compared with younger adults (140). Similar phenomena are observed in the context of periodontal disease where macrophages from older individuals produce higher levels of prostaglandin $E_2$ in response to *P. gingivalis* than those from younger controls (75, 131). Although the property of prostaglandin $E_2$ to inhibit interleukin-12 may compromise the capacity of older individuals to clear infection (150), prostaglandin $E_2$ is also heavily implicated in periodontal tissue destruction [127] and thus its elevated levels could account, at least in part, for the increased inflammation observed in older individuals.

Natural killer cells are a type of cytotoxic lymphocytes belonging to the group of innate lymphoid cells also critical to the innate immune system. Infiltration of the gingival tissues by natural killer cells has been demonstrated in periodontal disease, whereby natural killer cells are activated in chronic periodontal lesions (129). Specifically, interferon-gamma production by natural killer cells has been shown to be triggered by co-culture stimulation of dendritic cells with *P. gingivalis* or *Aggregatibacter actinomycetemcomitans* (93, 94). In addition, a recent report describes natural killer cell-specific recognition and activation by the periodontal pathogen *Fusobacterium nucleatum* (29). In humans, natural killer cells can be broadly divided into a $CD56^{low}$ population with cytotoxic activity and a $CD56^{hi}$ population that is responsible for cytokine production (25). In older humans, cytotoxicity of the $CD56^{low}$ population is decreased on a per-cell basis as a result of a defect in the mobilization of perforin to the immunological synapse (81, 166). Similarly, an age-dependent decrease in cytokine expression by $CD56^{hi}$ natural killer cells has also been observed (166). Interestingly, there is an expansion of the $CD56^{low}$ natural killer cell compartment concomitant with a diminished number of $CD56^{hi}$ natural killer cells upon aging (166).

Interestingly, some of the earliest studies addressing questions associated with aging-related innate immune responses within the periodontium were specifically focused on epithelial cells and gingival fibroblasts; both cell types have recently been shown to be active players in innate immunity. An increased proliferative activity with aging of various periodontal tissues was reported in earlier studies using a short-lived mouse strain, in particular within the gingival epithelium and connective tissue of the crevicular region (119, 169). However, further studies did not find significant differences in cell proliferation or apoptosis in gingival tissues from older mice by immunohistochemistry (163). Rather than changes in cell proliferation, senescent human gingival fibroblasts appear to exhibit important functional changes in the production of important immunoinflammatory mediators. Thus, aged gingival fibroblasts (i.e. cells after 17–20 population doublings) produced higher amounts of interleukin-6 in response to lipopolysaccharide from *Campylobacter rectus in vitro* compared with young cells (five to six population doublings) (135). Similarly, increases in the production of prostaglandin $E_2$ by aged gingival fibroblasts, with respect

to the young counterpart, were shown to be associated with higher levels of interleukin-1beta produced in response to lipopolysaccharide from *C. rectus* (176). These observations were later confirmed *ex vivo* by comparing the interleukin-1beta and prostaglandin $E_2$ responses of gingival fibroblasts from old (20 months of age) and young (6 weeks of age) rats (136). Most recently, variations in the magnitude of transcriptional and translational responses of gingival fibroblasts from C57BL/6 mice in response to *P. gingivalis* (W83 strain) were reported. In particular, decreased transcription of interleukin-6, chemokine (C-X-C motif) ligand 1, vascular endothelial growth factor, keratinocyte growth factor, fibroblast growth factor-2, bone morphogenetic protein-2, tissue inhibitor of metalloproteinase-1 and tissue inhibitor of metalloproteinase-2 was observed, whereas transforming growth factor-beta, matrix metalloproteinase-2, matrix metalloproteinase-3, and matrix metalloproteinase-13 were up-regulated in cells from aged animals (38). These results reinforce the hypothesis that age-related changes in the ability of gingival cells to respond to bacterial challenges and orchestrate tissue repair could be involved in a higher risk of periodontitis with aging.

Recently, the nonhuman primate (Rhesus) model of periodontal infection and inflammation has been used to document the aging-associated gingival transcriptome changes of different innate immune response pathways. This model has been used for several decades to understand the mechanisms associated with periodontal disease (44, 83, 115, 139, 148, 164), and represents an excellent model for studies in aging (21, 43, 160, 162). In summary, the transcriptome of aged gingival tissues in nonhuman primates exhibits significant differences in innate immune pathways that are critical for maintaining a symbiotic relationship with the oral microbiome, and could predispose to dysbiosis and periodontitis (Fig. 4). In particular, aging seems to enhance a gingival environment that can enrich for anaerobic species and increases the likelihood for pathogenic species to invade and persist inside the cells, ultimately leading to a dysregulated and persistent immunoinflammatory response. Importantly, mechanistic studies targeting these pathways/genes to validate their role in increasing the risk of periodontitis with aging need to be developed.

As a whole, these data describe age-associated alterations in innate immunity and their consequences, which results in the paradox of suppressed innate immune function in the face of the observed systemic low-grade chronic inflammation associated with aging and periodontal disease. However, these data also reflect that the effects of aging on innate immune function are complex, with evidence for both inappropriately impaired and augmented responses to pattern recognition receptor engagement that reflects ligand, cell type, cell-activation state and tissue-context specificity. In general, evidence suggests that aging significantly impacts critical innate cellular and molecular responses of gingival tissues that could be contributing to a higher risk for periodontitis with age. Specifically, the ability of gingival tissues/cells to sense and respond to microbes (in particular invasive pathogens), appears to be critically compromised with aging. In addition, important negative regulatory mechanisms for inflammation and tissue repair are also affected by aging. It is unclear at this point if these changes are related to a specific cell type (epithelial cells, fibroblasts or immune cells) or if it is a general characteristic for the entire periodontium. Importantly, whether these age-related transcriptional changes are caused by intrinsic factors (cellular senescence) leading to disease susceptibility, or reflect alterations that are driven by environmental factors (oral microbiome, diet or oral hygiene), remains to be elucidated.

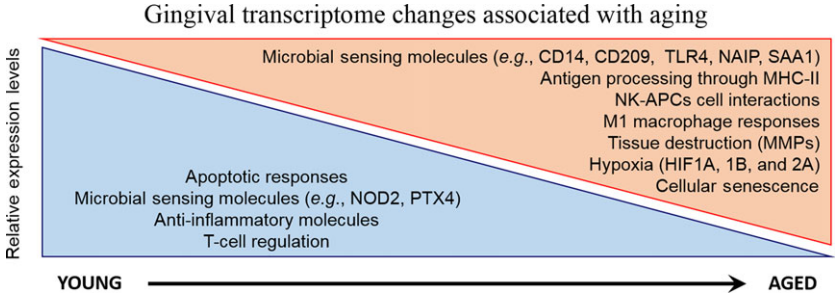


**Fig. 4. Summary of various aspects of the host innate and adaptive immune systems that have been determined to be altered in aging, leading to increases in the expression and severity of periodontitis. APC, antigen-presenting cell; CD, cluster of differentiation; HIF, hypoxia inducible factor; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NAIP, Baculoviral IAP repeat-containing protein 1; NK, natural killer; NOD, Nucleotide-Binding Oligomerization Domain; PTX, pentraxin; SAA1, serum amyloid A1; TLR, toll-like receptor.**

## Adaptive immune-cell compartment

As previously alluded to under the condition of gingival health, there is some minimal inflammation, including the presence of adaptive immune cells (Fig. 5). The adaptive immune system is equipped with two key facets: diverse antigen-recognizing lymphocyte populations (naïve lymphocytes); and very-long-lived antigen-experienced lymphocytes (memory lymphocytes). If the innate immune system is unsuccessful in eliminating the antigenic stimulus or stressor, it induces and recruits cells of the adaptive immune response. Here, T-cells and B-cells interact with antigen-presenting cells, become activated, proliferate and migrate to the site of inflammation. B-cells produce antibodies that target extracellular pathogens. T-cell populations include $CD8^+$ cytotoxic T-cells that lyse infected cells and $CD4^+$ T-helper cells that secrete a wide range of cytokines and activate other components of both the innate and the adaptive immune response. Despite a continuous decline in the generation and maintenance of adaptive immune+e-cell populations after puberty, the adaptive immune system adjusts to these age-associated changes and protects the body successfully against most insults during nearly all of adult life (188). Only in the late phase of life does the progressive decline of immune function lead to morbidity and mortality in the aged (188) (Fig. 5). The main alteration responsible for immunosenescence is the overall decline of antigen-specific immunity caused by a substantial decrease in the number of naïve lymphocytes as a result of a reduction in thymic output of T-cells and the generation of progenitor B-cells in the bone marrow (109, 188). In the periodontium, a low number of these lymphocytes are suggested to be critical for maintaining a homeostasis between the host periodontal tissues and the bacterial plaque, similar to that of the neutrophil population (42). Thus, it can be envisioned that a decrease in the frequency of these cells in the aged can lead to a decrease in the ability to keep subgingival infection at bay in older individuals.

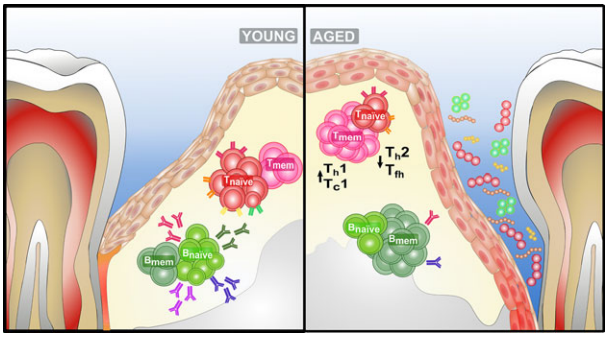


**Fig. 5. Age-associated adaptive immunity alterations in periodontal disease. Age-related decreases in the percentage and number of naïve and regulatory T- and B- cell lymphocytes, coupled with an increase in the size of the memory T-cell pool, results in a decrease in the diversity of both the T- and B-cell antigen-recognition repertoire. In addition, T-cells from older individuals have a more T helper-1 and Tc1 cytotoxic effector phenotype, producing an intrinsic change to a more inflammatory/cytotoxic environment. This age-induced narrowing and skewing of the lymphocyte compartment results in impaired and augmented responses, respectively. Thus, the heightened inflammation observed in the periodontium of aged individuals may be a secondary effect arising from the inability to elicit appropriate and effective adaptive immune responses as a result of age-specific alterations that would normally effectively control microbial overgrowth in the periodontal tissues. $B_{mem}$, memory B-cells; $B_{naïve}$, naïve B-cells; $T_c1$, T cytotoxic cell; $T_{fh}$, T follicular helper cell; $T_h1$, T-helper 1 cell; $T_h2$, T-helper 2 cell; $T_{mem}$, memory T-cells; $T_{naïve}$, naïve T-cells.**

Age-associated thymic involution involves reduction in size of the organ and an increase in its adipocyte content, resulting in a decreased output of naïve and regulatory T-lymphocytes, which is concomitant with an increase in the size of the memory T-cell pool (57, 96, 108, 109, 142, 143, 196). The B-cell compartment is similarly affected by aging, whereby there is an age-related decrease in the percentage and number of B-cells in the blood of humans, including naïve B-cells, again concomitant with an accumulation of antigen-experienced memory B-cells (4, 5, 62–64, 66, 89, 125). This is specifically related to the pre-B-cell-receptor developmental stage that normally signals several rounds of proliferation resulting in an expanded population, leading to a complex set of B-cell-specific epigenetic changes with allelic exclusion of the heavy chain locus and activation of the light chain loci for crucial V(D)J recombination and construction of the effector antibody repertoire (71). The aging output in B-cell development has been suggested to reflect cellular pathways that may be progressively diverted into a pre-B-cell-receptor compromised pathway, minimizing these final stages of B-cell maturation and antibody heterogeneity (161). Although B-cell production and differentiation in the bone marrow declines with age in mice, whether this is the mechanism associated with the observed decrease in humans is unclear (5). Together this results in an age-dependent decrease in the diversity of both the T- and B-cell antigen-recognition repertoire. This phenomenon is also reflected in the periodontal lesion of older individuals with chronic periodontitis in that (i) the macrophage to T-cell ratio is increased; and (ii) the infiltrating T-helper and

cytotoxic T-cell populations have a more memory-like phenotype (42, 45). In addition, under conditions of health, immunoglobulins of all isotypes are generally present at low levels in the gingival crevicular fluid (42), yet certain subclasses of IgG appear to be specifically elevated in the gingival crevicular fluid in active sites of aged individuals with chronic periodontitis (158). In addition, the specificity of the antibodies present under conditions of periodontal health are directed toward a myriad of microorganisms associated with the normal microbiota (both supragingival and subgingival species) (42), whereas under conditions of disease these antibodies are specific for a smaller number of microorganisms, including *P. gingivalis* and/or *A. actinomycetemcomitans* (42). This contributes, in part, to the decrease in activation-induced cytidine deaminase, a critical enzyme for immunoglobulin class switch recombination and somatic hypermutation, both of which are required for antibody diversity and maturation leading to the generation of protective memory responses. These findings, coupled with identification of elevations in tumor necrosis factor-alpha within the 'inflammaging' processes, have been shown to predispose B-cells to respond less well to initial stimulation, resulting in molecular changes that impact affinity maturation and production of less effective antibody (17). Together these data suggest that aging-induced narrowing and skewing of both the T- and B-cell repertoire and diversity occurs in chronic periodontal diseases.

Along with alterations in frequency, there is also a functional decline in adaptive immunity with age. Specifically, naïve $CD4^+$ T-cells from aged individuals display decreased *in vitro* responsiveness to T-cell-receptor stimulation and altered profiles of cytokine secretion when compared with those from younger adults (4, 174). This change in cytokine-secretion profile reduces the B-cell helper function of these T-cells (174). Specifically, naïve CD4 T-cells from aged individuals are less responsive to interleukin-6 signaling and this reduces their ability to become T-helper-2 and/or T-follicular helper effector cells, each of which provides B-cell help (196). Instead, naïve CD4 T-cells from older individuals still differentiate into T-helper-1 cell and CD4 cytotoxic effector subsets, producing an intrinsic change to a more inflammatory/cytotoxic environment (59). Indeed following vaccination, aged mice and humans develop similar levels of IgM as younger cohorts, but lower levels of total IgG and all the IgG isotypes (170, 187). These alterations in IgG responses are partly the result of age-related impaired class-switching, most likely caused by the decreased induction of activation-induced cytidine deaminase (27, 65) and/or an intrinsic variable and heavy chain repertoire shift (97, 101). Again, this myriad of aging-associated phenomena are manifested in chronic periodontal diseases whereby chronic periodontitis lesions are enriched for $CD8^+$ T-cells where there is an imbalance toward T-helper-1 cell and Tc1 polarization and the T-helper-2 cytokine profiles (i.e. those that would provide help for antibody production) are less prominent (177). This imbalance of cytokine production not only affects soft-tissue destruction but also promotes the induction of bone resorption, the hallmark of chronic periodontal disease (177).

Using the nonhuman primate gingival transcriptome data, Gonzalez et al. have also been documenting changes within adaptive immune system pathways. A principal finding in aging healthy gingival tissues was significant increases in gene expression in the major histocompatibility complex-II pathway, including the intracellular molecules that are required for processing native proteins into short peptide fragments (74). Parallel to the up-regulation of the exogenous antigen-presentation pathway was a down-regulation of major histocompatibility complex-I pathway genes in aging healthy gingival tissue. The results demonstrated no changes in genes related to interactions with $CD4^+$ helper T-cells and a minimal effect on $CD8^+$ T-cells in healthy aging tissues. However, increases in expression of selected genes related to natural killer cell interactions with antigen-presenting cells in the aging healthy gingival tissues suggested the potential for down-regulating the function of these cells in the aged tissues.

As a whole, these data describe age-associated alterations in adaptive and innate immunity and their consequences, which again results in the paradox of less robust adaptive immunity in the face of the observed systemic low-grade chronic inflammation associated with periodontal disease. However, these data also reflect that the effects of aging on the narrowing and skewing of the lymphocyte compartment result in impaired and augmented responses, respectively, which have the ability to contribute to the progression of periodontal disease progression in the aged.

## Osteoimmunology compartment

The effects of aging on periodontal tissues have been suggested to be based on molecular changes in the array of cells of the periodontium, the combination of which is thought to intensify alveolar bone resorption in elderly individuals. These effects are considered to

be reflective of: (i) altered differentiation/proliferation of cells for coupling bone biological processes (osteoblasts, osteoclasts); (ii) enhanced responses to the oral microbiota, modified by environmental stressors leading to the secretion of cytokines/chemokines involved in osseous resorption; and (iii) systemic endocrine alterations related to host responses and physiologic/pathologic bone responses with aging (85) (Fig. 6). Numerous immune and nonimmune cells within the periodontium communicate with both osteoblasts and osteoclasts, modulating the balance of physiologic bone formation and resorption, whereby bone metabolism can also be modulated by multiple factors, including aging (48, 175). In particular, aging affects the maintenance of normal bone remodeling and metabolism, which leads to increased bone resorption, inherent changes in bone architecture and less resistance to fracture (37). In addition, aging enhances both RANK expression on osteoclast progenitors and RANKL expression in the supporting mesenchymal stromal cells, leading to a pro-osteoclastogenic environment shifting the balance toward potentiated bone resorption (31, 33). Similarly, advances in the field of osteoimmunology clearly show that cytokines produced as a result of the associated innate and adaptive host responses also contribute to the disruption of local bone homeostasis. Here, select proinflammatory cytokines (e.g. prostaglandin $E_2$, tumor necrosis factor-alpha, interleukin-1beta, interleukin-6 and interleukin-17) appear to play a critical role in enhancing osteoclastogenesis (20), whereas other molecules [e.g. interferon-beta, interleukin-4, interleukin-10 and chemokine (C-C motif) ligand 22] provide a molecular feedback dampening the bone-loss processes (9). Similarly, whilst T- and B-cells have both been shown to be a major source of RANKL in the diseased periodontium (91), T-cell responses do not necessarily have deleterious outcomes as T-cell-secreted cytokines, such as interferon-gamma, interleukin-4 and transforming growth factor-beta have also been shown to inhibit osteoclastogenesis (134, 179). Thus, one can imagine how previously discussed age-related alterations in the function of both innate and adaptive immune cells can directly affect bone metabolism and remodeling involved in periodontal disease.

As previously mentioned, a transcriptomic study was conducted using a nonhuman primate model of naturally occurring periodontitis in which the profile of the genes associated with osteogenic potential during aging and periodontal disease was assessed (J. L. Ebersole, unpublished). Data generated here suggest a transcriptome environment that reflects a more general osteogenic homeostasis in aging healthy tissues, with the potential to regulate osteoclast and osteoblast functions to help control inappropriate microbial stimulation from the accretion of oral biofilms. Alterations of gene expression in periodontitis, particularly in aged animals, were skewed toward creating an environment with substantial osteoclastogenic potential, consistent with the bone resorption in periodontitis. Specifically, genes regulating osteoclast function were up-regulated in healthy gingival tissues with aging, which include bone morphogenetic protein-3, matrix metalloproteinase-8 and

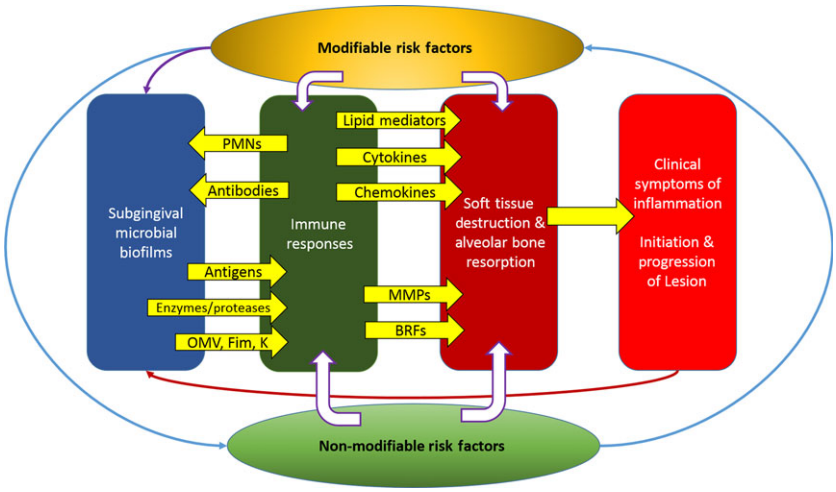


**Fig. 6. Interrelationships of bacterial biofilm stimulation, host responses and collateral effects on host tissues in periodontitis. Modifiable factors include diet and smoking, and nonmodifiable factors focus on genetic predisposition to response characteristics and soft tissue and bone biology of an individual (Adapted from *Carranza's Clinical Periodontology*, 10th ed. WB Saunders Company). BRF, bone resorbing factors; Fim, bacterial fimbriae; K, bacterial capsules; MMPs, matrix metalloproteinases; OMV, outer membrane vesicles; PMN, polymorphonuclear neutrophil.**

secreted phosphoprotein 1. Interestingly, a number of genes changing their expression with periodontitis, overlapped with those changing with aging in healthy tissues, including secreted phosphoprotein 1 and toll-like receptor-4. Secreted phosphoprotein 1 encodes osteopontin, which is a major constituent of mineralized matrix and a ligand for integrin αvβ3 (104). It is also involved in enhancing production of interferon-gamma and interleukin-12. High osteopontin levels have been previously noted in inflammation and in sepsis, as inflammatory cytokines (such as tumor necrosis factor-alpha and interleukin-1beta) modulate its expression (193) and is known to induce T-helper-1 cytokines and recruitment of inflammatory cells (113). In addition, up-regulation of genes for alkaline phosphatase, proto-oncogene c-Fos and matrix metalloproteinases also indicate an environment conducive to enhanced bone metabolism. Together these data indicate that local bone-resorptive processes are up-regulated under the condition of aging, creating a tissue-destructive environment.

## Genetic and epigenetic regulation of immune aging

Throughout life, the human body attempts to integrate key biological responses in order to generate a comprehensive defense network that protects us against threats to our survival. These integrated responses are often influenced by a person's genetics, as well as behavioral choices. Genetic variations, especially those that slow the process of aging, can in part explain why some individuals 'age well'. Centenarians are individuals who live to the age of 100 and beyond and thus represent a highly unique group of people that can be studied to gain a better understanding of the genetics influencing the aging process. To date, centenarian studies have identified several key genes and/or genetic variations that appear to influence longevity in humans, many of which lie in pathways that influence the overall defense network for survival, such as DNA damage repair, immunity, inflammation and host defense to cancer (Fig. 7; Table 1). One important take-home lesson from these findings is that aging is a complex phenotype, which is influenced by the entire genome that one inherits, typically not by a single gene or genetic variation. Thus, whilst one individual may have a combination of genetic variations that promote longevity, reduce inflammaging and/or provide enhanced disease protection, another individual may arrive at the same outcomes with a completely different combination of genetic variations. Moreover, in some cases the same genetic variation can lead to different results when comparing different ethnic groups and/or genders. A few of the key genetic variations identified in centenarian studies that can influence the processes of aging as they relate to inflammation are described below.

### Interferon gamma

Genetic variations within the interferon-gamma gene have been shown to be associated with reduction in protein production and thus enhanced longevity.

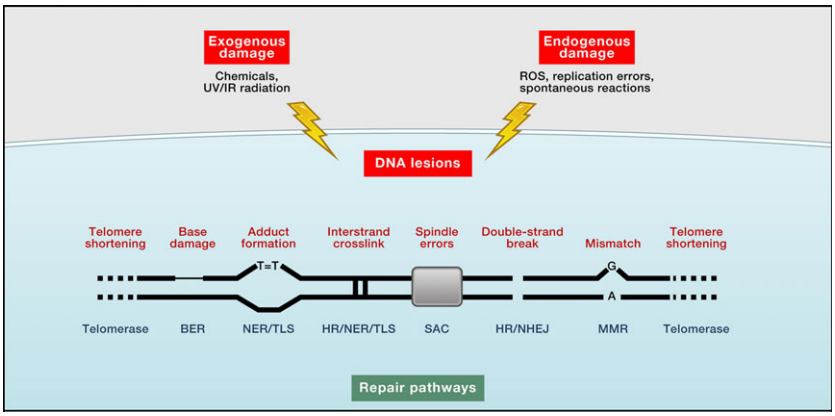


**Fig. 7. Genomic and epigenomic alterations (112). (Used with permission.) Genomic instability and telomere attrition. Endogenous or exogenous agents can stimulate a variety of DNA lesions that are schematically represented on one single chromosome. Such lesions can be repaired by a variety of mechanisms. Excessive DNA damage or insufficient DNA repair favors the aging process. Note that both nuclear DNA and mitochondrial DNA (not represented here) are subjected to age-associated genomic alterations. BER, base excision repair; HR, homologous recombination; IR, infrared; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end-joining; ROS, reactive oxygen species; SAC, spindle assembly; TLS, translesion synthesis; UV, ultraviolet.**

**Table 1.** Human gene polymorphisms that are related to aging/longevity, and immune-response genes affected by aging.

| Single nucleotide polymorphism | Chromosome | Near or within gene | Population and/or phenotype connected | Author/Reference |
|---|---|---|---|---|
| **Tumor suppressor genes** | | | | |
| rs2802292 (G/T) | 6q21 | Forkhead box O3 transcription factor | Japanese male centenarians<br>Southern Italian centenarians<br>German centenarians<br>G allele – longer lifespan<br>T allele – shorter lifespan | Willcox et al. (191)<br>Anselmi et al. (6)<br>Flachsbart et al. (51) |
| rs1935949 (C/T) | 6q21 | Intronic single nucleotide polymorphism in the forkhead box O3 gene | Longevity among women | Pawlikowska et al. (146) |
| Rs1042522 (C/G)<br>Arg72Pro | 17p13.1 | Tumor protein p53 | CC genotype – extended lifespan<br>GG genotype – less effective response to chemotherapy for cancer<br>C allele – risk allele for breast cancer | Johnson et al. (90)<br>Bojesen & Nordestgaard (18)<br>Han et al. (79)<br>Kim et al. (95) |
| **Immune response/inflammation related** | | | | |
| rs62559044 (T/A) | 9p21.3 | Interferon gamma | Proinflammatory response<br>A allele – reduced interferon gamma production; increased odds of longevity in women | See text |
| rs1800795 (−174; G/C) | 7p15.3 | Interleukin-6 | Proinflammatory response<br>G allele – more interleukin-6 production<br>C allele – less interleukin-6 production<br>CC genotype – reduced longevity; increased chronic inflammation | See text |
| rs1800629 (−308; A/G) | 6p21.33 | Tumor necrosis factor alpha | Proinflammatory response<br>A allele – higher levels of tumor necrosis factor production | See text |
| rs361525 (−238; A/G) | 6p21.33 | Tumor necrosis factor alpha | Proinflammatory response | See text |
| rs2069762 (−330) | 4q26 | Interleukin-2 | Immunomodulation<br>TT genotype – increased interleukin-2 production | See text |

**Table 1.** (*Continued*)

| Single nucleotide polymorphism | Chromosome | Near or within gene | Population and/or phenotype connected | Author/Reference |
|---|---|---|---|---|
| rs4986790 (+896; A/G) Asp299Gly | 9q33.1 | Toll-like receptor-4 | Immune system activation; detection of lipopolysaccharide from gram-negative bacteria<br>A allele associated with myocardial infarction<br>G allele associated with longevity | See text |
| rs1800896 (−1082; G/A) | 1q32.1 | Interleukin-10 | Anti-inflammatory response<br>A allele – reduced interleukin-10 production | See text |
| rs3803304 (C/G) | 14q32.33 | AKT1 | Intracellular signaling; serine/threonine-specific protein kinase; longevity | Pawlikowska et al. (146) |

Specifically, the 'A' allele at the single nucleotide polymorphism location +874 within the interferon-gamma gene (rs62559044; T/A) is associated with reduced interferon-gamma production when compared with the T allele (152). In addition, in studies of Italian centenarians (110), the 'A' allele significantly increased a woman's chance of achieving increased longevity, whereas the TT genotype at this single nucleotide polymorphism locus has also been associated with less desirable kidney allograft outcomes (34). Together these data support the notion that the T allele contributes to a stronger proinflammatory response than does the A allele, potentially resulting in improved longevity.

## Interleukin-6

Inflammaging is also characterized by increased serum levels of interleukin-6 (59, 69, 72, 190, 195), whereby high plasma levels of interleukin-6 in older individuals can serve as a marker of reductions in muscle strength and increased frailty risk (12, 46). Studies of the interleukin-6 promoter variation (−174), rs1800795, have shown that individuals who inherit the rs1800795 C-allele tend to have elevated interleukin-6 transcription and thus increased protein expression compared with individuals who inherit the rs1800795 G-allele (15, 50). To this end, genetic studies have demonstrated that the CC genotype at this locus is associated with medical conditions such as reduced longevity [19, 84], predisposition to atherosclerosis (87), reductions in favorable clinical outcomes following coronary revascularization surgery (16), an increased risk of organ rejection after allograft transplant (128, 145), Alzheimer's disease (105) and severe periodontitis (10, 126).

## Tumor necrosis factor-alpha

The human tumor necrosis factor-alpha single nucleotide polymorphisms, rs1800629 (−308 promoter polymorphism; A/G) and rs361525 (−238, promoter polymorphism; A/G), have the potential to influence transcription factor binding, and ultimately expression of the tumor necrosis factor-alpha gene (155). Specifically, the single nucleotide polymorphism rs1800629 A-allele has been associated with higher levels of expression of tumor necrosis factor-alpha, whereby the risk of acute kidney allograft rejection was shown to be increased in individuals with the A allele (144). This single nucleotide polymorphism variation has also been connected to autoimmune disease, cardiovascular disease and

chronic inflammatory disease (7, 49, 122, 137). Together these observations suggest a genetic predisposition associated with an augmented tumor necrosis factor-alpha response.

## Interleukin-10

Studies of the interleukin-10 single nucleotide polymorphism rs1800896 (−1082 promoter mutation; G/A) have suggested that variation at this site can influence longevity in a gender-specific manner. Here, the rs1800896 A-allele results in low levels of interleukin-10 production, whereas the GG genotype is associated with high interleukin-10 production. The latter genotype has been associated with centenarian men but not with centenarian women (111), as well as with longevity in Jordanian men (92); the reason for the gender bias is unknown. Conversely, the single nucleotide polymorphism rs1800896 A-allele has been associated with a high risk of developing prostate and colorectal cancer (183, 186). Both types of cancer have higher prevalence later in life, and appear to have inflammatory components. Again, these observations suggest a genetic predisposition associated with altered inflammatory responses linked to inflammaging and longevity.

Based on evidence generated by studying monozygotic (identical) twins, we now know that changes in a person's epigenetic landscape also play a role in aging. In addition, aging itself contributes to the initiation of deleterious processes within cells, including epigenetic modification of gene expression that can affect normal cellular functions (178). In Spain, researchers have demonstrated that identical (monozygotic) twins show very similar epigenetic landscapes at birth and in early life (55). As the twins age, however, they can be differentially exposed to dietary and other environmental factors, and can differentially develop disease, even with their essentially identical DNA sequences (genome). It was originally thought that environmental factors directly affect the differential development of diseases in identical twins, but now there is a growing appreciation of how environmental factors can differentially affect the 'epigenome' of the monozygotic twins, resulting in potentially vastly different patterns of gene expression through mechanisms such as methylation, microRNAs, noncoding RNAs and histone acetylation (Fig. 8).

Specific data show that changes in DNA methylation (the 'methylome') are among the mechanisms contributing to the aging process, including the immune system (156). There was hypermethylation of 8,540 DNA CpG sites associated with aging in 146 nonagenarians (individuals in their 90s) compared with 30 young controls (19-30 years old) in an array analysis. The transcription start sites of the majority of human genes are overlapped by groups ('islands') of CpG sites. The methylation level of 377 of the CpG sites was also associated with variation in the expression of genes associated with immune-system functions, particularly the cellular immune response and phagocytosis (118). Another study of the change in DNA methylation with aging indicated that master regulators of genome-wide gene activity may be altered, amplifying the effect (194). Massively parallel ('next-generation') sequencing of human CpG-enriched DNA areas has also demonstrated a significant pattern of change in methylation of a number of genes with age (120).

Perhaps, aging-related transcriptional/translational changes in innate and adaptive immunity could

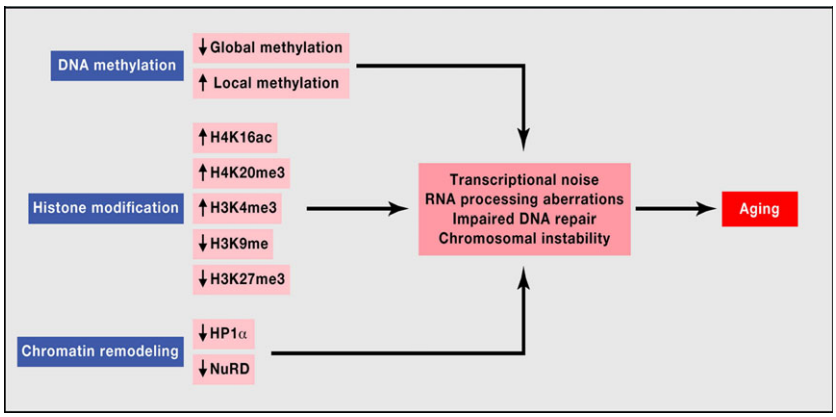


**Fig. 8. Genomic and epigenomic alterations (112). (Used with permission.) Epigenetic alterations. Alterations in the methylation of DNA or in the acetylation and methylation of histones, as well as of other chromatin-associated proteins, can induce epigenetic changes that contribute to the aging process.**

involve epigenetic changes, in which several environmental factors may play a role. A better understanding of the cellular and molecular mechanisms associated with transcriptional and post-transcriptional regulation [epigenetics, microRNAs] could contribute, in future strategies, to modulate specific gingival innate and adaptive immune responses that are impaired with aging with a goal of reducing the risk for periodontitis in this rapidly growing subgroup of the population. Although environmental factors, including 'lifestyle choices' have been discussed in regard to the effect on the epigenome, genome-wide studies have found that there is a stochastic DNA methylation drift secondary to imperfect maintenance of epigenetic status. That is, changes in some areas of the genome in aging stem or other progenitor cells could limit their ability to respond, leading to a lack of cell replenishment of aging or dying tissue (86). In addition to specific lifestyles that are associated with longevity and increasingly with an association with the epigenome, the development of medications or other treatments to reverse or modify the epigenetic changes that occur as a hallmark of aging and diseases of aging are becoming a focus. Several specific compounds that target enzymes responsible for epigenetic changes have been developed and are in clinical use or in clinical trials to be tested for several diseases associated with aging. Future health care may confront the process of aging as much as the specific diseases associated with age (22).

## Concluding remarks

It is clear that periodontitis increases in extent and severity across a large proportion of the human population with aging. These clinical changes are coincident with the host innate and adaptive immune-response systems recognizing the microbial transitions in the biofilms at sites of disease, resulting in altered levels of cellular and humoral immune-effector cells and molecules reactive with various pathogenic microbial species. The effects of aging on the immune system include distinct alterations in both innate and adaptive immune-cell function and effector biomolecules, resulting in processes reflecting immunosenescence, immunoactivation and inflammaging. It is important to note that the aging-associated *in vivo* microenvironment is likely to contribute additionally to the complexity of both innate immune and adaptive functional defects. This is highlighted by the fact that the same cytokines which seem suppressed *in vitro* are the same cytokines that are detected at high levels

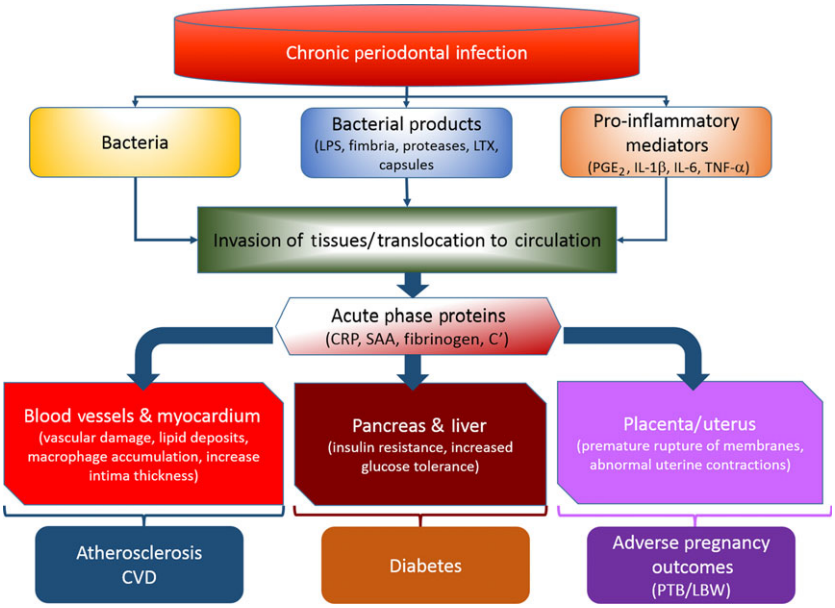


**Fig. 9. Schematic of pathways of chronic periodontal infections stimulating local host responses and leading to breakdown of integrity of the periodontium. Subsequently, there is enhanced translocation of bacteria to the systemic circulation with accompanying increases in systemic inflammation and acute-phase responses affecting distant tissues. The resulting alterations in these distant tissues provide a biologic basis for the linkage between periodontitis and adverse systemic health effects with the underlying principle of chronic inflammation. C', complement; CRP, C-reactive protein; CVD, cardiovascular disease; IL, interleukin; LBW, low birth weight; LPS, lipopolysaccharide; LPX, lipoxin; $PGE_2$, prostaglandin $E_2$; PTB, preterm birth; SAA, serum amyloid A; TNF-a, tumor necrosis factor-alpha.**

in the plasma of aged mice or humans (99, 172). A plausible interpretation in the context of periodontal disease is that, *in vivo*, these innate and adaptive immune cells interact with stromal cells within the periodontal tissues, resulting in different outcomes following similar stimulation (77). Alternatively or in addition to, the heightened inflammation observed in the periodontium of aged individuals may be a secondary effect arising from the inability to elicit appropriate and effective innate and adaptive immune responses as a result of age-specific alterations that would normally effectively control microbial overgrowth in the periodontal tissues. This ineffective immunity leads to chronic persistence of these pathogens and unresolved destructive inflammation with contributions from both arms of immunity (77).

In this model of chronic colonization/infection and responses to oral commensal and pathogenic bacteria, the aging host immune system is interacting with and responding to 'bacterial antigens' that are part of the autochthonous microbiome and have been evolving with the individual host-response system over many decades, reflecting oral health or associated with disease processes. Thus, how aging impacts these types of 'memory', 'recall' or 'tolerizing' responses to oral bacteria and the profile of isotypes, levels, specificity and functionality remain undefined in humans. Critical to the current paradigm of the chronic infection and persistent inflammation of periodontitis are the robust epidemiologic and experimental data supporting the capacity of this local dysregulation of responses. Attempting to re-establish local homeostasis can contribute to an array of systemic health conditions related to bacterial translocation through damaged tissues and the resulting chronic elevation in systemic inflammatory responses that leverage with other risk factors for cardiovascular disease, diabetes and adverse pregnancy outcomes (78, 107) (Fig. 9). Clearly, the compendium of these health issues that increase with aging emphasizes the importance of a fundamental understanding of the details of altered host responses to the oral microbial challenge within the aging process. As the discipline of periodontology becomes more fully engaged in P4 medicine (predictive, preventive, personalized and participatory; https://www.systemsbiology.org/research/p4-medicine/) there is an expectation of new approaches to documenting individual specificity of disease and more tailored preventive/intervention strategies that would couple extrinsic biologic therapeutics with improved management/control of intrinsic response systems across the lifespan.

Understanding the biological basis for altered innate and adaptive immunity and inflammation in aging as it relates to periodontal disease is a challenge. Despite the challenges of aging and periodontal research, it is crucial to understand the mechanisms responsible for age-related changes in the immune system in order to develop strategies to maintain good health in the aging population, especially within the periodontium. Importantly, it is becoming increasingly clear that these strategies must harness both the innate and adaptive immune systems. This undertaking must utilize well-characterized human cohorts to offer the possibility of linking alterations in immune function with biological mechanisms. Integrative systems biology approaches, utilizing computational approaches together with model systems, will be crucial to allow for the appreciation of the complexity of the immune system and its relationship to macro- and micro-environmental cues and stresses.

## References


1. Agrawal A, Agrawal S, Cao JN, Su H, Osann K, Gupta S. Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinositide 3-kinase-signaling pathway. *J Immunol* 2007: **178**: 6912–6922.
2. Agrawal A, Sridharan A, Prakash S, Agrawal H. Dendritic cells and aging: consequences for autoimmunity. *Expert Rev Clin Immunol* 2012: **8**: 73–80.
3. Agrawal A, Tay J, Ton S, Agrawal S, Gupta S. Increased reactivity of dendritic cells from aged subjects to self-antigen, the human DNA. *J Immunol* 2009: **182**: 1138–1145.
4. Allman D, Miller JP. The aging of early B-cell precursors. *Immunol Rev* 2005: **205**: 18–29.
5. Allman D, Miller JP. B cell development and receptor diversity during aging. *Curr Opin Immunol* 2005: **17**: 463–467.
6. Anselmi CV, Malovini A, Roncarati R, Novelli V, Villa F, Condorelli G, Bellazzi R, Puca AA. Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res* 2009: **12**: 95–104.
7. Antonicelli R, Olivieri F, Cavallone L, Spazzafumo L, Bonafe M, Marchegiani F, Cardelli M, Galeazzi R, Giovagnetti S, Perna GP, Franceschi C. Tumor prostaglandin factor-alpha gene -308G>A polymorphism is associated with ST-elevation myocardial infarction and with high plasma levels of biochemical ischemia markers. *Coron Artery Dis* 2005: **16**: 489–493.
8. Aprahamian T, Takemura Y, Goukassian D, Walsh K. Ageing is associated with diminished apoptotic cell clearance in vivo. *Clin Exp Immunol* 2008: **152**: 448–455.
9. Araujo-Pires AC, Vieira AE, Francisconi CF, Biguetti CC, Glowacki A, Yoshizawa S, Campanelli AP, Trombone AP, Sfeir CS, Little SR, Garlet GP. IL-4/CCL22/CCR4 axis controls regulatory T-cell migration that suppresses inflam-

matory bone loss in murine experimental periodontitis. *J Bone Miner Res* 2015: **30**: 412–422.
10. Babel N, Cherepnev G, Babel D, Tropmann A, Hammer M, Volk H-D, Reinke P. Analysis of tumor necrosis factor-α, transforming growth factor-β, interleukin-10, IL-6, and interferon-γ gene polymorphisms in patients with chronic periodontitis. *J Periodontol* 2006: **77**: 1978–1983.
11. Baggio G, Donazzan S, Monti D, Mari D, Martini S, Gabelli C, Dalla Vestra M, Previato L, Guido M, Pigozzo S, Cortella I, Crepaldi G, Franceschi C. Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors. *FASEB J* 1998: **12**: 433–437.
12. Barbieri M, Ferrucci L, Ragno E, Corsi A, Bandinelli S, Bonafe M, Olivieri F, Giovagnetti S, Franceschi C, Guralnik JM, Paolisso G. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. *Am J Physiol Endocrinol Metab* 2003: **284**: E481–E487.
13. Beerman I, Bhattacharya D, Zandi S, Sigvardsson M, Weissman IL, Bryder D, Rossi DJ. Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. *Proc Natl Acad Sci USA* 2010: **107**: 5465–5470.
14. Beli E, Clinthorne JF, Duriancik DM, Hwang I, Kim S, Gardner EM. Natural killer cell function is altered during the primary response of aged mice to influenza infection. *Mech Ageing Dev* 2011: **132**: 503–510.
15. Bennermo M, Held C, Stemme S, Ericsson CG, Silveira A, Green F, Tornvall P. Genetic predisposition of the interleukin-6 response to inflammation: implications for a variety of major diseases? *Clin Chem* 2004: **50**: 2136–2140.
16. Bittar MN, Carey JA, Barnard J, Fildes JE, Pravica V, Yonan N, Hutchinson IV. Interleukin 6 G-174C polymorphism influences outcome following coronary revascularization surgery. *Heart Surg Forum* 2005: **8**: E140–E145; discussion E145.
17. Blomberg BB, Frasca D. Age effects on mouse and human B cells. *Immunol Res* 2013: **57**: 354–360.
18. Bojesen SE, Nordestgaard BG. The common germline Arg72Pro polymorphism of p53 and increased longevity in humans. *Cell Cycle* 2008: **7**: 158–163.
19. Bonafe M, Olivieri F, Cavallone L, Giovagnetti S, Mayegiani F, Cardelli M, Pieri C, Marra M, Antonicelli R, Lisa R, Rizzo MR, Paolisso G, Monti D, Franceschi C. A gender–dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. *Eur J Immunol* 2001: **31**: 2357–2361.
20. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature* 2003: **423**: 337–342.
21. Branch-Mays GL, Dawson DR, Gunsolley JC, Reynolds MA, Ebersole JL, Novak KF, Mattison JA, Ingram DK, Novak MJ. The effects of a calorie-reduced diet on periodontal inflammation and disease in a non-human primate model. *J Periodontol* 2008: **79**: 1184–1191.
22. Brunet A, Berger SL. Epigenetics of aging and aging-related disease. *J Gerontol A Biol Sci Med Sci* 2014: **69**: S17–S20.
23. Butcher SK, Chahal H, Nayak L, Sinclair A, Henriquez NV, Sapey E, O'Mahony D, Lord JM. Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. *J Leukoc Biol* 2001: **70**: 881–886.
24. Butcher S, Chahel H, Lord JM. Review article: ageing and the neutrophil: no appetite for killing? *Immunology* 2000: **100**: 411–416.
25. Caligiuri MA. Human natural killer cells. *Blood* 2008: **112**: 461–469.
26. Canaday DH, Amponsah NA, Jones L, Tisch DJ, Hornick TR, Ramachandra L. Influenza-induced production of interferon-alpha is defective in geriatric individuals. *J Clin Immunol* 2010: **30**: 373–383.
27. Cancro MP, Hao Y, Scholz JL, Riley RL, Frasca D, Dunn-Walters DK, Blomberg BB. B cells and aging: molecules and mechanisms. *Trends Immunol* 2009: **30**: 313–318.
28. Chatta GS, Price TH, Stratton JR, Dale DC. Aging and marrow neutrophil reserves. *J Am Geriatr Soc* 1994: **42**: 77–81.
29. Chaushu S, Wilensky A, Gur C, Shapira L, Elboim M, Halftek G, Polak D, Achdout H, Bachrach G, Mandelboim O. Direct recognition of *Fusobacterium nucleatum* by the NK cell natural cytotoxicity receptor NKp46 aggravates periodontal disease. *PLoS Pathog* 2012: **8**: e1002601.
30. Cho RH, Sieburg HB, Muller-Sieburg CE. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. *Blood* 2008: **111**: 5553–5561.
31. Chung PL, Zhou S, Eslami B, Shen L, LeBoff MS, Glowacki J. Effect of age on regulation of human osteoclast differentiation. *J Cell Biochem* 2014: **115**: 1412–1419.
32. Clark HL, Banks R, Jones L, Hornick TR, Higgins PA, Burant CJ, Canaday DH. Characterization of MHC-II antigen presentation by B cells and monocytes from older individuals. *Clin Immunol* 2012: **144**: 172–177.
33. Collin-Osdoby P, Yu X, Zheng H, Osdoby P. RANKL-mediated osteoclast formation from murine RAW 264.7 cells. *Methods Mol Med* 2003: **80**: 153–166.
34. Crispim JC, Wastowski IJ, Rassi DM, Mendes-Junior Silva CT, Bassi C, Castelli EC, Costa RS, Saber LT, Silva TG, Donadi EA. Interferon-gamma +874 polymorphism in the first intron of the human interferon-gamma gene and kidney allograft outcome. *Transpl Proc* 2010: **42**: 4505–4508.
35. Cuervo AM, Macian F. Autophagy and the immune function in aging. *Curr Opin Immunol* 2014: **29**: 97–104.
36. De Martinis M, Modesti M, Ginaldi L. Phenotypic and functional changes of circulating monocytes and polymorphonuclear leucocytes from elderly persons. *Immunol Cell Biol* 2004: **82**: 415–420.
37. Demontiero O, Vidal C, Duque G. Aging and bone loss: new insights for the clinician. *Ther Adv Musculoskelet Dis* 2012: **4**: 61–76.
38. Domon H, Tabeta K, Nakajima T, Yamazaki K. Age-related alterations in gene expression of gingival fibroblasts stimulated with *Porphyromonas gingivalis*. *J Periodontal Res* 2014: **49**: 536–543.
39. van Duin D, Mohanty S, Thomas V, Ginter S, Montgomery RR, Fikrig E, Allore HG, Medzhitov R, Shaw AC. Age-associated defect in human TLR-1/2 function. *J Immunol* 2007: **178**: 970–975.
40. Dunston CR, Griffiths HR. The effect of ageing on macrophage Toll-like receptor-mediated responses in the fight against pathogens. *Clin Exp Immunol* 2010: **161**: 407–416.
41. Dykstra B, Olthof S, Schreuder J, Ritsema M, de Haan G. Clonal analysis reveals multiple functional defects of aged

murine hematopoietic stem cells. *J Exp Med* 2011: **208**: 2691–2703.
42. Ebersole JL, Dawson DR 3rd, Morford LA, Peyyala R, Miller CS, Gonzalez OA. Periodontal disease immunology: 'double indemnity' in protecting the host. *Periodontol 2000* 2000: **62**: 163–202.
43. Ebersole JL, Steffen MJ, Gonzalez-Martinez J, Novak MJ. Effects of age and oral disease on systemic inflammatory and immune parameters in nonhuman primates. *Clin Vaccine Immunol* 2008: **15**: 1067–1075.
44. Ebersole JL, Steffen MJ, Reynolds MA, Branch-Mays GL, Dawson DR, Novak KF, Gunsolley JC, Mattison JA, Ingram DK, Novak MJ. Differential gender effects of a reduced-calorie diet on systemic inflammatory and immune parameters in nonhuman primates. *J Periodontal Res* 2008: **43**: 500–507.
45. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. *Periodontol 2000* 1994: **5**: 112–141.
46. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med* 2000: **51**: 245–270.
47. Eskan MA, Jotwani R, Abe T, Chmelar J, Lim JH, Liang S, Ciero PA, Krauss JL, Li F, Rauner M, Hofbauer LC, Choi EY, Chung KJ, Hashim A, Curtis MA, Chavakis T, Hajishengallis G. The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nat Immunol* 2012: **13**: 465–473.
48. Feng X, McDonald JM. Disorders of bone remodeling. *Annu Rev Pathol* 2011: **6**: 121–145.
49. Ferreira AC, Almeida S, Tavares M, Canedo P, Pereira F, Regalo G, Figueiredo C, Trindade E, Seruca R, Carneiro F, Amil J, Machado JC, Tavarela-Veloso F. NOD2/CARD15 and TNFA, but not IL1B and IL1RN, are associated with Crohn's disease. *Inflamm Bowel Dis* 2005: **11**: 331–339.
50. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998: **102**: 1369–1376.
51. Flachsbart F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A. Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA* 2009: **106**: 2700–2705.
52. Fortin CF, Larbi A, Dupuis G, Lesur O, Fulop T Jr. GM-CSF activates the Jak/STAT pathway to rescue polymorphonuclear neutrophils from spontaneous apoptosis in young but not elderly individuals. *Biogerontology* 2007: **8**: 173–187.
53. Fortin CF, Lesur O, Fulop T Jr. Effects of aging on triggering receptor expressed on myeloid cells (TREM)-1-induced PMN functions. *FEBS Lett* 2007: **581**: 1173–1178.
54. Fortin CF, McDonald PP, Lesur O, Fulop T Jr. Aging and neutrophils: there is still much to do. *Rejuvenation Res* 2008: **11**: 873–882.
55. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA* 2005: **102**: 10604–10609.
56. Franceschi C. Cell proliferation, cell death and aging. *Aging (Milano)* 1989: **1**: 3–15.
57. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* 2000: **908**: 244–254.
58. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014: **69**(Suppl 1): S4–S9.
59. Franceschi C, Olivieri F, Marchegiani F, Cardelli M, Cavallone L, Capri M, Salvioli S, Valensin S, De Benedictis G, Di Iorio A, Caruso C, Paolisso G, Monti D. Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. *Mech Ageing Dev* 2005: **126**: 351–361.
60. Franceschi C, Valensin S, Bonafe M, Paolisso G, Yashin AI, Monti D, De Benedictis G. The network and the remodeling theories of aging: historical background and new perspectives. *Exp Gerontol* 2000: **35**: 879–896.
61. Fransson C, Berglundh T, Lindhe J. The effect of age on the development of gingivitis. Clinical, microbiological and histological findings. *J Clin Periodontol* 1996: **23**: 379–385.
62. Frasca D, Blomberg BB. Aging affects human B cell responses. *J Clin Immunol* 2011: **31**: 430–435.
63. Frasca D, Blomberg BB. Aging impairs murine B cell differentiation and function in primary and secondary lymphoid tissues. *Aging Dis* 2011: **2**: 361–373.
64. Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. Age effects on B cells and humoral immunity in humans. *Ageing Res Rev* 2011: **10**: 330–335.
65. Frasca D, Landin AM, Riley RL, Blomberg BB. Mechanisms for decreased function of B cells in aged mice and humans. *J Immunol* 2008: **180**: 2741–2746.
66. Frasca D, Riley RL, Blomberg BB. Humoral immune response and B-cell functions including immunoglobulin class switch are downregulated in aged mice and humans. *Semin Immunol* 2005: **17**: 378–384.
67. Fulop T, Larbi A, Douziech N, Fortin C, Guerard KP, Lesur O, Khalil A, Dupuis G. Signal transduction and functional changes in neutrophils with aging. *Aging Cell* 2004: **3**: 217–226.
68. Gaffen SL, Hajishengallis G. 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* 2008: **87**: 817–828.
69. Gangemi S, Basile G, Monti D, Merendino RA, Di Pasquale G, Bisignano U, Nicita-Mauro V, Franceschi C. Age-related modifications in circulating IL-15 levels in humans. *Mediators Inflamm* 2005: **2005**: 245–247.
70. Garbe K, Bratke K, Wagner S, Virchow JC, Lommatzsch M. Plasmacytoid dendritic cells and their Toll-like receptor 9 expression selectively decrease with age. *Hum Immunol* 2012: **73**: 493–497.
71. Geier JK, Schlissel MS. Pre-BCR signals and the control of Ig gene rearrangements. *Semin Immunol* 2006: **18**: 31–39.
72. Giacconi R, Cipriano C, Albanese F, Boccoli G, Saba V, Olivieri F, Franceschi C, Mocchegiani E. The -174G/C poly-

morphism of IL-6 is useful to screen old subjects at risk for atherosclerosis or to reach successful ageing. *Exp Gerontol* 2004: **39**: 621–628.
73. Gomez CR, Nomellini V, Faunce DE, Kovacs EJ. Innate immunity and aging. *Exp Gerontol* 2008: **43**: 718–728.
74. Gonzalez OA, Novak MJ, Kirakodu S, Stromberg A, Orraca L, Gonzalez-Martinez J, Ebersole JL. Comparative analysis of gingival tissue antigen presentation pathways in aging and periodontitis. *J. Clin Periodontol* 2014: **41**: 327–39.
75. Goronzy JJ, Weyand CM. T cell development and receptor diversity during aging. *Curr Opin Immunol* 2005: **17**: 468–475.
76. Gupta S, Agrawal A. Inflammation & autoimmunity in human ageing: dendritic cells take a center stage. *Indian J Med Res* 2013: **138**: 711–716.
77. Hajishengallis G. Too old to fight? Aging and its toll on innate immunity. *Mol Oral Microbiol* 2010: **25**: 25–37.
78. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. *Nat Rev Immunol* 2015: **15**: 30–44.
79. Han JY, Lee GK, Jang DH, Lee SY, Lee JS. Association of p53 codon 72 polymorphism and MDM2 SNP309 with clinical outcome of advanced nonsmall cell lung cancer. *Cancer* 2008: **113**: 799–807.
80. Hastings KT, Cresswell P. Disulfide reduction in the endocytic pathway: immunological functions of gamma-interferon-inducible lysosomal thiol reductase. *Antioxid Redox Signal* 2011: **15**: 657–668.
81. Hazeldine J, Hampson P, Lord JM. Reduced release and binding of perforin at the immunological synapse underlies the age-related decline in natural killer cell cytotoxicity. *Aging Cell* 2012: **11**: 751–759.
82. Hearps AC, Martin GE, Angelovich TA, Cheng WJ, Maisa A, Landay AL, Jaworowski A, Crowe SM. Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. *Aging Cell* 2012: **11**: 867–875.
83. Holt SC, Ebersole J, Felton J, Brunsvold M, Kornman KS. Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of periodontitis. *Science* 1988: **239**: 55–57.
84. Hurme M, Lehtimäki T, Jylhä M, Karhunen PJ, Hervonen A. Interleukin-6 −174G/C polymorphism and longevity: a follow-up study. *Mech Ageing Dev* 2005: **126**: 417–418.
85. Huttner EA, Machado DC, de Oliveira RB, Antunes AG, Hebling E. Effects of human aging on periodontal tissues. *Spec Care Dentist* 2009: **29**: 149–155.
86. Issa J-P. Aging and epigenetic drift: a vicious cycle. *J Clin Invest* 2014: **124**: 24–29.
87. Jenny NS, Tracy RP, Ogg MS, Luong LA, Kuller LH, Arnold AM, Sharrett AR, Humphries SE. In the elderly, interleukin-6 plasma levels and the −174 g>c polymorphism are associated with the development of cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2002: **22**: 2066–2071.
88. Jing Y, Shaheen E, Drake RR, Chen N, Gravenstein S, Deng Y. Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. *Hum Immunol* 2009: **70**: 777–784.
89. Johnson SA, Cambier JC. Ageing, autoimmunity and arthritis: senescence of the B cell compartment – implications for humoral immunity. *Arthritis Res Ther* 2004: **6**: 131–139.
90. Johnson N, Fletcher O, Palles C, Rudd M, Webb E, Sellick G, dos Santos Silva I, McCormack V, Gibson L, Fraser A. Counting potentially functional variants in BRCA1, BRCA2 and ATM predicts breast cancer susceptibility. *Hum Mol Genet* 2007: **16**: 1051–1057.
91. Kawai T, Matsuyama T, Hosokawa Y, Makihira S, Seki M, Karimbux NY, Goncalves RB, Valverde P, Dibart S, Li YP, Miranda LA, Ernst CW, Izumi Y, Taubman MA. B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. *Am J Pathol* 2006: **169**: 987–998.
92. Khabour OF, Barnawi JM. Association of longevity with IL-10 −1082 G/A and TNF-α−308 G/A polymorphisms. *Int J Immunogenet* 2010: **37**: 293–298.
93. Kikuchi T, Hahn CL, Tanaka S, Barbour SE, Schenkein HA, Tew JG. Dendritic cells stimulated with *Actinobacillus actinomycetemcomitans* elicit rapid gamma interferon responses by natural killer cells. *Infect Immun* 2004: **72**: 5089–5096.
94. Kikuchi T, Willis DL, Liu M, Purkall DB, Sukumar S, Barbour SE, Schenkein HA, Tew JG. Dendritic-NK cell interactions in *P. gingivalis*-specific responses. *J Dent Res* 2005: **84**: 858–862.
95. Kim JG, Sohn SK, Chae YS, Song HS, Kwon K-Y, Do YR, Kim MK, Lee KH, Hyun MS, Lee WS. TP53 codon 72 polymorphism associated with prognosis in patients with advanced gastric cancer treated with paclitaxel and cisplatin. *Cancer Chemother Pharmacol* 2009: **64**: 355–360.
96. Kirkwood TB, Franceschi C. Is aging as complex as it would appear? New perspectives in aging research. *Ann N Y Acad Sci* 1992: **663**: 412–417.
97. Klinman NR, Kline GH. The B-cell biology of aging. *Immunol Rev* 1997: **160**: 103–114.
98. Kornman KS. Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. *Am J Clin Nutr* 2006: **83**: 475S–483S.
99. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. *Exp Gerontol* 2004: **39**: 687–699.
100. Larbi A, Douziech N, Dupuis G, Khalil A, Pelletier H, Guerard KP, Fulop T Jr. Age-associated alterations in the recruitment of signal-transduction proteins to lipid rafts in human T lymphocytes. *J Leukoc Biol* 2004: **75**: 373–381.
101. LeMaoult J, Szabo P, Weksler ME. Effect of age on humoral immunity, selection of the B-cell repertoire and B-cell development. *Immunol Rev* 1997: **160**: 115–126.
102. Li G, Smithey MJ, Rudd BD, Nikolich-Zugich J. Age-associated alterations in CD8alpha+ dendritic cells impair CD8 T-cell expansion in response to an intracellular bacterium. *Aging Cell* 2012: **11**: 968–977.
103. Liang S, Domon H, Hosur KB, Wang M, Hajishengallis G. Age-related alterations in innate immune receptor expression and ability of macrophages to respond to pathogen challenge in vitro. *Mech Ageing Dev* 2009: **130**: 538–546.
104. Liaw L, Almeida M, Hart CE, Schwartz SM, Giachelli CM. Osteopontin promotes vascular cell adhesion and spreading and is chemotactic for smooth muscle cells in vitro. *Circ Res* 1994: **74**: 214–224.
105. Licastro F, Grimaldi LME, Bonafè M, Martina C, Olivieri F, Cavallone L, Giovanietti S, Masliah E, Franceschi C. Interleukin-6 gene alleles affect the risk of Alzheimer's disease

and levels of the cytokine in blood and brain. *Neurobiol Aging* 2003: **24**: 921–926.
106. Lindborg CM, Propert KJ, Pignolo RJ. Conservation of prolongevity genes among mammals. *Mech Ageing Dev* 2015: **146–148**: 23–27.
107. Linden GJ, Lyons A, Scannapieco FA. Periodontal systemic associations: review of the evidence. *J Periodontol* 2013: **84**: S8–S19.
108. Linehan E, Fitzgerald DC. Ageing and the immune system: focus on macrophages. *Eur J Microbiol Immunol (Bp)* 2015: **5**: 14–24.
109. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. *Nat Immunol* 2004: **5**: 133–139.
110. Lio D, Scola L, Crivello A, Bonafe M, Franceschi C, Olivieri F, Colonna-Romano G, Candore G, Caruso C. Allele frequencies of +874T–>A single nucleotide polymorphism at the first intron of interferon-gamma gene in a group of Italian centenarians. *Exp Gerontol* 2002: **37**: 315–319.
111. Lio D, Scola L, Crivello A, Colonna-Romano G, Candore G, Bonafe M, Cavallone L, Franceschi C, Caruso C. Gender-specific association between -1082 IL-10 promoter polymorphism and longevity. *Genes Immun* 2002: **3**: 30–33.
112. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013: **153**: 1194–1217.
113. Lund SA, Giachelli CM, Scatena M. The role of osteopontin in inflammatory processes. *J Cell Commun Signal* 2009: **3**: 311–322.
114. Mabbott NA, Kobayashi A, Sehgal A, Bradford BM, Pattison M, Donaldson DS. Aging and the mucosal immune system in the intestine. *Biogerontology* 2015: **16**: 133–145.
115. Madden TE, Caton JG. Animal models for periodontal disease. *Methods Enzymol* 1994: **235**: 106–119.
116. Man AL, Gicheva N, Nicoletti C. The impact of ageing on the intestinal epithelial barrier and immune system. *Cell Immunol* 2014: **289**: 112–118.
117. Mari D, Mannucci PM, Coppola R, Bottasso B, Bauer KA, Rosenberg RD. Hypercoagulability in centenarians: the paradox of successful aging. *Blood* 1995: **85**: 3144–3149.
118. Marttila S, Kananen L, Häyrynen S, Jylhävä J, Nevalainen T, Hervonen A, Jylhä M, Nykter M, Hurme M. Ageing-associated changes in the human DNA methylome: genomic locations and effects on gene expression. *BMC Genom* 2015: **16**: 179.
119. Marwah AS, Meyer J, Weinmann JP. Mitotic rate of gingival epithelium in two age groups. *J Invest Dermatol* 1956: **27**: 237–247.
120. McClay JL, Aberg KA, Clark SL, Nerella S, Kumar G, Xie LY, Hudson AD, Harada A, Hultman CM, Magnusson PK. A methylome-wide study of aging using massively parallel sequencing of the methyl-CpG-enriched genomic fraction from blood in over 700 subjects. *Hum Mol Genet* 2014: **23**: 1175–1185.
121. McElhaney JE, Zhou X, Talbot HK, Soethout E, Bleackley RC, Granville DJ, Pawelec G. The unmet need in the elderly: how immunosenescence, CMV infection, co-morbidities and frailty are a challenge for the development of more effective influenza vaccines. *Vaccine* 2012: **30**: 2060–2067.
122. Medica I, Kastrin A, Maver A, Peterlin B. Role of genetic polymorphisms in ACE and TNF-[alpha] gene in sarcoidosis: a meta-analysis. *J Hum Genet* 2007: **52**: 836–847.
123. Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, Cesari M, Nourhashemi F. Proinflammatory cytokines, aging, and age-related diseases. *J Am Med Dir Assoc* 2013: **14**: 877–882.
124. Miller RA. The aging immune system: primer and prospectus. *Science* 1996: **273**: 70–74.
125. Miller JP, Allman D. Linking age-related defects in B lymphopoiesis to the aging of hematopoietic stem cells. *Semin Immunol* 2005: **17**: 321–329.
126. Moreira PR, Lima PMA, Sathler KOB, Imanishi SAW, Costa JE, Gomez RS, Gollob KJ, Dutra WO. Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. *Clin Exp Immunol* 2007: **148**: 119–126.
127. Moretto MM, Lawlor EM, Khan IA. Aging mice exhibit a functional defect in mucosal dendritic cell response against an intracellular pathogen. *J Immunol* 2008: **181**: 7977–7984.
128. Müller-Steinhardt M, Härtel C, Müller B, Kirchner H, Fricke L. The interleukin-6 [sup -174] promoter polymorphism is associated with long-term kidney allograft survival. *Kidney Int* 1824: **2002**: 62.
129. Muthukuru M. Technical advance: decreased helper T cells and increased natural killer cells in chronic periodontitis analyzed by a novel method for isolating resident lymphocytes. *J Leukoc Biol* 2012: **92**: 683–692.
130. Niwa Y, Kasama T, Miyachi Y, Kanoh T. Neutrophil chemotaxis, phagocytosis and parameters of reactive oxygen species in human aging: cross-sectional and longitudinal studies. *Life Sci* 1989: **44**: 1655–1664.
131. Noguchi K, Ishikawa I. The roles of cyclooxygenase-2 and prostaglandin E2 in periodontal disease. *Periodontol 2000* 2007: **43**: 85–101.
132. Nyugen J, Agrawal S, Gollapudi S, Gupta S. Impaired functions of peripheral blood monocyte subpopulations in aged humans. *J Clin Immunol* 2010: **30**: 806–813.
133. O'Connor JE, Herrera G, Martinez-Romero A, de Oyanguren FS, Diaz L, Gomes A, Balaguer S, Callaghan RC. Systems Biology and immune aging. *Immunol Lett* 2014: **162**: 334–345.
134. O'Gradaigh D, Compston JE. T-cell involvement in osteoclast biology: implications for rheumatoid bone erosion. *Rheumatology (Oxford)* 2004: **43**: 122–130.
135. Ogura N, Matsuda U, Tanaka F, Shibata Y, Takiguchi H, Abiko Y. In vitro senescence enhances IL-6 production in human gingival fibroblasts induced by lipopolysaccharide from Campylobacter rectus. *Mech Ageing Dev* 1996: **87**: 47–59.
136. Okamura H, Yamaguchi M, Abiko Y. Enhancement of lipopolysaccharide-stimulated PGE2 and IL-1beta production in gingival fibroblast cells from old rats. *Exp Gerontol* 1999: **34**: 379–392.
137. O'Rielly DD, Roslin NM, Beyene J, Pope A, Rahman P. TNF-α −308 G/A polymorphism and responsiveness to TNF-α blockade therapy in moderate to severe rheumatoid arthritis: a systematic review and meta-analysis. *Pharmacogenomics J* 2009: **9**: 161–167.
138. Orsini G, Legitimo A, Failli A, Massei F, Biver P, Consolini R. Enumeration of human peripheral blood dendritic cells throughout the life. *Int Immunol* 2012: **24**: 347–356.
139. Oz HS, Puleo DA. Animal models for periodontal disease. *J Biomed Biotechnol* 2011: **2011**: 754857.

140. Panda A, Qian F, Mohanty S, van Duin D, Newman FK, Zhang L, Chen S, Towle V, Belshe RB, Fikrig E, Allore HG, Montgomery RR, Shaw AC. Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. *J Immunol* 2010: **184**: 2518–2527.
141. Pang WW, Price EA, Sahoo D, Beerman I, Maloney WJ, Rossi DJ, Schrier SL, Weissman IL. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc Natl Acad Sci USA* 2011: **108**: 20012–20017.
142. Pawelec G. When T cells get old. *Sci Aging Knowledge Environ* 2005: **205**: pe39.
143. Pawelec G, Akbar A, Caruso C, Solana R, Grubeck-Loebenstein B, Wikby A. Human immunosenescence: is it infectious? *Immunol Rev* 2005: **205**: 257–268.
144. Pawlik A, Domanski L, Rozanski J, Florczak M, Dabrowska-Zamojcin E, Dutkiewicz G, Gawronska-Szklarz B. IL-2 and TNF-alpha promoter polymorphisms in patients with acute kidney graft rejection. *Transpl Proc* 2005: **37**: 2041–2043.
145. Pawlik A, Domanski L, Rozanski J, Florczak M, Wrzesniewska J, Dutkiewicz G, Dabrowska-Zamojcin E, Gawronska-Szklarz B. The cytokine gene polymorphisms in patients with chronic kidney graft rejection. *Transpl Immunol* 2005: **14**: 49–52.
146. Pawlikowska L, Hu D, Huntsman S, Sung A, Chu C, Chen J, Joyner AH, Schork NJ, Hsueh WC, Reiner AP. Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Aging Cell* 2009: **8**: 460–472.
147. Pereira LF, de Souza AP, Borges TJ, Bonorino C. Impaired in vivo CD4+ T cell expansion and differentiation in aged mice is not solely due to T cell defects: decreased stimulation by aged dendritic cells. *Mech Ageing Dev* 2011: **132**: 187–194.
148. Persson GR, Engel D, Whitney C, Darveau R, Weinberg A, Brunsvold M, Page RC. Immunization against *Porphyromonas gingivalis* inhibits progression of experimental periodontitis in nonhuman primates. *Infect Immun* 1994: **62**: 1026–1031.
149. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005: **366**: 1809–1820.
150. Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. *Aging Cell* 2004: **3**: 161–167.
151. Plowden J, Renshaw-Hoelscher M, Gangappa S, Engleman C, Katz JM, Sambhara S. Impaired antigen-induced CD8+ T cell clonal expansion in aging is due to defects in antigen presenting cell function. *Cell Immunol* 2004: **229**: 86–92.
152. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. *Hum Immunol* 2000: **61**: 863–866.
153. Qian F, Wang X, Zhang L, Chen S, Piecychna M, Allore H, Bockenstedt L, Malawista S, Bucala R, Shaw AC, Fikrig E, Montgomery RR. Age-associated elevation in TLR5 leads to increased inflammatory responses in the elderly. *Aging Cell* 2012: **11**: 104–110.
154. Qian F, Wang X, Zhang L, Lin A, Zhao H, Fikrig E, Montgomery RR. Impaired interferon signaling in dendritic cells from older donors infected in vitro with West Nile virus. *J Infect Dis* 2011: **203**: 1415–1424.
155. Qidwai T, Khan F. Tumour prostaglandin factor gene polymorphism and disease prevalence. *Scand J Immunol* 2011: **74**: 522–547.
156. Rang FJ, Boonstra J. Causes and Consequences of Age-Related Changes in DNA Methylation: A Role for ROS? *Biology* 2014: **3**: 403–25.
157. Ratts RB, Weng NP. Homeostasis of lymphocytes and monocytes in frequent blood donors. *Front Immunol* 2012: **3**: 271.
158. Reinhardt RA, McDonald TL, Bolton RW, DuBois LM, Kaldahl WB. IgG subclasses in gingival crevicular fluid from active versus stable periodontal sites. *J Periodontol* 1989: **60**: 44–50.
159. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. *J Immunol* 2002: **169**: 4697–4701.
160. Reynolds MA, Dawson DR, Novak KF, Ebersole JL, Gunsolley JC, Branch-Mays GL, Holt SC, Mattison JA, Ingram DK, Novak MJ. Effects of caloric restriction on inflammatory periodontal disease. *Nutrition* 2009: **25**: 88–97.
161. Riley RL. Impaired B lymphopoiesis in old age: a role for inflammatory B cells? *Immunol Res* 2013: **57**: 361–369.
162. Roth GS, Mattison JA, Ottinger MA, Chachich ME, Lane MA, Ingram DK. Aging in rhesus monkeys: relevance to human health interventions. *Science* 2004: **305**: 1423–1426.
163. Sakai T, Kiyoshima T, Kobayashi I, Moroi R, Ibuki T, Nagadome M, Terada Y, Sakai H. Age-dependent changes in the distribution of BrdU- and TUNEL-positive cells in the murine gingival tissue. *J Periodontol* 1999: **70**: 973–981.
164. Schou S, Holmstrup P, Kornman KS. Non-human primates used in studies of periodontal disease pathogenesis: a review of the literature. *J Periodontol* 1993: **64**: 497–508.
165. Seidler S, Zimmermann HW, Bartneck M, Trautwein C, Tacke F. Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults. *BMC Immunol* 2010: **11**: 30.
166. Shaw AC, Goldstein DR, Montgomery RR. Age-dependent dysregulation of innate immunity. *Nat Rev Immunol* 2013: **13**: 875–887.
167. Simell B, Vuorela A, Ekstrom N, Palmu A, Reunanen A, Meri S, Kayhty H, Vakevainen M. Aging reduces the functionality of anti-pneumococcal antibodies and the killing of *Streptococcus pneumoniae* by neutrophil phagocytosis. *Vaccine* 2011: **29**: 1929–1934.
168. Sridharan A, Esposo M, Kaushal K, Tay J, Osann K, Agrawal S, Gupta S, Agrawal A. Age-associated impaired plasmacytoid dendritic cell functions lead to decreased CD4 and CD8 T cell immunity. *Age (Dordr)* 2011: **33**: 363–376.
169. Stahl SS, Tonna EA, Weiss R. The effects of aging on the proliferative activity of rat periodontal structures. *J Gerontol* 1969: **24**: 447–450.
170. Steger MM, Maczek C, Berger P, Grubeck-Loebenstein B. Vaccination against tetanus in the elderly: do recommended vaccination strategies give sufficient protection. *Lancet* 1996: **348**: 762.
171. Steger MM, Maczek C, Grubeck-Loebenstein B. Peripheral blood dendritic cells reinduce proliferation in in vitro aged T cell populations. *Mech Ageing Dev* 1997: **93**: 125–130.

172. Stout RD, Suttles J. Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. *Immunol Rev* 2005: **205**: 60–71.
173. Stranks AJ, Hansen AL, Panse I, Mortensen M, Ferguson DJ, Puleston DJ, Shenderov K, Watson AS, Veldhoen M, Phadwal K, Cerundolo V, Simon AK. Autophagy Controls Acquisition of Aging Features in Macrophages. *J Innate Immun* 2015: **7**: 375–391.
174. Swain S, Clise-Dwyer K, Haynes L. Homeostasis and the age-associated defect of CD4 T cells. *Semin Immunol* 2005: **17**: 370–377.
175. Takayanagi H. Osteoimmunology and the effects of the immune system on bone. *Nat Rev Rheumatol* 2009: **5**: 667–676.
176. Takiguchi H, Yamaguchi M, Okamura H, Abiko Y. Contribution of IL-1 beta to the enhancement of Campylobacter rectus lipopolysaccharide-stimulated PGE2 production in old gingival fibroblasts in vitro. *Mech Ageing Dev* 1997: **98**: 75–90.
177. Taubman MA, Kawai T. Involvement of T-lymphocytes in periodontal disease and in direct and indirect induction of bone resorption. *Crit Rev Oral Biol Med* 2001: **12**: 125–135.
178. Taverna G, Seveso M, Giusti G, Hurle R, Graziotti P, Stifter S, Chiriva-Internati M, Grizzi F. Senescent remodeling of the innate and adaptive immune system in the elderly men with prostate cancer. *Curr Gerontol Geriatr Res* 2014: **2014**: 478126.
179. Teng YT, Nguyen H, Gao X, Kong YY, Gorczynski RM, Singh B, Ellen RP, Penninger JM. Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection. *J Clin Invest* 2000: **106**: R59–R67.
180. Tortorella C, Simone O, Piazzolla G, Stella I, Cappiello V, Antonaci S. Role of phosphoinositide 3-kinase and extracellular signal-regulated kinase pathways in granulocyte macrophage-colony-stimulating factor failure to delay fas-induced neutrophil apoptosis in elderly humans. *J Gerontol A Biol Sci Med Sci* 2006: **61**: 1111–1118.
181. Tseng CW, Kyme PA, Arruda A, Ramanujan VK, Tawackoli W, Liu GY. Innate immune dysfunctions in aged mice facilitate the systemic dissemination of methicillin-resistant *S. aureus*. *PLoS ONE* 2012: **7**: e41454.
182. Tseng CW, Liu GY. Expanding roles of neutrophils in aging hosts. *Curr Opin Immunol* 2014: **29**: 43–48.
183. Tsilidis KK, Helzlsouer KJ, Smith MW, Grinberg V, Hoffman-Bolton J, Clipp SL, Visvanathan K, Platz EA. Association of common polymorphisms in IL10, and in other genes related to inflammatory response and obesity with colorectal cancer. *Cancer Causes Control* 2009: **20**: 1739–1751.
184. van der Velden U. The onset age of periodontal destruction. *J Clin Periodontol* 1991: **18**: 380–383.
185. Vuorio R, Hirvas L, Vaara M. The Ssc protein of enteric bacteria has significant homology to the acyltransferase Lpxa of lipid A biosynthesis, and to three acetyltransferases. *FEBS Lett* 1991: **292**: 90–94.
186. Wang M-H, Helzlsouer KJ, Smith MW, Hoffman-Bolton JA, Clipp SL, Grinberg V, De Marzo AM, Isaacs WB, Drake CG, Shugart YY, Platz EA. Association of IL10 and other immune response- and obesity-related genes with prostate cancer in CLUE II. *Prostate* 2009: **69**: 874–885.
187. Weksler ME, Szabo P. The effect of age on the B-cell repertoire. *J Clin Immunol* 2000: **20**: 240–249.
188. Weng NP. Aging of the immune system: how much can the adaptive immune system adapt? *Immunity* 2006: **24**: 495–499.
189. Wenisch C, Patruta S, Daxbock F, Krause R, Horl W. Effect of age on human neutrophil function. *J Leukoc Biol* 2000: **67**: 40–45.
190. Wikby A, Nilsson BO, Forsey R, Thompson J, Strindhall J, Lofgren S, Ernerudh J, Pawelec G, Ferguson F, Johansson B. The immune risk phenotype is associated with IL-6 in the terminal decline stage: findings from the Swedish NONA immune longitudinal study of very late life functioning. *Mech Ageing Dev* 2006: **127**: 695–704.
191. Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA* 2008: **105**: 13987–13992.
192. Wong C, Goldstein DR. Impact of aging on antigen presentation cell function of dendritic cells. *Curr Opin Immunol* 2013: **25**: 535–541.
193. Yu XQ, Fan JM, Nikolic-Paterson DJ, Yang N, Mu W, Pichler R, Johnson RJ, Atkins RC, Lan HY. IL-1 up-regulates osteopontin expression in experimental crescentic glomerulonephritis in the rat. *Am J Pathol* 1999: **154**: 833–841.
194. Yuan T, Jiao Y, de Jong S, Ophoff RA, Beck S, Teschendorff AE. An integrative multi-scale analysis of the dynamic DNA methylation landscape in aging. *PLoS Genet* 2015: **11**: e1004996–e1004996.
195. Zanni F, Vescovini R, Biasini C, Fagnoni F, Zanlari L, Telera A, Di Pede P, Passeri G, Pedrazzoni M, Passeri M, Franceschi C, Sansoni P. Marked increase with age of type 1 cytokines within memory and effector/cytotoxic CD8+ T cells in humans: a contribution to understand the relationship between inflammation and immunosenescence. *Exp Gerontol* 2003: **38**: 981–987.
196. Zhang W, Brahmakshatriya V, Swain SL. CD4 T cell defects in the aged: causes, consequences and strategies to circumvent. *Exp Gerontol* 2014: **54**: 67–70.
197. Zhao J, Zhao J, Legge K, Perlman S. Age-related increases in PGD(2) expression impair respiratory DC migration, resulting in diminished T cell responses upon respiratory virus infection in mice. *J Clin Invest* 2011: **121**: 4921–4930.