J Clin Periodontol 2017; 44 (Suppl. 18): S153–S177 doi: 10.1111/jcpe.12675

Journal of Clinical Periodontology

Age-related changes in immune function (immune senescence) in caries and periodontal diseases: a systematic review

Philip M. Preshaw[1,2], Karsten Henne[3], John J. Taylor[1,2], Ruth A. Valentine[1,4] and Georg Conrads[3]

[1]School of Dental Sciences, Newcastle University, Newcastle upon Tyne, UK; [2]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK; [3]Division of Oral Microbiology and Immunology, Department of Operative Dentistry, Periodontology and Preventive Dentistry, RWTH Aachen University Hospital, Aachen, Germany; [4]Human Nutrition Research Centre, Newcastle University, Newcastle upon Tyne, UK

Preshaw PM, Henne K, Taylor JJ, Valentine RA, Conrads G. Age-related changes in immune function (immune senescence) in caries and periodontal diseases: a systematic review. J Clin Periodontol 2017; 44 (Suppl. 18): S153–S177. doi: 10.1111/jcpe.12675.

Abstract

Aim: To systematically review the evidence regarding immune senescence in the pathogenesis of periodontitis and dental caries.

Methods: A systematic search of electronic databases utilizing medical subject headings (MeSH terms) supplemented by screening of review articles and other relevant texts was undertaken.

Results: Seventy-three articles were included (43 for periodontitis, 30 for caries). Study results were found to be generally heterogeneous. Regarding periodontitis, human studies suggest evidence for altered neutrophil function and increased production of pro-inflammatory mediators (e.g. interleukin-1β, interleukin-6 and prostaglandin E_2) in older compared to younger subjects, and animal experiments suggest increased expression of genes that contribute to a pro-inflammatory state in older compared to younger animals. Regarding dental caries, research relating to changes in immune functioning and the impact of ageing is in its infancy. A small number of studies have reported components of innate and adaptive immunity that affect the composition of saliva and dental biofilms with possible impacts on caries progression.

Conclusion: There is evidence that immune functioning related to periodontitis and (less investigated) dental caries alters with increasing age. In both conditions, age-associated mechanistic changes in immune functioning are complex and incompletely understood and it is not clear how these relate to disease susceptibility.

Key words: caries; immune function; immune senescence; mucosal immunity; periodontitis

Accepted for publication 23 December 2016

Immune senescence refers to the process of altered immune functioning that accompanies increasing age. The cellular and molecular mechanisms that characterize the ageing process are, as yet, incompletely defined, but it is considered that immune senescence results in increased susceptibility of older individuals to infections. Our environment contains a wide range of potential infectious agents, including viruses, bacteria, archaea, fungi, protozoa and parasites, many of which may reside within the oral cavity. Some are transient whereas others are acquired in different phases of life and remain as an element of the human microbiome. These microbes may cause and/or contribute to the aetiology of two of the most

Conflict of interest and source of funding statement

Dr. Conrads reports Other from LCL biokey GmbH company, outside the submitted work. In addition, Dr. Conrads has a patent 'Detektionsverfahren für kariogene Keime, Method for detection of cariogenic bacteria. Deutsches Patent- und Markenamt, Nr. 101 09 012' issued. Dr. Taylor reports Grants from Philips Research, outside the submitted work. Dr. Preshaw reports Grants from Colgate-Palmolive, grants from Philips Research, outside the submitted work. Dr. Henne and Dr. Valentine have nothing to disclose.

prevalent diseases known to the human race, periodontitis and dental caries. The immune system plays a fundamental role in combatting microbial infections, but with increasing age, there is evidence that aspects of immune function may alter, such that susceptibility to infections increases. Ageing results in alterations to both the adaptive and innate arms of the immune system, not necessarily leading to immune deficiency, but rather to dysregulation of immune responses (Gomez et al. 2008, Hajishengallis 2010). The term "inflammaging" has been used to refer to an increased systemic chronic inflammatory status in older individuals (Franceschi et al. 2000, Franceschi 2007), which may result from both an increase in pro-inflammatory status and a reduced ability to control infections in older age.

Periodontal diseases include a number of conditions affecting the supporting structures of the teeth, with chronic periodontitis being highly prevalent, particularly in older populations. In this review, the term periodontitis is used to indicate chronic periodontitis, unless otherwise specified. In general terms, an estimated 10–15% of adult populations are affected by severe periodontitis, and it has been reported that severe periodontitis is the sixth most prevalent condition worldwide, with prevalence gradually increasing with age (Kassebaum et al. 2014). Periodontitis is a chronic inflammatory condition in which tissue damage results from dysregulated and prolonged inflammatory responses to the persisting subgingival biofilm. It is therefore relevant to consider whether changes in immune functioning as a result of increasing age may be relevant in the pathogenesis of periodontitis. Furthermore, changes in immune function in general, and particularly in mucosal immune function, might be relevant not only for periodontitis, but also, albeit less explored, for tooth decay. Dental caries is a destructive process causing decalcification of the teeth leading to continued destruction of enamel and dentine. Similar to periodontitis, dental caries is a highly prevalent condition, affecting 60–90% of schoolchildren and the vast majority of adults in most industrialized countries according to World Health Organization (WHO) statistics (http://www.who.int/oral_health/disease_burden/global/en/ accessed 21 October 2016). The frequency of dietary sugar consumption (especially sucrose) is key to the major differences in caries prevalence rates that have been observed around the world. However, caries is a multifactorial condition as the disease process also involves other carbohydrates, oral microorganisms (especially *Streptococcus mutans*, lactobacilli, bifidobacteria and yeasts), acids from food and beverages, differing susceptibilities of different teeth/surfaces, and salivary flow and salivary compounds (Sheiham & James 2015). One of the main reasons for failing to control dental caries globally is that most researchers and healthcare planners have focused on preventing caries in children. However, the extent of caries (in both individuals and populations) increases progressively as people get older (Sheiham & James 2015). According to National Institutes of Health (NIH) statistics (http://www.nidcr.nih.gov/DataStatistics/FindDataByTopic/DentalCaries/ accessed 21 October 2016), dental caries, both treated and untreated, in seniors aged 65 years and older declined from the early 1970s until 1999–2004. But, despite this decline, prevalence is still high: 93% of adults aged ≥65 years and 92% of adults aged 20–64 years have dental caries (treated or untreated). Caries, once seen as a disease of childhood, is clearly a lifelong disease and factors associated with ageing can also increase the risk of dental caries (Miyazaki et al. 1992, Peltola et al. 2004, Batchelor 2015, Baumgartner et al. 2015).

The aim of this systematic review was to consider the evidence for a role for immune senescence in the pathogenesis of both periodontitis and dental caries.

Materials and Methods

Literature search

A systematic search strategy was developed, utilizing medical subject headings (MeSH terms) to search the relevant literature electronically in Medline/PubMed up to 30 May 2016. The systematic search strategy and search terms are shown in Fig. 1. Bibliographies of review articles, relevant texts such as WHO and NIH documents, books and book chapters were also screened. The search strategy was employed consistently for both the periodontitis and caries search terms other than at the final stages of the search, when context-specific terms were utilized for periodontitis and caries (as shown in Fig. 1).

Criteria for considering publications for this review

After identification, relevant articles were selected based on the PRISMA flow diagram (Moher et al. 2009). The decision was made *a priori* to be as inclusive as possible during the search to identify relevant literature that would inform this topic. Given the wide variety of different study types in this subject area, it was considered not appropriate to define stringent criteria for selecting studies to be reported in this review, but to adopt broad inclusion/exclusion criteria as shown below.

Inclusion criteria:

- studies in which immune function and/or immune senescence and/or ageing had been considered in the context of periodontitis or caries
- human studies of all types (including laboratory-based studies)
- animal studies of all types

Exclusion criteria:

- reviews (although these were nonetheless obtained to permit review of reference lists as part of the hand-searching process)
- case reports
- letters/editorials
- conference abstracts
- articles not in English and unable to be translated or not available online or by inter-library loans.

Selection of studies

Titles derived from the searches were screened based on the inclusion and exclusion criteria to assess potential eligibility. Abstracts of titles that were considered potentially eligible were obtained and independently screened by two authors for further

Step 1: Search for terms relating to general immune function	"immunity"[MeSH Terms] OR "immunity"(All Fields) OR "adaptive immunity"[MeSH Terms] OR "adaptive immunity"[All Fields] OR "immunity, innate"[MeSH Terms] OR "innate immunity"[All Fields] OR "immune system processes"[MeSH Terms] OR "immune system"[MeSH Terms] OR "immune system"[All Fields] OR "immune function"[All Fields] OR "immune$"[All Fields] OR "inflammation"[MeSH Terms] OR "inflammat$"[All Fields] OR "leukocytes"[MeSH Terms] OR "leukocyte$" [All Fields] OR "leukocytes, mononuclear"[MeSH Terms] OR "phagocytes"[MeSH Terms] OR "phagocytosis"[MeSH Terms] OR "phagocyt$"[All Fields] OR "macrophages"[MeSH Terms] OR "macrophage$"[All Fields] OR "monocytes"[MeSH Terms] OR "monocyte$"[All Fields] OR "neutrophils"[MeSH Terms] OR "neutrophil$"[All Fields] OR "granulocytes"[MeSH Terms] or "granulocyte$"[All Fields] OR "mast cells"[MeSH Terms] OR "mast cell$"[All Fields] OR "antigen-presenting cells"[MeSH Terms] OR "antigen-presenting cell$"[All Fields] OR "dendritic cells"[MeSH Terms] OR "dendritic cell$"[All Fields] OR "fibroblasts"[MeSH Terms] OR "fibroblast$"[All Fields] OR "osteoblasts"[MeSH Terms] OR "osteoblast$"[All Fields] OR "osteoclasts"[MeSH Terms] OR "osteoclast$"[All Fields] OR "keratinocytes"[MeSH Terms] or "keratinocyte$"[All Fields] OR "epithelial cells"[MeSH Terms] OR "epithelial cell$"[All Fields] OR "periodontal ligament cell$"[All Fields] OR "endothelial cells"[MeSH Terms] OR "endothelial cell$"[All Fields] OR "stem cells"[MeSH Terms] OR "stem cell$"[All Fields] OR "pluripotent stem cells"[MeSH Terms] OR "pluripotent stem cell$"[All Fields] OR "lymphocytes"[MeSH Terms] OR "lymphocyte$"[All Fields] OR "T-lymphocytes"[MeSH Terms] OR "T-lymphocyte$"[All Fields] OR "T-cell$"[All Fields] OR "B-lymphocytes"[MeSH Terms] OR "B-lymphocyte$"[All Fields] OR "B-cell$"[All Fields] OR "plasma cells"[MeSH Terms] OR "plasma cell$"[All Fields] OR "major histocompatibility complex"[MeSH Terms] OR "major histocompatibility complex"[All Fields] OR "MHC"[All Fields] OR "HLA antigens"[MeSH Terms] OR "HLA antigens"[All Fields] OR "antigens"[MeSH Terms] OR "antigen$"[All Fields] OR "antibodies"[MeSH Terms] OR "antibod$"[All Fields] OR "cytokines"[MeSH Terms] OR "cytokine$"[All Fields] OR "chemokines"[MeSH Terms] OR "chemokine$"[All Fields] OR "chemotaxis"[MeSH Terms] OR "chemotaxis"[All Fields] OR "chemotaxis, leukocyte"[MeSH Terms] OR "interleukins"[MeSH Terms] OR "interleukin$"[All Fields] OR "apoptosis"[MeSH Terms] OR "apoptosis"[All Fields] OR "programmed cell death"[All Fields] OR "reactive oxygen species"[MeSH Terms] OR "reactive oxygen species"[All Fields] OR "repair"[All Fields] OR "resolution"[All Fields]	
Step 2: Search for terms relating to ageing	"aging"[MeSH Terms] OR "aging"[All Fields] OR "ageing"[All Fields] OR "aged"[MeSH Terms] OR "aged"[All Fields] OR "elderly"[All Fields] OR "older"[All Fields] OR "geriatric"[All Fields] OR "old age"[All Fields] OR "older age"[All Fields] OR "age changes"[All Fields]	
Step 3: Combine the results for Step 1 and Step 2 using an AND operation		
Step 4: Search for terms relating to immune senescence	"immunosenescence"[MeSH Terms] OR "immunosenescence"[All Fields] OR "immune senescence"[All Fields] OR "senescence"[All Fields] OR "cell senescence"[All Fields] OR "cell aging"[MeSH Terms] OR "cell aging"[All Fields] OR "telomere"[MeSH Terms] OR "telomere"[All Fields] OR "telomere shortening"[MeSH Terms] OR "telomere shortening"[All Fields]	
Step 5: Combine the results for Step 3 and Step 4 using an OR operation		
Step 6a: Search for terms relating to periodontitis		**Step 6b: Search for terms relating to dental caries**
"periodontitis"[MeSH Terms] OR "periodontitis"[All Fields] OR "chronic periodontitis"[MeSH Terms] OR "chronic periodontitis"[All Fields] OR "aggressive periodontitis"[MeSH Terms] OR "aggressive periodontitis"[All Fields] OR "periodontal diseases"[MeSH Terms] OR "periodontal disease$"[All Fields] OR "periodontal"[All Fields] OR "gingival diseases"[MeSH Terms] OR "gingival disease$"[All Fields] OR "gingivitis"[MeSH Terms] OR "gingivitis"[All Fields] OR "gingiva$"[All Fields]		"caries"[MeSH Terms] OR "caries"[All Fields] OR "tooth decay"[MeSH Terms] OR "tooth decay"[All Fields] OR "carious"[MeSH Terms] OR "carious"[All Fields] OR "cariogenic"[MeSH Terms] OR "cariogenic"[All Fields]
Step 7a: Combine the results for Step 5 and Step 6a using an AND operation for terms relating to periodontal disease and immune senescence		
Step 7b: Combine the results for Step 5 and Step 6b using an AND operation for terms relating to caries and immune senescence		

Fig. 1. Search strategy for identifying papers that investigated the role of immune senescence in the pathogenesis of periodontitis and dental caries.

consideration for inclusion. Following this, the full text of selected articles was obtained and assessed for inclusion in the review, with any disagreements resolved by discussion. The number of articles assessed at each selection point for the periodontitis and caries searches is shown in Fig. 2. In the caries search, it became obvious that no specific articles were available for many aspects of the search (e.g. dental caries AND B cells). However, we utilized information from recently published reviews addressing those topics. Articles addressing pulpitis or periapical periodontitis, anticaries vaccinations or immune-stimulating probiotics were not further considered. Articles addressing hyposalivation, oral candidiasis, root caries and nutritional deficiencies were found to be very important for explaining the caries susceptibility of older adults but were included for discussion only.

Assessment of quality

Given the wide variety of identified studies, it was not possible to adopt the PRISMA statement or develop a focused (PICO) question for review. None of the selected studies were randomized controlled trials, and none of them included any form of intervention relevant to ageing or immune senescence. Mostly, the studies were descriptive reports of observational studies or experimental procedures in humans or experimental animals of different ages. Given these constraints, it was not possible to perform an assessment of quality of the identified papers.

Results

Search results

The flow of articles through the literature review process is presented in Fig. 2. The electronic and manual search strategies revealed a combined total of 1689 titles of which 1424 articles were excluded following title review, leaving 265 abstracts for screening. Of these, the full texts of 159 papers were obtained and finally 73 articles were considered to be eligible for inclusion in the review (43 for periodontitis and 30 for caries). The reasons for excluding articles are presented in Fig. 2.

Immune Senescence and Periodontitis

Study characteristics

Of the 43 articles identified from the review process, 23 related to studies in humans and 20 related to studies in experimental animals. The principal findings from the studies and brief interpretations are presented in Tables 1 and 2. A wide range of experimental designs and methodologies were used by researchers to study the effect of immune senescence and ageing on periodontitis.

Observations – human studies

Three studies evaluated leucocyte telomere length (LTL) in patients with and without advanced periodontitis (Masi et al. 2011, 2014, Sanders et al. 2015). These studies identified that patients with periodontitis had shorter LTL than controls, after adjusting for confounding variables including age. In the single longitudinal study that investigated changes in LTL over time (Sanders et al. 2015), there was no evidence of any difference in the rate of shortening of LTL between the periodontitis patients and the controls, suggesting that LTL shortening in periodontitis patients may have occurred in earlier life, or alternatively that individuals

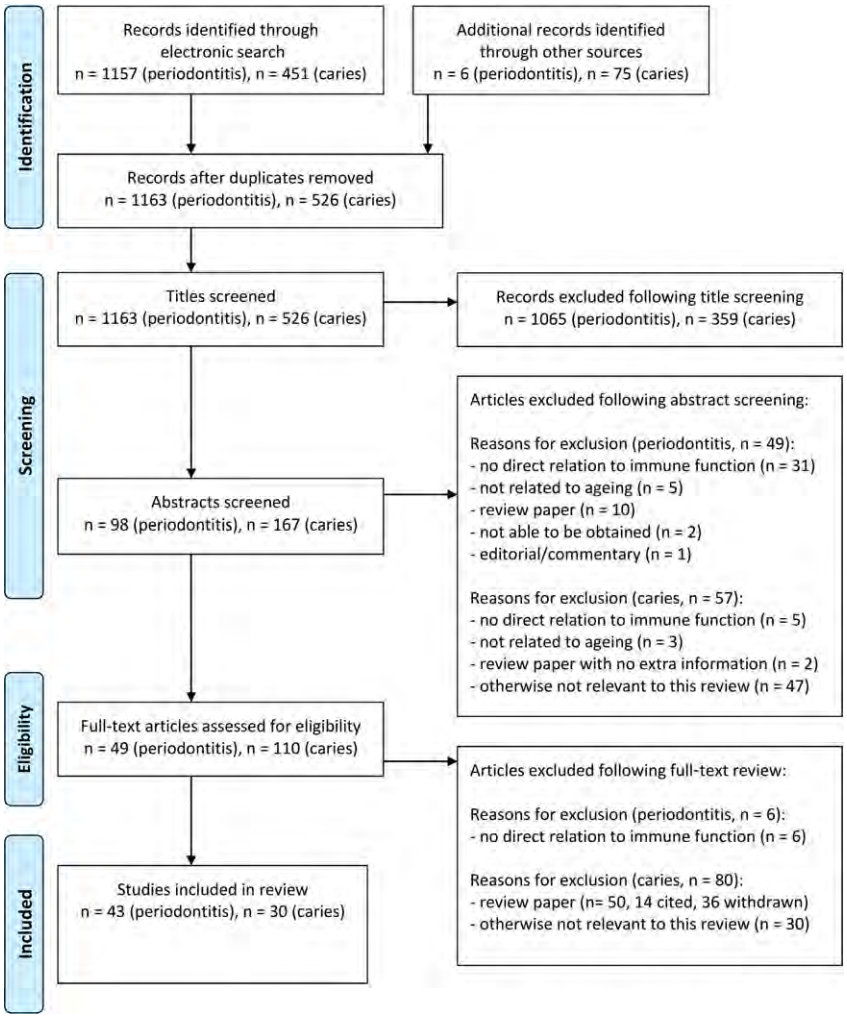

Fig. 2. Flow of articles through the search (based on the PRISMA checklist).

with shorter LTL were more likely to develop periodontitis.

Immune functioning in older patients with and without periodontitis was assessed in a study that evaluated neutrophil extracellular trap (NET) formation (Hazeldine et al. 2014). This study reported a significant age-related decrease in NET formation, suggesting possible increased susceptibility to infection in older individuals. However, the study also revealed that expression of IL-8 receptors and Toll-like receptor (TLR)-4 was similar on neutrophils from old and young patients. A further study identified a decrease in the ratio of CD4+ T-lymphocyte subsets and alterations in the ratios of antigen-presenting cells in older patients with periodontitis compared to younger patients with periodontitis (Bodineau et al. 2009).

A number of studies used the experimental gingivitis model to assess changes in gingival inflammation in young and older participants (Fransson et al. 1996, 1999, Tsalikis et al. 2002). Broadly, these studies indicated that there are differences in the inflammatory response that develops following biofilm accumulation between young and old subjects, with evidence of increased production of interleukin (IL)-1β, α2-macroglobulin and immunoglobulin Ig (G3) in the older subjects, together with elevated clinical signs of gingival inflammation. The studies are difficult to interpret, however, given that there were inconsistent changes in plaque levels between the younger and older participants in the different studies.

A large number of studies used an *ex vivo* design in which fibroblasts isolated from periodontal ligament (PDL) or gingiva underwent a variable number of passages to derive cells of different "ages", which were then exposed to either mechanical stress or challenge with lipopolysaccharide (LPS) from various periodontal pathogens (Kent et al. 1996, Ogura et al. 1996, Takiguchi et al. 1996, 1997, Shimizu et al. 1997, Abiko et al. 1998, Mochizuki et al. 1999, Ohzeki et al. 1999, Miura et al. 2000). The older cells were found to produce increased levels of plasminogen activator, prostaglandin E_2 (PGE_2), IL-1β and IL-6 compared to the younger cells. A further study in which gingival biopsies were obtained and assayed for inflammatory cytokines identified a variable pattern of cytokine levels, with levels generally higher in inflamed tissues, and with older subjects generally having higher levels of IL-1β and IL-6 compared to younger subjects (Yakovlev et al. 1996).

A number of studies reported no clear differences in inflammatory status between young and older participants. One study reported no differences in serum IL-6 levels between 70- and 80-year-old patients with periodontitis; however, there was no control group of younger patients in this study (Murata et al. 2001). Furthermore, studies of leucocyte subsets in peripheral blood samples and anti-*Prevotella intermedia* IgG levels in serum samples did not reveal evidence of consistent or significant differences between young subjects without periodontitis and older patients with mild-to-severe periodontitis (McArthur et al. 1995, 1996).

Observations – animal studies

A number of studies have evaluated gene expression profiles in a variety of different animal models, utilizing animals of different ages. Broadly, these suggest increased expression of genes that contribute to a pro-inflammatory state in older animals, although there was often a variable pattern of gene expression that can be difficult to interpret. Furthermore, the ages of experimental animals are difficult to align to human ageing. Increased age and the presence of periodontitis were associated with a differential pattern of

Table 1. Principal findings of the studies identified from the systematic review regarding periodontitis and immune senescence (human studies, $n = 23$)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Abiko et al. (1998)	Ex vivo human gingival fibroblasts (GFs) and PDL fibroblasts (PLFs) from young subjects (10–12 years), older subjects (18–23, and 26–37 years), Down syndrome patients (17–22 years) and young (4 weeks) and old (60 weeks) rats.	In vitro ageing achieved by passaging cells 5–6 times (young) or 18–20 times (old). Cultured GFs were exposed to LPS, and cultured PLFs were exposed to cyclic tension forces.	LPS-stimulated PGE_2, IL-1β, IL-6 and plasminogen activator production was higher in old human GFs than younger cells. RT-PCR showed higher gene expression of COX-2, IL-1β, IL-6 and tissue-type plasminogen activator in old cells than young cells. Cyclic tension applied to PLFs stimulated increased production of IL-1β, PGE_2 and plasminogen activator.	Ageing of gingival and PDL fibroblasts may be relevant in the pathogenesis of periodontal diseases through increased production of inflammatory mediators in response to LPS and mechanical stress.
Bodineau et al. (2009)	Chronic periodontitis patients aged > 75 years ($n = 8$), and chronic periodontitis patients aged 50–60 years ($n = 8$).	Gingival tissue specimens harvested and immunohistochemistry performed.	The CD4 + T lymphocytes/CD45RB+ ratio was significantly decreased, and the ratios of antigen-presenting dendritic cell (DC) SIGN+/CD45RB+ and DC-LAMP+/CD45RB+ cells were significantly increased, in the older patients.	In older periodontitis patients, there was a decrease in the ratio of gingival CD4 + lymphocytes, and an increase in the ratios of antigen-presenting cells, particularly mature DC-LAMP+ dendritic cells, suggesting altered immune functioning in older patients with periodontitis
de Arruda Cardoso Smith et al. (2004)	18 participants aged 5–54 years with Down syndrome and 11 controls aged 69–87 years.	Fluorescence in situ hybridization (FISH) used to investigate the integrity of two chromosome 21 regions (21q telomere and the 21q22.13-q22.2 region) in gingival fibroblasts.	No significant differences in terms of missing FISH signals between the patients and controls, and no significant correlations between missing FISH signals and age in the Down syndrome patients.	No evidence of impact of age on telomere integrity in chromosome 21.
Fransson et al. (1996)	Young (age 20–25 years) and older (age 65–80 years) periodontally healthy adults ($n = 5$ in both groups).	Three week experimental gingivitis model with assessment of plaque and gingival indices and gingival biopsy at days 0, 7 and 21.	Plaque accumulation was similar in both groups, but older subjects developed more gingival inflammation (higher gingival index scores, higher GCF volumes and larger inflammatory lesion in gingival biopsies) compared to the younger subjects.	Older subjects developed more gingivitis than younger subjects in a 3-week experimental gingivitis model.
Fransson et al. (1999)	Young (age 20–25 years) and older (age 65–80 years) periodontally healthy adults ($n = 5$ in both groups).	Three week experimental gingivitis model with assessment of α2-macroglobulin and IgG3 in GCF, and immunohistochemical analysis of gingival biopsies.	GCF samples from older individuals had higher levels of α2-macroglobulin and IgG3 compared to young subjects, and gingival biopsies from the older individuals contained a higher proportion of B cells and lower density of PMNs compared to the younger subjects.	There are differences in the inflammatory response that develops in response to biofilm accumulation in young and older individuals.

Table 1. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Hazeldine et al. (2014)	39 healthy young subjects (age 25.5 ± 4.2 years) and 38 healthy old subjects (age 69.9 ± 5.3 years) who did not have periodontitis. Plus 20 patients with chronic periodontitis (age 42.2 ± 7.1 years) and 20 age- and gender-matched controls who did not have periodontitis.	Peripheral blood neutrophils isolated and assessment of neutrophil extracellular trap (NET) formation assessed by immunofluorescence microscopy.	LPS- and IL-8-induced NET formation exhibited a significant age-related decline. Generation of reactive oxygen species (ROS, required for formation of NETs) was also reduced in neutrophils from older donors. Expression of IL-8 receptors (CXCR1 and CXCR2) and TLR-4 was similar on neutrophils from old and young subjects, and neutrophils challenged with PMA showed no age-associated differences in ROS or NET production. When neutrophils from young and old patients with chronic periodontitis were exposed to PMA and HOCL, NET formation to HOCL (but not PMA) was lower in older periodontitis patients, but not in comparison to age-matched controls.	These data suggest that a defect in proximal signalling is responsible for age-associated differences in ROS and NET production. Impaired NET formation is a defect of innate immunity in older adults, but does not contribute to increased incidence of periodontitis in older adults.
Kent et al. (1996)	Ex vivo human gingival fibroblasts (GFs) cell line.	Cells were passaged up to 10 times and cytokine levels in supernatants assayed.	IL-6 levels were detected in culture supernatants, and levels gradually decreased with increasing passage.	Not clear if reducing IL-6 levels were due to lower number of cells present in culture or a decrease in IL-6 mRNA or IL-6 protein synthesis.
Masi et al. (2011)	Case–control study of 356 subjects with periodontitis (n = 285 with chronic periodontitis and n = 71 with aggressive periodontitis) and 207 without periodontitis.	Leucocyte telomere length (LTL) was measured using RT-PCR after peripheral blood sample and leucocyte isolation. Assays were also performed for oxidative stress (reactive oxygen metabolites) and biological antioxidant potential, as well as for hsCRP.	Patients with periodontitis had significantly higher hsCRP and reactive oxygen metabolites than controls. Overall, periodontitis patients had shorter LTL than controls, independent of age, gender, ethnicity and smoking. When subdivided by periodontitis type, only chronic periodontitis patients displayed shorter LTL. LTL was negatively correlated with age, oxidative stress and severity of periodontitis.	Chronic inflammation could be the main driver of shorter LTL in patients with periodontitis. Shorter LTL was not observed in the aggressive periodontitis patients, but was in the chronic periodontitis patients. This may be because only long-term exposure to inflammation (such as might be experienced by chronic periodontitis patients) may affect the rate of telomere shortening. The aggressive periodontitis patients were slightly (but significantly) younger than the chronic periodontitis patients in this study.

Table 1. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Masi et al. (2014)	371 patients with type 2 diabetes and 259 with type 1 diabetes with either gingivitis, moderate or advanced chronic periodontitis, or who were edentulous.	Leucocyte telomere length (LTL) was measured using RT-PCR after peripheral blood sample and leucocyte isolation. Endotoxaemia was assessed using limulus amoebocyte lysate method. Periodontal status was determined using the single highest Basic Periodontal Examination score present (gingivitis = BPE 1 and 2; moderate periodontitis = BPE 3; advanced periodontitis = BPE 4).	Presence of periodontitis (BPE codes 3 and 4) was associated with shorter LTL ($p = 0.04$ when adjusted for age). A negative association was identified between LTL and endotoxaemia in the severe periodontitis and type 2 diabetes groups ($p = 0.01$ for both). Shorter LTL was associated with increased extent of periodontitis ($p = 0.01$), based on the cumulative sum of BPE score in all 6 sextants.	LTL is associated with endotoxaemia and presence of periodontitis in people with diabetes. A role for LTL shortening in pathogenic processes that may link periodontitis and diabetes remains to be determined.
McArthur et al. (1995)	Young subjects (25–35 years) without periodontitis and older subjects (65–75 years) with mild-to-severe periodontitis.	Assessment of total IgG in serum samples collected from the patients.	Levels of anti-*Prevotella intermedia* IgG in the elderly subjects were lower than those in the younger subjects. There were no differences between groups for six other IgG types against periodontal pathogens.	Periodontal disease in the elderly was not associated with significant or consistent changes in serum IgG levels to periodontal pathogens.
McArthur et al. (1996)	Young subjects (25–35 years) without periodontitis and older subjects (65–75 years) with mild-to-severe periodontitis.	Leucocyte subsets from peripheral blood samples determined using flow cytometry.	Other than increased numbers of leucocytes in the elderly group with severe periodontitis, no other alterations in the leucocyte parameters tested were identified.	Periodontal disease in the elderly was not associated with changes in leucocyte subsets in the peripheral blood due to ageing.
Miura et al. (2000)	Ex vivo human PDL-derived fibroblasts from 3 healthy subjects.	Mechanical tension applied to cultured fibroblasts that had undergone 5–7 passages (young cells) or 24–26 passages (aged cells).	Aged cells produced significantly more plasminogen activator in response to mechanical tension than young cells.	Ageing of PDL cells may affect the severity of inflammation and subsequent tissue degradation by producing increased quantities of plasminogen activator in response to forces caused by trauma from occlusion.
Mochizuki et al. (1999)	Ex vivo human gingival fibroblasts (GFs) from 5 healthy subjects, and gingival fibroblasts from 5 young (6 weeks old) and 5 older (20 months old) male Wistar rats.	Human GFs cells cultured for 5–6 passages (young cells) or 17–20 passages (old cells). Old and young human GFs and rat GFs exposed to LPS and plasminogen activator activity measured.	Older GFs (human and rat) produced higher plasminogen activator activity following LPS stimulation than did younger GFs.	The ageing of GFs may result in increased severity of inflammation and tissue breakdown by producing increased quantities of plasminogen activator in response to LPS.
Murata et al. (2001)	70- and 80-year-old patients with moderate or severe chronic periodontitis	Cross-sectional study of serum IL-6 levels.	No significant differences in serum IL-6 levels between patients with differing severity of periodontitis, or between the 70- and 80-year-old patients.	No evidence of an impact of age in serum IL-6 levels. All patients were of older age and serum levels of IL-6 were generally low.
Ogura et al. (1996)	Ex vivo human gingival fibroblasts (GFs) from 3 healthy subjects.	In vitro ageing achieved by passaging cells 5–6 times (young) or 17–20 times (old), then exposed to LPS.	LPS-stimulated IL-6 production was higher in old GFs than young GFs	Ageing of GFs may be relevant in periodontal pathogenesis through increased production of inflammatory mediators in response to LPS.

Table 1. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Ohzeki et al. (1999)	Ex vivo human PDL-derived fibroblasts from 3 healthy subjects.	Mechanical tension applied to cultured fibroblasts that had undergone 5–7 passages (young cells) or 24–26 passages (aged cells).	Aged cells produced significantly more PGE_2 in response to mechanical tension than young cells. The COX-2 mRNA level was higher in aged cells whereas COX-1 mRNA remained unchanged.	Ageing of PDL cells may affect the severity of inflammation and subsequent tissue degradation by producing increased quantities of PGE_2 in response to forces caused by trauma from occlusion.
Sanders et al. (2015)	178 patients with severe chronic periodontitis and 178 controls with no/mild chronic periodontitis.	Case–control study with leucocyte telomere length (LTL) measured from DNA collected at two time points, 6 years apart. Multiple linear regression used to evaluate associations between LTL at baseline, at follow-up, and change scores with severe periodontitis.	Periodontitis patients had shorter LTL at baseline ($p = 0.03$) and follow-up ($p = 0.04$) compared to controls, after adjusting for confounding. Overall, LTL reduced over time ($p = 0.02$). The rate of LTL shortening over time did not differ between cases and controls.	Periodontitis is associated with shorter LTL (as observed at baseline and follow-up). The finding of no impact of periodontitis on the rate of LTL shortening suggests that LTL shortening in the periodontitis cases may have occurred earlier in life, or alternatively, that individuals with shorter LTL were more likely to develop periodontitis.
Shimizu et al. (1997)	Ex vivo human PDL fibroblasts from 3 healthy subjects.	In vitro ageing achieved by passaging cells 5–6 times (young) or 18–20 times (old), then exposed to cyclic tension forces.	Old PDL fibroblasts subjected to mechanical tension produced twice as much IL-1β as did young cells.	Ageing of PDL fibroblasts may be relevant in the pathogenesis of periodontal diseases through increased production of inflammatory mediators in response to mechanical stress.
Takiguchi et al. (1996)	Ex vivo human gingival fibroblasts (GFs) from 3 healthy subjects.	In vitro ageing achieved by passaging cells 5–6 times (young) or 17–20 times (old), then exposed to LPS.	LPS-stimulated PGE_2 production was higher in old GFs than young GFs.	Ageing of GFs may be relevant in periodontal pathogenesis through increased production of inflammatory mediators in response to LPS.
Takiguchi et al. (1997)	Ex vivo human gingival fibroblasts (GFs) from 3 healthy subjects.	In vitro ageing achieved by passaging cells 5–6 times (young) or 17–20 times (old), then exposed to LPS.	LPS-stimulated IL-1β and PGE_2 production was higher in old GFs than young GFs.	Ageing of GFs may be relevant in periodontal pathogenesis through increased production of inflammatory mediators in response to LPS.
Tsalikis et al. (2002)	Young (age 20–22 years) and older (age 61–65 years) periodontally healthy adults ($n = 5$ in both groups).	Three week experimental gingivitis model with assessment of IL-1α and IL-1β levels in GCF at baseline, day 21 (at end of plaque accumulation phase) and day 28 (1 week after resuming oral hygiene).	IL-1α levels increased in a similar fashion from day 0 to day 21 and then decreased to day 28 with no significant differences between the groups. IL-1β levels appeared to increase only in the older patients at day 21 and were significantly higher than those in the young patients at this time point. Other than this, there were no significant differences between the groups.	Interpretation is difficult, as although gingival inflammation (gingival index) and inflammatory cytokines appear to be higher in the older participants at day 21, plaque levels were also higher in the older group at this time point.

Table 1. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Vincent-Bugnas et al. (2013)	20 patients with chronic periodontitis and 10 controls who did not have periodontitis.	Periodontal pocket epithelial cells harvested using a curette from deep and shallow pockets in the periodontitis patients and from the sulcus in healthy patients.	Epithelial cells harvested from the pockets/sulci were commonly infected with EBV. The basal level of EBV infection in epithelial cells was significantly increased in periodontitis compared to controls and correlated with disease severity. EBV-infected epithelial cells produced larger amounts of the chemokine CCL20 and appeared more prone to apoptosis.	Periodontal/gingival epithelial cells may serve as a latent source of EBV-infected cells and contribute to EBV persistence in healthy carriers. Latently infected epithelial cells in the healthy sulcus may represent a baseline of infection and subsequent changes in the gingival environment may lead to increased EBV infection. In turn, EBV may exacerbate inflammation by inducing cell death and promoting a pro-inflammatory response.
Yakovlev et al. (1996)	Individuals with and without gingivitis in age groups 6–14, 18–35, 36–54, ≥55 years.	Gingival biopsies obtained and tissues assayed for inflammatory cytokines.	Variable pattern of cytokine levels, with levels typically higher in inflamed tissues. Generally, older subjects had higher levels of IL-1β and IL-6 than younger subjects.	Older individuals have elevated levels of pro-inflammatory cytokines present in the gingival tissues.

macrophage development that tended towards a more tissue-destructive pro-inflammatory macrophage phenotype, as well as increased expression of genes for chemokines and receptors including TLR-4 and CD14 (Gonzalez et al. 2015). In a mouse model of cultured macrophages challenged with *Porphyromonas gingivalis*, a variable pattern of gene expression was observed when comparing older and younger mice, with evidence of increased expression of genes for pro-inflammatory mediators such as IL-1α, IL-1β, IL-6, CCL3 and CXCL10, but decreased expression of genes for mediators such as IL-10 and TNF-α in the older mice. There was also evidence of a trend for reduced expression of TLR-pathway-associated genes and genes involved in intracellular signalling in older mice (Shaik-Dasthagirisaheb et al. 2015). Similarly, in a study in which mouse gingival fibroblasts were challenged with *P. gingivalis*, a variable pattern of increased and decreased gene expression was again noted when comparing young and older mice. However, there were some contrasting findings compared to the previous study of mouse macrophages, such as decreased expression of genes for pro-inflammatory mediators such as IL-6, CXCL1, TLR-2 and TLR-4, and increased expression of genes for MMP-3 and MMP-13 in older mice compared to younger mice (Domon et al. 2014). In a study of macrophages isolated from the peritoneal cavity of young and old mice that were cultured with *P. gingivalis*, various age-dependent increases and decreases in gene expression were noted, including increased expression of receptors that amplify inflammation (such as C5a anaphylatoxin receptor and TREM-1) in older mice, although no differences in bacterial killing were noted between the young and older mice (Liang et al. 2009). In apparent contrast, a further study of gingival fibroblasts from old and young rats reported decreased expression of matrix metalloproteinase (MMP)-9 in the older rats, together with evidence of cell cycle dysregulation (Kim et al. 2009).

Histological studies have reported reduced collagen density and increased collagen degradation in the

Table 2. Principal findings of the studies identified from the systematic review regarding periodontitis and immune senescence (animal studies, $n = 20$)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Domon et al. (2014)	Young (8 weeks) and older (≥24 months) C57BL/6 mice.	Gingival fibroblasts (GFs) were stimulated with *P. gingivalis* LPS or live *P. gingivalis* strain W83. Expression of genes coding for cytokines, chemokines, immune receptors, growth factors and MMPs was assessed using RT-PCR, and enzyme-linked immunosorbent assay (ELISA) for IL-6 and TGF-β1.	GFs from older mice exhibited significantly decreased gene expression of *Il-6, Cxcl1, Tlr2, Tlr4, Irak3, Kgf, Timp1, Timp3* and *Rankl* and increased expression of *TgfB1, Mmp3, Mmp13* and *Opg*. Basal IL-6 production was significantly lower and TGF-β1 production was significantly higher in older mouse GFs. *P. gingivalis* LPS significantly upregulated IL-6 and TGF-β1 production in old and young GFs. Live *P. gingivalis* downregulated IL-6 production whereas TGF-β1 production was not affected in young and old GFs. *P. gingivalis* W83 upregulated *Vegf, Fgf-2* and *Bmp2* expression in both young and old GFs, but this stimulatory effect was lower in older GFs.	There are age-related alterations in the gene expression of various cytokines, chemokines, immune receptors, growth factors, MMPs and TIMPs in mouse GFs. This suggests that alterations in gene expression could play a role in the pathogenesis and progression of periodontitis in older individuals. However, further studies are warranted because transcript levels do not necessarily reflect protein levels or activation status.
Ebersole et al. (2008)	Rhesus monkeys aged from <1 year to >24 years, divided into age groups <3, ≥3–8, 8–15 and >15 years.	Serum samples obtained for analysis of CRP, IL-8, MCP-1, RANTES, MMP-1, MMP-2, MMP-9, PGE_2, LBP and BPI (bactericidal permeability-inducing factor).	A variable presentation of differences in serum inflammatory mediator levels was observed between the age group categories. In broad terms, serum BPI, RANTES, MMP-1 and MMP-9 were significantly higher in younger animals compared to older animals. Serum antibody responses to periodontal pathogens were generally lower in the younger animals.	The explanation for the higher levels of inflammatory mediators in the younger animals is not clear. However, it is clear that there are age-associated variations in levels of systemic inflammatory mediators.
Eskan et al. (2012)	Young (8–10 weeks) and older (18 months) C57BL/6 mice.	A ligature-induced, single-tooth split-mouth, experimental periodontitis model was utilized. Assessment of Del-1 (an endogenous inhibitor of neutrophil adhesion dependent on the integrin LFA-1) expression in excised gingival tissue was performed using immunohistochemistry and quantitative PCR. Alveolar bone loss was assessed by direct measurement.	Del-1 mRNA and protein were expressed in gingival tissues in young and old mice, but at a much lower level in older mice. The lower expression of Del-1 in the gingival tissues of old mice was associated with higher expression of IL-17, and more neutrophil infiltration than that in young mice. A significant inverse correlation was noted between Del-1 expression and alveolar bone loss in old mice. Local administration of Del-1 inhibited IL-17 production, neutrophil accumulation and bone loss.	Older age in mice was associated with reduced levels of Del-1 in the gingival tissues, which may contribute to dysregulated or enhanced neutrophil recruitment and bone loss in the older animals. Del-1 as an endogenous anti-inflammatory factor may be a potential therapeutic for inflammatory diseases such as periodontitis.
Gonzalez et al. (2011)	Periodontally healthy Rhesus monkeys aged ≤3 (young), 12–15 (adult) and 18–22 years (aged), and Rhesus monkeys with periodontitis aged 12–15 and 18–22 years.	Gingival biopsies obtained from healthy and diseased sites and analysed for expression of genes related to apoptosis.	Younger animals with healthy periodontal tissues had greater expression of pro-apoptotic and lower expression of anti-apoptotic genes compared to older animals with healthy tissues. Genes differentially expressed in healthy and periodontitis tissues in adult animals were mutually exclusive to genes expressed in tissues from aged animals.	Apoptotic events which occur normally in the gingival tissues could be reduced in ageing, although the interpretation of changes in apoptotic pathways associated with ageing is difficult given the differing expression profiles observed in adult and aged animals.

Table 2. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Gonzalez et al. (2013)	Periodontally healthy Rhesus monkeys aged ≤3 (young), 3–7 (adolescent), 12–15 (adult) and 18–22 years (aged).	Gingival biopsies obtained from periodontally healthy sites and gene expression in apoptotic pathways assessed.	Significant positive correlations with ageing were noted for 12 genes, and significant negative correlations with 5 genes. Altered gene expression related to apoptosis receptor levels, apoptotic pathways, cytokine effects on apoptotic effects and cell cycle.	Both the positively and negatively correlated genes indicate that healthy tissues in older animals exhibit decreased apoptotic potential compared to younger animals.
Gonzalez et al. (2014)	Rhesus monkeys from 3 to 23 years old.	Buccal gingival sample obtained from healthy or periodontitis sites, RNA isolated and microarray analysis to characterize the transcriptome.	There was increased transcription of genes related to MHC class II and negative regulation of NK cells with ageing in healthy gingival tissues. In adult and ageing periodontitis tissues, there was decreased transcription of genes for MHC class II antigens, and upregulation of MHC class I-associated genes.	Transcriptional changes in this animal model suggest a response of healthy ageing tissues through the class II pathway, whereas there are altered responses in periodontitis that could reflect host-associated self-antigens, or targeting intracellular microbial pathogens.
Gonzalez et al. (2015)	Rhesus monkeys with periodontal health (in age groups ≤3, 3–7, 12–16, 18–23 years), and Rhesus monkeys aged 12–16 and 18–23 years with periodontitis.	Buccal gingival samples obtained from healthy or periodontitis-affected gingival tissues. RNA extraction and analysis of gene expression in relation to macrophage phenotype undertaken to identify genes that were differentially expressed across the age groups and in animals affected by periodontitis.	Significant increase in M1/M2 macrophage phenotype gene expression was observed in periodontally healthy aged tissues, with increased expression of genes for chemokines and receptors such as CCR2, CCR7, TLR-4 and CD14. Similarly, in periodontitis tissues, expression of M1/M2 genes was increased, including factors for T-cell communication (CCL5, CCL19), B-cell communication (CCL19, CCR7) and macrophage-specific factors such as scavenger receptors SRA and CD163.	Increasing age and periodontitis are associated with a differential pattern of macrophage infiltration, differentiation and maturation that tends towards an increased inflammatory and tissue-destructive macrophage phenotype (M1/M2).
Gonzalez et al. (2016)	Periodontally healthy Rhesus monkeys in age groups ≤ 3, 3–7, 12–16, 18–23 years).	RNA isolated from gingival biopsies and transcriptome analysed.	A subset of 159 genes were differentially expressed across at least one of the age categories. Distinct communities of functionally related genes were associated with immune/inflammatory responses.	Gene expression in younger animals was weighted towards host responses associated with anti-inflammatory mediators or those linked with T-cell regulation of responses.
Irie et al. (2014)	Male Fischer 344 rats (n = 24) that were 4 or 8 months old divided into two groups: experimental and control.	A dentifrice containing anti-oxidative, anti-inflammatory and antibacterial agents was applied into the gingival sulcus in the experimental group 5 days per week for 2 months. Histological analysis was performed to assess collagen density, and serum reactive oxygen metabolites were assayed.	In the control group, gingival collagen density decreased with ageing, but did not change in the experimental group. Both groups showed an increase in serum oxidative stress with ageing, but this was less in the experimental group than the control group (p = 0.008).	Ageing is associated with increased collagen degradation, and this was reduced by the application of the dentifrice.

Table 2. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Kato & Mikami (2011)	BCG-immunized mice aged 6–7 weeks and 12–14 months.	Mice were injected intraperitoneally with *P. gingivalis* strain 381, then sacrificed after injection. Peritoneal lavage fluid was assayed for IFN-γ, IL-12, IL-4, IL-1β, IL-6 and TNF-α using ELISA. *P. gingivalis* DNA in peritoneal cell exudates was assayed by PCR.	In BCG-immunized young mice, *P. gingivalis* was eliminated within 24 h of inoculation whereas *P. gingivalis* elimination occurred more slowly in the BCG-immunized old mice. In young mice, this reduction in *P. gingivalis* was accompanied by increased IFN-γ and IL-12 levels, and nitric oxide (NO) was continuously produced. The BCG vaccine was more effective in augmenting bactericidal activity in the young mice compared to the old mice.	BCG inoculation was an effective immunostimulant in young mice, but not in the older mice. IFN-γ production was rapidly increased following *P. gingivalis* inoculation in the young BCG-immunized mice (probably to activate macrophages to clear the *P. gingivalis* infection), but this did not occur in the old mice.
Kim et al. (2006)	Young (6 months old) and older (24 months old) pathogen-free Sprague-Dawley rats.	Gingival tissues harvested and analysed for expression of chemokines and receptors.	RANTES and CCR5 mRNA and protein levels were significantly elevated in the aged rat gingival tissues compared to the younger rats. Old rats had enhanced mitogen-activated protein kinase (MAPK) activities: extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK.	Age-related increases in RANTES and CCR5 expression are associated with increased NF-κB and MAPK activity in gingival tissues.
Kim et al. (2007)	Young (6 months old) and older (24 months old) pathogen-free Sprague-Dawley rats.	Gingival tissues harvested and analysed to study effects of kaempferol on ROS and glutathione (GSH) oxidative status.	Older rats had significantly higher ROS levels and significantly lower GSH levels compared to the younger rats.	There is evidence for increased oxidative stress in the gingival tissues of old rats compared to younger rats.
Kim et al. (2009)	Young (6 weeks old) and older (16 months old) male Wistar rats.	Gingival fibroblasts (GFs) isolated from gingival tissues and cultured and G1 phase cell cycle regulation and MMP-9 expression assessed.	G1 cell cycle protein levels were significantly reduced in response to IL-1β stimulation with increasing in vitro age. TNF-α-induced MMP-9 expression was also reduced in aged GFs compared to young GFs.	Cell cycle dysregulation and downregulation of MMP-9 expression in rat GFs may play a role in disease progression in older animals.
Liang et al. (2009)	Young (8–10 weeks old) and aged ($\geq$ 18 months old) BALB/cByJ mice.	Macrophages isolated from peritoneal cavity, cultured and incubated with *P. gingivalis*. Quantitative real-time PCR used to assess gene expression, and flow cytometry to assess surface receptor expression, in resting or activated macrophages.	Various age-dependent alterations in gene expression were observed, including both reductions and increases in immune activity. Surface expression of receptors that amplify inflammation, C5a anaphylatoxin receptor (C5aR) and triggering receptor expressed on myeloid cells-1 (TREM-1), was elevated in macrophages from aged mice. No significant changes were observed in phagocytosis and killing of *P. gingivalis* between the young and aged mice.	Immune functioning is altered in aged mice compared with young mice, with evidence of both increased and decreased expression of mediators and receptors that are involved in immunity. Increased surface expression of C5aR and TREM-1 by macrophages in older mice may contribute to increased inflammation associated with age.
Liang et al. (2010)	Young (8–10 weeks old) and aged ($\geq$18 months old) BALB/cByJ mice.	Histological assessment of alveolar bone resorption, and quantitative real-time PCR to assess mRNA expression of inflammatory cytokines and other mediators in excised gingival tissues.	Older mice displayed significantly more alveolar bone loss than young mice, as well as significantly elevated expression of mRNA for IL-1β, TNF-α, IL-17A, TLR-2, CD14, CD11b, CD18, complement C5a receptor and triggering receptor expressed on myeloid cells-3 (TREM-3).	Older mice have naturally occurring periodontitis with increased alveolar bone loss and increased expression of pro-inflammatory cytokines and innate immune receptors involved in the induction or amplification of inflammation.

Table 2. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Pandruvada et al. (2016)	Rhesus monkeys in age groups 12–16 years (adult) and 18–23 years (aged) with health and periodontitis in both groups.	Gingival tissue samples analysed for gene expression profiles relevant to osteoclast/osteoblast proliferation, adhesion and function.	Healthy aged tissues had a gene profile suggestive of enhanced osteoclastic adhesion, proliferation and function, and impaired osteoblastic activity. The gingival transcriptome in animals with periodontitis was indicative of a local inflammatory response driving towards bone resorption.	Periodontitis is associated with a pro-bone resorptive gingival transcriptome irrespective of age. However, a greater bone-destructive environment is associated with ageing in healthy tissues.
Shaik-Dasthagirisaheb et al. (2010)	Young (2 months) and aged (1 and 2 years) mice.	Bone marrow-derived macrophages were cultured and exposed to *P. gingivalis* strain 381 and levels of various cytokines, chemokines, nitric oxide (NO) and PGE_2 were determined in culture supernatants using multiplex immunoassays.	*P. gingivalis* induced robust production of TNF-α, IL-6, IL-10, neutrophil chemoattractant protein, MCP-1, MIP-1α, RANTES, NO and PGE_2 from the macrophages of young mice. Macrophages from the 2-year-old mice produced significantly less TNF-α, IL-6, NO, MCP-1 and MIP-1α compared to the young or 1-year-old mice. There were no differences in the levels of IL-1β, IL-10, PGE_2 or RANTES between the age groups. Macrophages from 2-month- and 1-year-old mice produced similar levels of chemokines except for MCP-1, which was reduced in the 1-year-old mice.	Immunological function alters with increasing age, with clear evidence of a reduced immune response in older mice. This suggests that senescence plays a role in changing immune responsiveness to pathogens such as *P. gingivalis*.
Shaik-Dasthagirisaheb et al. (2015)	2- and 12-month-old mice of three types: wild-type, MyD88 knockout and $Trif^{Lps2}$ (mice with a mutation in the *Lps2* gene that renders TRIF non-functional).	Microarray analysis of gene expression in bone marrow-derived macrophages cultured with *P. gingivalis* to investigate the contributions of the TLR adaptor molecules MyD88 and TRIF and ageing on expression of TLR-pathway-associated mRNAs.	There was a variable pattern of gene expression in the 12-month-old wild-type mice compared to the 2-month-old wild-type mice, including increased expression of some genes (e.g. *Il1a, Il1b, Il6, Ccl3, Cxcl10*) and decreased expression of others (e.g. *Il10, Il12a, Tnf, Ifnb, Ifng*), although these changes did not necessarily achieve statistical significance. There was a trend for reduced expression of TLR-pathway-associated genes, and of genes involved in intracellular signalling in the older mice. Age also influenced the expression of genes in the MyD88 knockout and $Trif^{Lps2}$ mice following challenge with *P. gingivalis*.	Age has an influence on the immune responses elicited by *P. gingivalis* with a variable pattern of increased and decreased expression of genes that are relevant in periodontal immunobiology.
Shimizu et al. (2000)	Young (6 weeks old) and older (60 weeks old) male Wistar rats ($n = 5$ in each group).	Mechanical tension applied to cultured PDL cells harvested from the rats.	Cell deformation associated with twofold increase in PGE_2 and IL-1β production by old PDL cells compared with that by young cells, although constitutive levels were similar in both groups.	Increased production of PGE_2 and IL-1β produced by PDL cells subject to mechanical forces may subsequently lead to increased alveolar bone resorption.

Table 2. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation
Yoneda et al. (2013)	Male Fischer 344 rats aged 2 (n = 6) and 4 months (n = 18)	Applied topical reduced co-enzyme Q10 (rCoQ10) or vehicle only in the 4-month-old rats for 2 months. Histological assessments were performed and serum assay of 8-OHdG (oxidized derivative of deoxyguanosine, one of the major products of DNA oxidation) was undertaken using ELISA.	The vehicle-treated rats showed an age-dependent increase in circulating oxidative stress (8-OHdG), and these levels were significantly lower in the experimental group than the control group at 6 months of age. rCoQ10 decreased oxidative DNA damage and tartrate-resistant acid-phosphatase (TRAP)-positive osteoclasts in the periodontal tissues at 6 months of age compared to controls, and lowered gene expression of caspase-1 and IL-1β in the periodontal tissue.	Ageing was associated with increases in circulating oxidative stress, and rCoQ10 suppressed age-related inflammatory reactions and osteoclast differentiation by inhibiting oxidative stress.

gingival tissues of old rats compared to younger rats (Irie et al. 2014), and ageing has been associated with increased oxidative stress in rats (Kim et al. 2007, Yoneda et al. 2013). Increased alveolar bone loss and elevated expression of mRNA for inflammatory cytokines and other pro-inflammatory mediators have been reported in old mice compared to young mice (Liang et al. 2010), but, on the other hand, *P. gingivalis*-challenged macrophages from older mice produced significantly less tumour necrosis factor (TNF)-α, IL-6, NO, monocyte chemoattractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α compared to younger mice, although there were no differences in the levels of IL-1β, IL-10, PGE_2 and RANTES (regulated on activation, normal T cell expressed and secreted, also known as CCL5) between the young and old animals (Shaik-Dasthagirisaheb et al. 2010). In 24-month-old rat gingival tissues, RANTES (CCL5) and C-C chemokine receptor 5 (CCR5) mRNA and protein levels were significantly higher compared to those in younger rats, as well as ERK, JNK and p38 MAPK, suggesting that increased RANTES and CCR5 expression are associated with increased NF-κB and MAPK activity (Kim et al. 2006). Similar to studies conducted using human tissue samples, cultured PDL cells from older rats that were exposed to mechanical tension produced increased levels of PGE_2 and IL-1β compared to cells from younger rats (Shimizu et al. 2000). In apparent contrast, a study of systemic inflammation (serum analysis of a variety of pro-inflammatory mediators) reported the serum bactericidal permeability-inducing factor (BPI), RANTES (CCL5), MMP-1 and MMP-9 were significantly higher in the serum samples of younger primates compared to older primates, whereas serum antibody responses to periodontal pathogens were generally lower in the younger animals (Ebersole et al. 2008).

Immune Senescence and Dental Caries

Whereas a number of articles have specifically addressed immune senescence in relation to periodontitis, this is not the case for caries and immune senescence. However, there are immune processes affecting the dental biofilm which have an impact on caries susceptibility, and studies have evaluated components of the innate and the adaptive immune responses which have an impact on caries risk. Information regarding senescence of these components was identified from the literature and is discussed below, with key findings from the studies presented in Table 3. As the field of caries and immune senescence is relatively unexplored (this article aims to encourage researchers to work in this area), some conclusions might, at this stage, be considered more theoretical.

Innate immune response

Ageing affects the phenotype and functions of cells and secretory components (e.g. antimicrobial peptides, cytokines) of innate immunity. For example, the expression and function of innate receptors as well as subsequent signal transduction in phagocytes and dendritic cells is altered in the aged. Furthermore, production of interferon I/III and other cytokines by leucocytes (e.g. neutrophils, dendritic cells) is decreased in the elderly (Solana et al. 2012). Although little is known regarding senescence with respect to salivary antimicrobial factors, it can be presumed that reduced saliva flow in many elderly individuals could reduce their concentration in the oral cavity. A caries-protective role of mucins and cathelicidin LL-37, together with age-related changes in concentrations, has been demonstrated (Baughan et al. 2000, Davidopoulou et al. 2012, Culp et al. 2015), and it has been reported that levels of salivary antimicrobial peptides are variable over the lifetime and between individuals and are linked with bacterial colonization (Malcolm et al. 2014).

The Toll-like receptors TLR-2 and TLR-4 have been shown to recognize the peptidoglycan of Gram-positive and the LPS of Gram-negative bacteria, respectively, either alone or in association with the common coreceptor CD14 (Zhao et al. 2014). Microbial invasion of dentin has been shown to upregulate

Table 3. Principal findings of the studies identified from the systematic review regarding caries and a variety of immune senescence-related factors (n = 30)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation of authors [comment of review authors about relevance for elderly]
Alstad et al. (2008)	92 (44 female, 48 male) 71-year-old subjects in Sweden consecutively chosen from a representative cohort study.	The subjects were examined for: (1) caries-related status, (2) oral function, (3) salivary conditions, (4) cariogenic microorganisms and (5) oral sugar clearance.	The latent variables from the factor analyses were used and an association found between clearance and caries in multivariate regression models.	Oral sugar clearance appears to be independently associated with the prevalence of dental caries in the elderly.
Baughan et al. (2000)	Twenty-four subjects between the ages of 65 and 82 years (average age 73 years) with no active caries lesions were recruited for the study.	Potential relationships between *Streptococcus mutans* titres in the oral cavity and mucin concentrations, saliva flow rates, DMFS index (decayed, missing and filled surfaces) and age were studied.	The best model for predicting *S. mutans* category contained log mucin MG2 as a predictor variable for all of its parameter estimates. No other sets of parameter estimates were statistically significant.	Age-related reduced levels of mucins and especially MG2 might serve as an important predictor variable in caries risk assessment for older adults.
Belda-Ferre et al. (2015)	17 donors (8 caries-free subjects and 9 caries-active subjects) were selected for sampling of supragingival plaque.	The human oral metaproteome was investigated using HILIC chromatography, followed by LC-MS/MS analysis.	Among the immunity-associated proteins identified, a statistically significant overexpression of azurocidin, complement C3, pIgr, RAC-2 and hASC-3 was seen in healthy volunteers, whereas in diseased patients only Ig alpha and mu chains were over-represented, pointing to a wider variety of defence mechanisms in healthy individuals.	The proteome differs between caries-free and caries-active subjects including those proteins associated with immune functions. [An impact on elderly patients can be assumed, however, it was not the subject of this study and remains to be demonstrated].
Bergandi et al. (2007)	The study was carried out on 20 healthy caries-free children and 20 age-matched patients affected by two to eight carious lesions.	Western blotting analysis of saliva samples to identify the presence of the soluble form of CD14.	CD14 was present in healthy controls but completely absent in the saliva of caries-affected patients. Interestingly, it re-appeared in patients' saliva a few weeks after dental restoration.	The absence of salivary soluble CD14 could indicate caries activity. [An age-dependent effect of sCD14 can be assumed, however, it was not the subject of this study and remains to be demonstrated].
Biria et al. (2010)	The study was carried out on 40 healthy children, of whom 20 were caries-free and 20 had early childhood caries, within the ages of 36 to 71 months.	A subgroup of seven children with early childhood caries received caries treatment. The sCD14 levels in salivary samples were analysed by enzyme-linked immunosorbent assay (ELISA) and found to be higher among children with caries than caries-free subjects (57.82 ng/ml vs. 31.92 ng/ml).	After three months, the mean concentration of sCD14 among the treated children decreased to 11.38 ng/ml, which was significantly lower than that of children with caries before intervention ($p < 0.001$), and also caries-free children ($p < 0.05$).	The increased levels of sCD14 can be considered as a marker of inflammation and innate immune response during early childhood caries. [An impact on elderly patients can be assumed, however, it was not the subject of this study and remains to be demonstrated].

Table 3. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation of authors [comment of review authors about relevance for elderly]
Challacombe & Lehner (1976), Rose et al. (1994), Chia et al. (2000), Nikfarjam et al. (2004), Chawda et al. (2011), Golpasand Hagh et al. (2013), Gomez et al. (2015), Colombo et al. (2016)	In these eight studies, 546 individuals (caries-free or caries-resistant, caries-active or caries-susceptible, of different age groups) were recruited.	Correlations between *S. mutans*-directed salivary secretory IgA (sIgA), serum IgG/IgM antibody levels and *S. mutans* counts in saliva as well as DMF (decayed, missing, filled) indices were investigated.	In summary, in of all the studies, sIgA (directed against various *S. mutans* antigens) decreased with increasing symptoms of caries activity. In contrast, IgG serum levels were found to be more positively correlated with caries activity. Serum IgM levels (if tested) were not clearly related to caries activity.	These results suggest that salivary sIgA antibodies to *S. mutans* may play a role in natural protection from dental caries whereas increased serum IgG might be more reactive and indicative for active caries. [An impact on elderly patients can be assumed, especially as the switch from IgM to IgA is compromised].
Culp et al. (2015)	Five mice of each sex and genotype (mucin 19 positive and negative).	Impact of mucin 19 deletion on cariogenicity in mice on a highly cariogenic diet and challenged with *S. mutans*.	When challenged with *S. mutans*, total smooth and sulcal surface lesions were more than 2- and 1.6-fold higher in Muc19(−/−) mice compared with wild-type mice, whereas the severity of lesions were up to six- and tenfold higher, respectively.	Lubricating mucins play a protective role against caries. [An impact on elderly patients can be assumed as mucin production is reduced among the elderly].
Davidopoulou et al. (2012)	Unstimulated whole saliva was collected from 49 systemically healthy and gingivitis-free children aged 2–18 years. Their caries activity was recorded.	The salivary cathelicidin LL-37 concentration was determined by ELISA and related to age.	A positive correlation of LL-37 concentration with increasing age was observed.	LL-37 is an important molecule of immunity in the oral environment with an age-related concentration and it seems to play a protective role against caries. [An impact on elderly patients can be assumed, however, it was not the subject of this study and remains to be demonstrated].
Ferro et al. (2008)	595 elderly residents from Northern Italy (mean age 83.2 ± 9.2 years).	Subjects were investigated for oral health status and treatment needs.	Poor oral hygiene was found in 86% of the patients, root caries in 51% and coronal caries in 46%.	Root caries (and other oral health problems) can be frequently found among the elderly.
Grimoud et al. (2003)	Population of 110 people in long-stay geriatrics units.	The prevalence of *Candida* colonization and the species distribution was investigated.	The oral cavity was colonized by *Candida* spp in 67% of cases with *C. albicans* and *C. glabrata* being the most frequent species.	The prevalence of [cariogenic] *Candida* species is high among seniors.
Hegde et al. (2013)	100 otherwise healthy adults divided into four groups: DMFT index (decayed, missing and filled teeth) 0, <3, <10 and >10.	The total antioxidant capacity of saliva and serum was estimated by the phosphomolybdenum method.	The mean total antioxidant capacity level of both saliva and serum increased highly significantly with the DMFT index.	Total antioxidant capacity of saliva has a linear relation with caries; that is, as the severity of caries increases, the total antioxidant capacity level also increases. [As the antioxidant capacity is known to decrease by age this caries-defence mechanism is compromised].

Table 3. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation of authors [comment of review authors about relevance for elderly]
Hirschfeld et al. (2015)	A pilot study was performed with supragingival biofilms and whole saliva from six healthy non-smoking subjects (26–50 years old).	Neutrophils obtained from blood were stimulated with twelve bacterial species isolated from biofilms (or with LPS) to monitor neutrophil extracellular trap (NET) formation.	Neutrophils, NETs, neutrophil-associated proteins, interleukin-1β and tumour necrosis factor were detected within plaque samples and saliva. Oral bacteria triggered NET formation.	The findings indicate that neutrophils are attracted towards dental biofilms, in which they become incorporated and where they are stimulated by microbes to release NETs and immunostimulatory proteins. [An age-dependent effect on neutrophil attraction can be assumed; however, it was not the subject of this study and remains to be demonstrated.]
Kamoda et al. (2008)	100 elderly people aged 77 years were examined.	Natural Killer (NK) cells were evaluated with flow cytometry together with bacterial counts for certain oral bacteria. The oral health status was examined.	A larger percentage of NK cells showed significant correlations to the isolation numbers of total streptococci, the species numbers of opportunistic pathogens, the numbers of decayed teeth and the amount of bridge work.	A higher proportion of CD69(+)NK cells is associated with the incidence of dental caries and the number of opportunistic pathogens and total streptococci in the oral cavity of the elderly.
Malcolm et al. (2014)	Dental plaque and saliva were collected from 57 children aged 12–24 months at baseline, of whom 23 children were followed up at 3 years of age.	Saliva was assessed for LL-37, human neutrophil peptides 1–3, calprotectin, lactoferrin, salivary IgA, total plaque bacteria and *S. mutans*.	Concentrations of antimicrobial peptides were highest in the saliva of 3-year-old children with the greatest burden of *S. mutans*.	Salivary antimicrobial peptides are variable over the lifetime and between individuals and are linked with bacterial colonization. [An impact on elderly patients can be assumed; however, it was not the subject of this study and remains to be demonstrated.]
Mila et al. (2012)	Multicentre observational study of a sample of 62 institutionalized (Spanish nursing home) people aged 68–96 years.	Dietary data were collected using the double weight method.	A large amount of potatoes were served (109.64 g/day) as were sweets and pastries (62.14 g/day).	There is a need to improve the residents' energy intake and to redistribute their energy and protein intake among the various food groups.
Natasasmita et al. (1989)	33 subjects with different caries experience.	The activity of serum IgG against the sonicated antigens of *S. mutans* was evaluated by micro-ELISA.	The serum IgG titre to *S. mutans* was significantly higher in the subjects with no detectable carious lesions than in the subjects with active caries.	The serum IgG antibodies to *S. mutans* were associated with the level of oral hygiene but not with age.
Power et al. (2014)	Two hundred and eight (94 males, 114 females) Irish community-dwelling subjects aged 64–93 years.	Nutritional status was assessed using the Mini Nutritional Assessment.	Older subjects ($\geq$ 75 years) consumed significantly ($p < 0.01$) more desserts/sweets than those aged 64–74 years.	The data indicate that the diet of elderly individuals is suboptimal with respect to nutrient intake, and excessive in terms of fat intake, with implications for the health status of this population group.

Table 3. (continued)

Author, year	Study population/experimental model	Study design	Main findings	Interpretation of authors [comment of review authors about relevance for elderly]
Sharma et al. (2014)	4 (young)-, 8-, 12- and 16 (senescent)-month-old mice (6 animals per group) were employed.	Mice were analysed for various immune parameters involving neutrophils, peripheral blood lymphocytes and "inflammaging" markers in plasma and humoral immune response.	Various changes were monitored during the ageing process with macrophages showing a remarkable decrease in TLR-2 and TLR-4 expression.	A decline in cell-mediated immune response was evident which suggests a skewed Th2 pathway during ageing. [The situation in the oral cavity was not the subject of this study and impact on sTLR-2 remains to be demonstrated.]
Sztajer et al. (2014)	In vitro study regarding communication between *S. mutans* and *C. albicans* cells.	Scanning electron microscopy, gas chromatography–mass spectrometry and transcriptome analysis of single or dual biofilms.	The complete quorum sensing system of *S. mutans* was stimulated by *C. albicans*, resulting in fundamentally changed virulence properties of the caries pathogen and more biomass.	*Candida* species [higher prevalence in the elderly] trigger *S. mutans* growth but reduce production of extracellular polysaccharides.
Tanida et al. (2001)	45 age-matched healthy volunteers and 60 patients (aged 32–86) with oral candidiasis.	The influence of ageing on candidal adhesion to keratinocytes, neutrophil functions and growth-inhibitory agents in saliva was investigated.	The generation of superoxide from neutrophils in saliva and their *Candida* killing activity decreased with age, especially in patients. Furthermore, a larger number of *Candida* adhered to oral keratinocytes were obtained from the elderly healthy controls than to those obtained from young controls.	The decreases in saliva flow rate and salivary anticandidal factors, suppression of salivary neutrophil function and the increase in candidal adhesion sites on keratinocytes predispose elderly individuals to oral candidiasis.
Taubman et al. (2007)	Rat experiments: 6–9 rats per group, two experiments with 3 or 5 groups.	DNA of periodontal pathogens was co-injected with *S. sobrinus* glucosyltransferase (GTF).	Animals receiving alum-GTF plus bacterial DNA (*P. gingivalis* in particular) demonstrated significantly reduced serum immunoglobulin G (IgG) antibody, salivary IgA antibody and T-cell proliferation to GTF compared to animals immunized with alum-GTF alone.	DNA from periodontal disease-associated bacteria [which increase in their abundance by age] did not enhance, but in fact suppressed, the immune response to a protein antigen from cariogenic streptococci, potentially through suppressor of cytokine signalling (SOCS) components triggered by innate mechanisms.
Toffanello et al. (2010)	Randomly stratified sample of 97 men and 94 women born between 1913 and 1918.	Nutrient and energy intake was monitored between 1988 and 1999.	Nutrient and energy intake remained fairly stable over a decade, despite changes in eating habits, with a higher intake of sweets and a lower consumption of soft drinks in both genders.	The increase in sweet-eating might be because ageing itself increases a person's sweet tooth, and this could be regarded as an age-related effect on dietary habits.
Zhao et al. (2014)	Unstimulated whole saliva was collected from 20 caries-free and 20 caries-active children between the ages of 5 and 13 years.	The concentration of sCD14 and sTLR-2 together with that of the cytokine IL-8 reported to be increased in dental caries was assessed by ELISA.	The sTLR-2 concentration (but not that of sCD14 or IL-8) in caries-active saliva was significantly higher than that in caries-free saliva.	The sTLR-2 in saliva is related to caries, serving as a potential biomarker for caries activity. [An age-dependent effect of sTLR-2 can be assumed, however, it was not the subject of this study and remains to be demonstrated].

TLR-4 in odontoblasts and mediate transforming growth factor-β (TGF-β) secretion facilitating collagen synthesis. Soluble TLR-2 and its coreceptor CD14 identified in saliva can bind to the cell wall components of cariogenic bacteria and modulate the disease process. Both were found to be either upregulated (Biria et al. 2010, Zhao et al. 2014) or absent (Bergandi et al. 2007) in children with early childhood caries. In mice, a reduction in the expression of TLR-2 and TLR-4 on macrophages during ageing has been described (Sharma et al. 2014). Other outcomes of immune senescence, such as decreases in activities of respiratory burst enzymes and neutrophil phagocytosis, or of CD28 expression in lymphocytes, have also been described for mice.

Release of NETs (a mixture of DNA, histones, antimicrobial proteins and granule content) as an antimicrobial defence strategy is gaining increasing attention. Neutrophils are attracted towards dental biofilms in which they become incorporated and stimulated, possibly by microbes, to release microbicidal NETs and immune-stimulatory proteins controlling biofilm growth (Hirschfeld et al. 2015). More compounds of leucocytes such as azurocidin, cysteine-rich secretory protein 3, cathelicidin LL-37, neutrophil defensin 1, cathepsin G, coronin 1A and BPI, integrin alpha-M, profilin-1, hASC-3, RAC-2 and CEACAM1, and alpha- and beta-2-macroglobulin are found in the biofilm of palatal and lingual tooth surfaces, and some have been correlated with caries progression (Belda-Ferre et al. 2015). As age-related decreases in chemotaxis, phagocytosis and intracellular killing of pathogens have been described (Solana et al. 2012), it is possible that NETs and other leucocyte compounds are less effective in controlling the biofilm in older people. Furthermore, the phenotype and function of natural killer (NK) cells from elderly individuals showed remodelling of the different subsets (decrease in CD56bright subpopulation and increase in the CD56dim cells) and decreased expression of activating natural cytotoxicity receptors. These changes also explain the decreased production of cytokines and the lower per-cell cytotoxicity observed in older individuals (Solana et al. 2012). A higher proportion of CD69(+)NK cells is associated with the incidence of dental caries and the number of opportunistic pathogens and total streptococci in the oral cavity of the elderly, and the proportionate number of CD69(+)NK cells has been suggested as a useful indicator for oral infections in elderly subjects (Kamoda et al. 2008).

Adaptive immune response

During an immune response, B cells can switch the expression of surface Ig from IgM to IgG, IgE or (salivary) secretory IgA (sIgA). The class switch recombination requires chromatin opening of the so-called S regions, recognition and cleavage of the target DNA by an endonuclease, and repair and ligation of the cleaved ends. Class switch recombination is extremely important for the humoral immune response, because it generates antibodies of the same specificity but with different effector functions. At least one contributing factor in B cells from older individuals responsible for their inability to respond well to vaccination or invading pathogens is a defect in these molecular events essential for the production of secondary isotypes (Frasca et al. 2011), among them anticariogenic IgG and IgA. The weakened class shift increases IgM isotype concentrations. As IgM affinity to antigens is lower than in secondary isotypes, the total affinity of immunoglobulins is reduced with ageing.

Naïve T cells from elderly persons exhibit numerous functional defects, including significantly shorter telomeres, a restricted T-cell receptor repertoire, reduced IL-2 production and impaired expansion and differentiation into effector cells, when compared with naïve cells from young people. As a consequence, their ability to mediate effective immune responses against new antigens is decreased (Weiskopf et al. 2009).

Taken together, senescence of the adaptive immune response, including reduced antibody affinity, reduced IgG and IgA production (Tanida et al. 2001) and defects of coordinating T cells, has a significant influence on B-cell function and humoral immune responses. As salivary sIgA specific to cariogenic microorganisms is believed to play an important role in the immune response against dental caries, a reduction in sIgA will ultimately permit increased risk of caries progression (Challacombe & Lehner 1976, Rose et al. 1994, Chia et al. 2000, Nikfarjam et al. 2004, Chawda et al. 2011, Golpasand Hagh et al. 2013, Gomez et al. 2015, Colombo et al. 2016). The same protective role might also be true for IgG (Natasasmita et al. 1989), but a more reactive role of anti-*S. mutans* IgG has also been demonstrated (Challacombe & Lehner 1976). Furthermore, proteolytic periodontal pathogens, which may have increasing abundance in saliva of elderly individuals, further suppress the immune response to cariogenic bacteria by two processes: (i) cleavage of immunoglobulins, complement and anticariogenic peptides, and (ii) DNA-based suppression of immune response to mutans streptococcal glucosyltransferase (Taubman et al. 2007).

Finally, oxidative stress which occurs as a result of an imbalance between free radical/reactive oxygen species and the antioxidant system has been implicated as one of the important contributory aetiological factors in many of the inflammatory oral pathologies, and dental caries is no exception. It seems to be important to upregulate antioxidant capacity (total antioxidant capacity, including peroxidases) in saliva to combat caries progression, but this might be impaired with ageing (Hegde et al. 2013).

Discussion

There are three key observations that suggest that the human immune system declines in effectiveness with age. Firstly, the incidence of infectious diseases increases in people over 65 years as compared to younger individuals (Boraschi et al. 2013), and there is increased autoimmunity and degenerative diseases that are associated with a constitutive low-grade inflammation (Goronzy et al. 2012, Boraschi et al. 2013). Secondly, the effectiveness of vaccination (e.g. to the influenza virus) is reduced in older age groups

(Goronzy & Weyand 2012). Thirdly, there is a delay in wound repair and healing with old age, processes linked with the function of immune cells such as macrophages.

Immune senescence and periodontitis

Alterations in innate immunity influence immediate defences against infections as well as compromising activation of adaptive immunity and lead to heightened inflammation. It is known that there is a functional decline in phagocytes such as neutrophils and macrophages with increasing age despite the fact that the numbers of these cells appear to be maintained throughout life. Functional variations may relate to the observed changes in receptor expression and signal transduction pathways (e.g. TLR signalling) in phagocytes that occur with increasing age (Hajishengallis 2010, Solana et al. 2012). In the context of periodontitis, age-related reductions in the formation of NETs have been reported, underscoring the fact that innate immunity does alter with increasing age (Hazeldine et al. 2014). Animal studies have also reported reduced apoptosis in association with ageing, suggesting that increasing age could be associated with increased risk of tissue damage as a result of dysregulated immune responses and persistence of inflammatory cells in the periodontal tissues.

The immune system is highly integrated, and thus, impaired macrophage function (e.g. attenuation of cytokine and chemokine secretion) compromises neutrophil release (from the bone marrow stores) and neutrophil chemotaxis. Studies of experimental animals have clearly identified altered gene expression (both increases and decreases) of a range of pro-inflammatory mediators in older animals with and without periodontitis compared to younger animals. Results from different experimental models can be difficult to interpret; for example, increased expression of TLR-4 was noted in the gingival tissues of aged Rhesus monkeys compared to young monkeys (Gonzalez et al. 2015), whereas reduced expression of TLR-pathway-associated genes was noted in bone marrow-derived macrophages of older mice compared to younger mice (Shaik-Dasthagirisaheb et al. 2015).

The effectiveness of the immune system in old age is compromised by other physiological changes, for example, in the skin (and other epithelial tissues), where there is decreased blood vessel density with increasing age which affects neutrophil diapedesis and reduces mitotic activity of constituent cells (e.g. epithelial cells and fibroblasts). This compromises opportunities for repair, and, linked to this, decreased collagen density and increased collagen degradation have been reported in the gingival tissues of older rats compared to younger animals (Irie et al. 2014). Cellular changes in ageing also result in an increased number of apoptotic cells and there is greater oxidative stress (Boraschi et al. 2013), a finding reflected in studies in this review, in which age-associated increases in oxidative stress in gingival tissues were observed (Yoneda et al. 2013, Irie et al. 2014). There is also evidence that dendritic cell populations are more constitutively activated in the elderly (Panda et al. 2010), a finding that was reflected in human studies of dendritic cell populations in patients with periodontitis (Bodineau et al. 2009).

Interestingly, the manufacture of lymphocytes (B and T cells) declines with age. Naïve T-cell production reduces as a direct result of thymic atrophy and this, combined with the decreased capacity to produce new T helper cells from memory cells and older naïve T cells, compromises the capacity of the immune system to mount both primary responses to novel antigens (such as a vaccine) and secondary responses to previously encountered pathogens (Weng 2006, Goronzy & Weyand 2012). At the cellular level, shifts in the balance of functional T-cell subsets in the elderly (e.g. increased dermal T_{reg} cells) potentially also influence immune responses (Vukmanovic-Stejic et al. 2008). There is now clear evidence for age-related intracellular molecular changes that lead to impaired T-cell receptor signalling and compromised CD4 T-cell function (Yu et al. 2012), a finding that was observed in older periodontitis patients (>75 years) who had reduced CD4 ratios compared to younger periodontitis patients (50–60 years) (Bodineau et al. 2009). There is a similar decline in naïve B-cell production from the bone marrow as well as in memory B-cell expansion with increasing age (Weng 2006). Furthermore, the B-cell repertoire decreases with age and is related to poor health (Gibson 2009). Critically, the decline in B-cell functionality and available T-cell help leads to an age-related decline in antibody function including reduced production of high-affinity antibodies (Weng 2006).

Compromised T-cell function and increased T-cell apoptosis are consequences of age-associated "telomeric erosion" as well as reduced DNA damage repair (Parish et al. 2010, Brunner et al. 2012). Reduced leucocyte telomere length (LTL) in chronic periodontitis patients compared to controls, even after accounting for age, was a consistent finding in the small number of studies that have investigated this area (Masi et al. 2011, 2014, Sanders et al. 2015). It has been postulated that periodontitis could accelerate LTL shortening as a result of increased systemic inflammation and oxidative stress (Steffens et al. 2013). However, another explanation could be that shorter LTL increases susceptibility to periodontitis; that is, younger individuals with periodontitis also have premature cell ageing (i.e. shorter telomeres, potentially as an inherited trait) which predisposes them to chronic diseases. On the other hand, a study of LTL in peripheral blood and gingival fibroblasts from aggressive periodontitis patients and age-matched periodontally healthy controls found no significant differences in LTL between the two groups (Takahashi et al. 2004), a finding that was also reported in a study of both chronic and aggressive periodontitis patients (Masi et al. 2011). In the single longitudinal study that investigated changes in LTL over time (Sanders et al. 2015), there was no evidence of any difference in the rate of shortening of LTL between chronic periodontitis patients and controls, suggesting that LTL shortening in periodontitis patients may have occurred in earlier life, or alternatively that individuals with shorter

LTL were more likely to develop periodontitis.

Taken collectively, the above studies suggest that there is altered immune responsiveness associated with ageing. The concept of inflammaging holds that there is a heightened state of basal activation of the immune system in old age, which is associated with inappropriate (and damaging) inflammatory responses (Franceschi et al. 2000, Franceschi 2007). However, it can be difficult to conceptualize inflammaging in the context of observations of decreased aspects of immune responsiveness in older age. It is clear from Tables 1 and 2 that a mixed pattern of age-associated increases and decreases in production of various inflammatory mediators by defence cells such as macrophages and neutrophils has been reported in the context of periodontal pathobiology. It is plausible that such alterations result in an inability to effectively control pathogens, which could lead to chronic persistence of pathogens and increased accumulation of defence cells in the periodontal tissues, in turn leading to increased tissue damage and disease.

Immune senescence and dental caries

The effect of senescence on salivary antimicrobial substances, TLRs, coreceptor CD14, neutrophils, NETs, immunostimulatory proteins and differential production of immunoglobulins with presumed impact on biofilm composition is important for caries susceptibility in the elderly patient. The 23 studies that have been discussed above (and summarized in Table 3) with regard to caries-related immune senescence each only contribute a small amount of information to the overall picture. However, as caries aetiology is so multifactorial, it is important to consider a few additional factors associated with ageing that further increase the risk of caries (studies also included in Table 3 summing up to 30 studies in total).

Firstly, the age-related progressive destruction of acinar cells leads to hyposalivation, which can be compounded by many of the chronic diseases of ageing and medications. With hyposalivation, the concentrations of certain lubricating mucins (e.g. MG2) which play a protective role against caries are reduced in saliva of the elderly (Baughan et al. 2000, Culp et al. 2015). However, elderly patients with normal saliva flow do exist and most non-immunoglobulin defence factors seem to act with full capacity over the entire life span (Tenovuo 1992). Secondly, saliva contains a variety of anticandidal proteins. The suppression of salivary neutrophil function and the increase in candidal adhesion sites on keratinocytes predispose elderly individuals to oral candidiasis (Tanida et al. 2001, Grimoud et al. 2003). The caries association of *Candida* species is, however, still controversial. The recently reported cross-feeding and inter-kingdom communication between *S. mutans* and *C. albicans* may support its aetiological importance (Sztajer et al. 2014). Thirdly, elderly persons are particularly at risk of root caries, which follows as a consequence of periodontitis (Ferro et al. 2008). Fourthly, the elderly, and especially the institutionalized elderly, are at increased risk of nutritional deficiencies. This is because the ability to smell and taste reduces with age, diminishing quality of life and impeding good nutrition. In nursing homes, a large amount of carbohydrate-containing foods are often served (Mila et al. 2012). Older subjects (≥75 years) seem to consume significantly more desserts/sweets than those aged 64–74 years (Power et al. 2014). Toffanello et al. investigated the dietary intake of free-living elderly people and found that ageing itself increases a person's sweet tooth and that dietary habits are age-related (Toffanello et al. 2010). In addition, oral sugar clearance, both salivary flow-dependent and salivary flow-independent, seems to be reduced among the elderly (Alstad et al. 2008). Therefore, diet clearly may contribute to increased caries risk among the elderly.

Diet and nutrition

Age-related changes in immune function are influenced not just by intrinsic factors but also by factors such as diet and nutritional status, medical conditions and stress. Immune responsiveness has been shown to be inadequate in those who are nutritionally compromised, with strong evidence indicating protein–energy malnutrition, deficiencies in several micronutrients, both vitamins and minerals (Maijo et al. 2014), or excessive intake of saturated trans-fatty acids in impairing aspects of immune function (Enos et al. 2013). Zinc homoeostasis influences immune functioning (although precise mechanisms are unclear), and zinc deficiency results in complex immune deficiencies affecting primary T cells, natural killer cells and reduced antibody formation (Rink & Haase 2007). Zinc is often deficient in the diets of the elderly, and preliminary evidence supports a role for zinc imbalance in periodontitis (Orbak et al. 2007). Notwithstanding these findings, the exact mechanisms of how diet may impact on immune senescence are unknown and further study in this area is required (Maijo et al. 2014).

Conclusions

Based on the studies included in this review, we conclude that there is evidence to support the assertion that immune functioning in the context of periodontitis alters with increasing age. Such alterations should not be regarded simply as a decline in immune functioning. It is also clear that the patterns of changes in immune functioning with age are complex and incompletely understood. Results from different studies that have utilized differing experimental designs are variable, with mixed patterns of increased and decreased expression of genes and pro-inflammatory mediators being reported. Human studies suggest evidence for altered neutrophil function and increased production of pro-inflammatory mediators such as interleukin-1β (IL-1β), IL-6 and prostaglandin E_2 (PGE_2) in older subjects with various periodontal conditions compared to younger subjects, and animal experiments broadly suggest increased expression of genes that contribute to a pro-inflammatory state in older animals compared to younger animals. With respect to dental caries, this review summarizes information on certain immune functions important for caries prevention and how these factors might change over lifetime. A

systematic search of the literature identified aspects of innate immunity (antimicrobial peptides, neutrophils and NETs, dendritic cells, natural killer cells, Toll-like receptors) and the adaptive immune response (B and T cells, secretory IgA and IgG), all of which may play a role in caries susceptibility with increasing age. For both periodontitis and dental caries, it is not clear how mechanistic changes in immune functioning that occur with increasing age may relate to susceptibility to disease in older age.

Recommendations for research

A concern about our understanding of the ageing immune system and its relation to oral diseases is that many studies have been carried out using mouse and other animal models, and translation of this knowledge to human biology is not straightforward. Furthermore, it is important that we adopt the perspective that the immune system in old age is naturally both quantitatively and qualitatively different to that found in younger, healthy adults rather than simply regarding age-related changes as a decline in immune function. Longitudinal prospective studies of oral health and development, such as the Department of Veterans Affairs Dental Longitudinal Study (VADLS) and the oral physiology component of the Baltimore Longitudinal Study of Ageing (BLSA), the Dunedin study (http://dunedinstudy.otago.ac.nz/ accessed 21 October 2016) or the more recently started KOALA study (https://www.koala-study.nl/ accessed 21 October 2016), will certainly shed more light on the interrelation between oral health and ageing.

Future research studies to investigate changes in immune functioning with increasing age in the context of periodontitis and dental caries are indicated. This is particularly important given the increases in the proportion of elderly persons in many populations of the world currently being observed. Studies are indicated to identify cells that play a role in senescence in periodontitis and caries, and how their phenotype and function contribute to disease. Studies involving human participants should aim to evaluate longitudinal changes in the elements of immunity and in immune functioning over time, and in a range of periodontal and dental conditions as well as in ageing individuals who are periodontally and dentally healthy. This information will be important to develop patient-specific diagnostic strategies such as biomarker analysis in addition to the development of adjuvant therapies. A non-reductionist approach to investigating immune function should be adopted, with awareness of the importance of considering immune functioning in broad terms rather than focusing on individual inflammatory mediators, proteins and receptors in isolation. Experimental designs and models should be used that are generalizable to the wider human population and that provide relevant information in the context of periodontitis and dental caries as well as considering the role of extrinsic factors that affect immune functioning such as nutritional and general health status.

Recommendations for clinical practice

Clinicians should be aware that immune functioning alters with increasing age. Ultimately, this may lead to changed susceptibility to periodontal disease and dental caries. However, the detail of how mechanistic changes in immune functioning relate to disease susceptibility is not clear. Clinicians should therefore remain vigilant to the signs of increased disease progression when reviewing and monitoring their ageing patients and implement appropriate preventive strategies as a matter of routine.

References

Abiko, Y., Shimizu, N., Yamaguchi, M., Suzuki, H. & Takiguchi, H. (1998) Effect of aging on functional changes of periodontal tissue cells. *Annals of Periodontology* **3**, 350–369.

Alstad, T., Holmberg, I., Osterberg, T. & Birkhed, D. (2008) Associations between oral sugar clearance, dental caries, and related factors among 71-year-olds. *Acta Odontologica Scandinavica* **66**, 358–367.

de Arruda Cardoso Smith, M., Borsatto-Galera, B., Feller, R. I., Goncalves, A., Oyama, R. S., Segato, R., Chen, E., Carvalheira, G. M., Filho, A. S., Burbano, R. R. & Payao, S. L. (2004) Telomeres on chromosome 21 and aging in lymphocytes and gingival fibroblasts from individuals with Down syndrome. *Journal of Oral Science* **46**, 171–177.

Batchelor, P. (2015) The changing epidemiology of oral diseases in the elderly, their growing importance for care and how they can be managed. *Age and Ageing* **44**, 1064–1070.

Baughan, L. W., Robertello, F. J., Sarrett, D. C., Denny, P. A. & Denny, P. C. (2000) Salivary mucin as related to oral *Streptococcus mutans* in elderly people. *Oral Microbiology & Immunology* **15**, 10–14.

Baumgartner, W., Schimmel, M. & Muller, F. (2015) Oral health and dental care of elderly adults dependent on care. *Swiss Dental Journal* **125**, 417–426.

Belda-Ferre, P., Williamson, J., Simon-Soro, A., Artacho, A., Jensen, O. N. & Mira, A. (2015) The human oral metaproteome reveals potential biomarkers for caries disease. *Proteomics* **15**, 3497–3507.

Bergandi, L., Defabianis, P., Re, F., Preti, G., Aldieri, E., Garetto, S., Bosia, A. & Ghigo, D. (2007) Absence of soluble CD14 in saliva of young patients with dental caries. *European Journal of Oral Sciences* **115**, 93–96.

Biria, M., Sattari, M., Vahid Golpayegani, M. & Kooshki, F. (2010) Association of salivary sCD14 concentration levels with early childhood caries. *Iranian Journal of Immunology* **7**, 193–197.

Bodineau, A., Coulomb, B., Tedesco, A. C. & Seguier, S. (2009) Increase of gingival matured dendritic cells number in elderly patients with chronic periodontitis. *Archives of Oral Biology* **54**, 12–16.

Boraschi, D., Aguado, M. T., Dutel, C., Goronzy, J., Louis, J., Grubeck-Loebenstein, B., Rappuoli, R. & Del Giudice, G. (2013) The gracefully aging immune system. *Science Translational Medicine* **5**, 185 ps188.

Brunner, S., Herndler-Brandstetter, D., Arnold, C. R., Wiegers, G. J., Villunger, A., Hackl, M., Grillari, J., Moreno-Villanueva, M., Burkle, A. & Grubeck-Loebenstein, B. (2012) Upregulation of miR-24 is associated with a decreased DNA damage response upon etoposide treatment in highly differentiated CD8(+) T cells sensitizing them to apoptotic cell death. *Aging Cell* **11**, 579–587.

Challacombe, S. J. & Lehner, T. (1976) Serum and salivary antibodies to cariogenic bacteria in man. *Journal of Dental Research* **55 Spec No**, C139–C148.

Chawda, J. G., Chaduvula, N., Patel, H. R., Jain, S. S. & Lala, A. K. (2011) Salivary SIgA and dental caries activity. *Indian Pediatrics* **48**, 719–721.

Chia, J. S., Chang, W. C., Yang, C. S. & Chen, J. Y. (2000) Salivary and serum antibody response to *Streptococcus mutans* antigens in humans. *Oral Microbiology & Immunology* **15**, 131–138.

Colombo, N. H., Pereira, J. A., da Silva, M. E., Ribas, L. F., Parisotto, T. M., Mattos-Graner Rde, O., Smith, D. J. & Duque, C. (2016) Relationship between the IgA antibody response against *Streptococcus mutans* GbpB and severity of dental caries in childhood. *Archives of Oral Biology* **67**, 22–27.

Culp, D. J., Robinson, B., Cash, M. N., Bhattacharyya, I., Stewart, C. & Cuadra-Saenz, G. (2015) Salivary mucin 19 glycoproteins: innate immune functions in *Streptococcus mutans*-induced caries in mice and evidence for expression in human saliva. *Journal of Biological Chemistry* **290**, 2993–3008.

Davidopoulou, S., Diza, E., Menexes, G. & Kalfas, S. (2012) Salivary concentration of the antimicrobial peptide LL-37 in children. *Archives of Oral Biology* **57**, 865–869.

Domon, H., Tabeta, K., Nakajima, T. & Yamazaki, K. (2014) Age-related alterations in

gene expression of gingival fibroblasts stimulated with *Porphyromonas gingivalis*. *Journal of Periodontal Research* **49**, 536–543.

Ebersole, J. L., Steffen, M. J., Gonzalez-Martinez, J. & Novak, M. J. (2008) Effects of age and oral disease on systemic inflammatory and immune parameters in nonhuman primates. *Clinal and Vaccine Immunology* **15**, 1067–1075.

Enos, R. T., Davis, J. M., Velazquez, K. T., McClellan, J. L., Day, S. D., Carnevale, K. A. & Murphy, E. A. (2013) Influence of dietary saturated fat content on adiposity, macrophage behavior, inflammation, and metabolism: composition matters. *Journal of Lipid Research* **54**, 152–163.

Eskan, M. A., Jotwani, R., Abe, T., Chmelar, J., Lim, J. H., Liang, S., Ciero, P. A., Krauss, J. L., Li, F., Rauner, M., Hofbauer, L. C., Choi, E. Y., Chung, K. J., Hashim, A., Curtis, M. A., Chavakis, T. & Hajishengallis, G. (2012) The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nature Immunology* **13**, 465–473.

Ferro, R., Besostri, A., Strohmenger, L., Mazzucchelli, L., Paoletti, G., Senna, A., Stellini, E. & Mazzoleni, S. (2008) Oral health problems and needs in nursing home residents in Northern Italy. *Community Dental Health* **25**, 231–236.

Franceschi, C. (2007) Inflammaging as a major characteristic of old people: can it be prevented or cured? *Nutrition Reviews* **65**, S173–S176.

Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E. & De Benedictis, G. (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences* **908**, 244–254.

Fransson, C., Berglundh, T. & Lindhe, J. (1996) The effect of age on the development of gingivitis. Clinical, microbiological and histological findings. *Journal of Clinical Periodontology* **23**, 379–385.

Fransson, C., Mooney, J., Kinane, D. F. & Berglundh, T. (1999) Differences in the inflammatory response in young and old human subjects during the course of experimental gingivitis. *Journal of Clinical Periodontology* **26**, 453–460.

Frasca, D., Diaz, A., Romero, M., Landin, A. M. & Blomberg, B. B. (2011) Age effects on B cells and humoral immunity in humans. *Ageing Research Reviews* **10**, 330–335.

Gibson, K. L., Wu, Y. C., Barnett, Y., Duggan, O., Vaughan, R., Kondeatis, E., Nilsson, B. O., Wikby, A., Kipling, D. & Dunn-Walters, D. K. (2009) B-cell diversity decreases in old age and is correlated with poor health status. *Aging Cell* **8**, 18–25.

Golpasand Hagh, L., Zakavi, F., Ansarifar, S., Ghasemzadeh, O. & Solgi, G. (2013) Association of dental caries and salivary sIgA with tobacco smoking. *Australian Dental Journal* **58**, 219–223.

Gomez, C. R., Nomellini, V., Faunce, D. E. & Kovacs, E. J. (2008) Innate immunity and aging. *Experimental Gerontology* **43**, 718–728.

Gomez, S. I., Jaramillo, L. M., Moreno, G. C., Roa, N. S. & Rodriguez, A. (2015) Differential reactivity of salivary IgA and IgG against *Streptococcus mutans* proteins in humans with different caries experience. *Acta Odontologica Latinoamericana* **28**, 3–12.

Gonzalez, O. A., Nagarajan, R., Novak, M. J., Orraca, L., Gonzalez-Martinez, J. A., Kirakodu, S. S. & Ebersole, J. L. (2016) Immune system transcriptome in gingival tissues of young nonhuman primates. *Journal of Periodontal Research* **51**, 152–163.

Gonzalez, O. A., Novak, M. J., Kirakodu, S., Orraca, L., Chen, K. C., Stromberg, A., Gonzalez-Martinez, J. & Ebersole, J. L. (2014) Comparative analysis of gingival tissue antigen presentation pathways in ageing and periodontitis. *Journal of Clinical Periodontology* **41**, 327–339.

Gonzalez, O. A., Novak, M. J., Kirakodu, S., Stromberg, A. J., Shen, S., Orraca, L., Gonzalez-Martinez, J. & Ebersole, J. L. (2013) Effects of aging on apoptosis gene expression in oral mucosal tissues. *Apoptosis* **18**, 249–259.

Gonzalez, O. A., Novak, M. J., Kirakodu, S., Stromberg, A., Nagarajan, R., Huang, C. B., Chen, K. C., Orraca, L., Martinez-Gonzalez, J. & Ebersole, J. L. (2015) Differential gene expression profiles reflecting macrophage polarization in aging and periodontitis gingival tissues. *Immunological Investigations* **44**, 643–664.

Gonzalez, O. A., Stromberg, A. J., Huggins, P. M., Gonzalez-Martinez, J., Novak, M. J. & Ebersole, J. L. (2011) Apoptotic genes are differentially expressed in aged gingival tissue. *Journal of Dental Research* **90**, 880–886.

Goronzy, J. J., Li, G., Yu, M. & Weyand, C. M. (2012) Signaling pathways in aged T cells – a reflection of T cell differentiation, cell senescence and host environment. *Seminars in Immunology* **24**, 365–372.

Goronzy, J. J. & Weyand, C. M. (2012) Immune aging and autoimmunity. *Cellular and Molecular Life Sciences* **69**, 1615–1623.

Grimoud, A. M., Marty, N., Bocquet, H., Andrieu, S., Lodter, J. P. & Chabanon, G. (2003) Colonization of the oral cavity by Candida species: risk factors in long-term geriatric care. *Journal of Oral Science* **45**, 51–55.

Hajishengallis, G. (2010) Too old to fight? Aging and its toll on innate immunity. *Molecular Oral Microbiology* **25**, 25–37.

Hazeldine, J., Harris, P., Chapple, I. L., Grant, M., Greenwood, H., Livesey, A., Sapey, E. & Lord, J. M. (2014) Impaired neutrophil extracellular trap formation: a novel defect in the innate immune system of aged individuals. *Aging Cell* **13**, 690–698.

Hegde, M. N., Hegde, N. D., Ashok, A. & Shetty, S. (2013) Evaluation of total antioxidant capacity of saliva and serum in caries-free and caries-active adults: an in-vivo study. *Indian Journal of Dental Research* **24**, 164–167.

Hirschfeld, J., Dommisch, H., Skora, P., Horvath, G., Latz, E., Hoerauf, A., Waller, T., Kawai, T., Jepsen, S., Deschner, J. & Bekeredjian-Ding, I. (2015) Neutrophil extracellular trap formation in supragingival biofilms. *International Journal of Medical Microbiology* **305**, 453–463.

Irie, K., Tomofuji, T., Ekuni, D., Endo, Y., Kasuyama, K., Azuma, T., Tamaki, N., Yoneda, T. & Morita, M. (2014) Anti-ageing effects of dentifrices containing anti-oxidative, anti-inflammatory, and anti-bacterial agents (Tomarina(R)) on gingival collagen degradation in rats. *Archives of Oral Biology* **59**, 60–65.

Kamoda, Y., Uematsu, H., Yoshihara, A., Miyazaki, H. & Senpuku, H. (2008) Role of activated natural killer cells in oral diseases. *Japanese Journal of Infectious Diseases* **61**, 469–474.

Kassebaum, N. J., Bernabe, E., Dahiya, M., Bhandari, B., Murray, C. J. & Marcenes, W. (2014) Global burden of severe periodontitis in 1990–2010: a systematic review and meta-regression. *Journal of Dental Research* **93**, 1045–1053.

Kato, C. & Mikami, M. (2011) Effect of aging on BCG immunostimulation of *Porphyromonas gingivalis* infection in mice. *Biomedical Research* **32**, 45–54.

Kent, L. W., Dyken, R. A., Rahemtulla, F., Allison, A. C. & Michalek, S. M. (1996) Effect of in vitro passage of healthy human gingival fibroblasts on cellular morphology and cytokine expression. *Archives of Oral Biology* **41**, 263–270.

Kim, H. K., Park, H. R., Lee, J. S., Chung, T. S., Chung, H. Y. & Chung, J. (2007) Down-regulation of iNOS and TNF-alpha expression by kaempferol via NF-kappaB inactivation in aged rat gingival tissues. *Biogerontology* **8**, 399–408.

Kim, H. K., Park, H. R., Sul, K. H., Chung, H. Y. & Chung, J. (2006) Induction of RANTES and CCR5 through NF-kappaB activation via MAPK pathway in aged rat gingival tissues. *Biotechnology Letters* **28**, 17–23.

Kim, S. J., Chung, Y. K., Chung, T. W., Kim, J. R., Moon, S. K., Kim, C. H. & Park, Y. G. (2009) Regulation of matrix metalloproteinase-9 expression between gingival fibroblast cells from old and young rats. *Biochemical and Biophysical Research Communications* **378**, 152–156.

Liang, S., Domon, H., Hosur, K. B., Wang, M. & Hajishengallis, G. (2009) Age-related alterations in innate immune receptor expression and ability of macrophages to respond to pathogen challenge in vitro. *Mechanisms of Ageing and Development* **130**, 538–546.

Liang, S., Hosur, K. B., Domon, H. & Hajishengallis, G. (2010) Periodontal inflammation and bone loss in aged mice. *Journal of Periodontal Research* **45**, 574–578.

Maijo, M., Clements, S. J., Ivory, K., Nicoletti, C. & Carding, S. R. (2014) Nutrition, diet and immunosenescence. *Mechanisms of Ageing and Development* **136–137**, 116–128.

Malcolm, J., Sherriff, A., Lappin, D. F., Ramage, G., Conway, D. I., Macpherson, L. M. & Culshaw, S. (2014) Salivary antimicrobial proteins associate with age-related changes in streptococcal composition in dental plaque. *Molecular Oral Microbiology* **29**, 284–293.

Masi, S., Gkranias, N., Li, K., Salpea, K. D., Parkar, M., Orlandi, M., Suvan, J. E., Eng, H. L., Taddei, S., Patel, K., Darbar, U., Donos, N., Deanfield, J. E., Hurel, S., Humphries, S. E. & D'Aiuto, F. (2014) Association between short leukocyte telomere length, endotoxemia, and severe periodontitis in people with diabetes: a cross-sectional survey. *Diabetes Care* **37**, 1140–1147.

Masi, S., Salpea, K. D., Li, K., Parkar, M., Nibali, L., Donos, N., Patel, K., Taddei, S., Deanfield, J. E., D'Aiuto, F. & Humphries, S. E. (2011) Oxidative stress, chronic inflammation, and telomere length in patients with periodontitis. *Free Radical Biology & Medicine* **50**, 730–735.

McArthur, W. P., Bloom, C., Taylor, M., Smith, J., Wheeler, T. & Magnusson, N. I. (1995) Antibody responses to suspected periodontal pathogens in elderly subjects with periodontal disease. *Journal of Clinical Periodontology* **22**, 842–849.

McArthur, W. P., Bloom, K., Taylor, M., Wheeler, T., Smith, J. & Magnusson, N. I. (1996) Peripheral blood leukocyte populations in the elderly with and without periodontal

disease. *Journal of Clinical Periodontology* **23**, 846–852.

Mila, R., Abellana, R., Padro, L., Basulto, J. & Farran, A. (2012) High consumption foods and their influence on energy and protein intake in institutionalized older adults. *Journal of Nutrition, Health & Aging* **16**, 115–122.

Miura, S., Yamaguchi, M., Shimizu, N. & Abiko, Y. (2000) Mechanical stress enhances expression and production of plasminogen activator in aging human periodontal ligament cells. *Mechanisms of Ageing and Development* **112**, 217–231.

Miyazaki, H., Shirahama, R., Ohtani, I., Shimada, N. & Takehara, T. (1992) Oral health conditions and denture treatment needs in institutionalized elderly people in Japan. *Community Dentistry and Oral Epidemiology* **20**, 297–301.

Mochizuki, K., Yamaguchi, M. & Abiko, Y. (1999) Enhancement of LPS-stimulated plasminogen activator production in aged gingival fibroblasts. *Journal of Periodontal Research* **34**, 251–260.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., The PRISMA Group. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine* **6**, e1000097.

Murata, T., Miyazaki, H., Senpuku, H. & Hanada, N. (2001) Periodontitis and serum interleukin-6 levels in the elderly. *Japanese Journal of Infectious Diseases* **54**, 69–71.

Natasasmita, S., Soemohatmoko, S. & Watanabe, H. (1989) Serum IgG level against *Streptococcus mutans* in subjects with caries experience. *Bulletin of Tokyo Medical and Dental University* **36**, 27–33.

Nikfarjam, J., Pourpak, Z., Shahrabi, M., Nikfarjam, L., Kouhkan, A., Moazeni, M. & Aghamohammadi, A. (2004) Oral manifestations in selective IgA deficiency. *International Journal of Dental Hygiene* **2**, 19–25.

Ogura, N., Matsuda, U., Tanaka, F., Shibata, Y., Takiguchi, H. & Abiko, Y. (1996) In vitro senescence enhances IL-6 production in human gingival fibroblasts induced by lipopolysaccharide from *Campylobacter rectus*. *Mechanisms of Ageing and Development* **87**, 47–59.

Ohzeki, K., Yamaguchi, M., Shimizu, N. & Abiko, Y. (1999) Effect of cellular aging on the induction of cyclooxygenase-2 by mechanical stress in human periodontal ligament cells. *Mechanisms of Ageing and Development* **108**, 151–163.

Orbak, R., Kara, C., Ozbek, E., Tezel, A. & Demir, T. (2007) Effects of zinc deficiency on oral and periodontal diseases in rats. *Journal of Periodontal Research* **42**, 138–143.

Panda, A., Qian, F., Mohanty, S., van Duin, D., Newman, F. K., Zhang, L., Chen, S., Towle, V., Belshe, R. B., Fikrig, E., Allore, H. G., Montgomery, R. R. & Shaw, A. C. (2010) Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. *Journal of Immunology* **184**, 2518–2527.

Pandruvada, S. N., Gonzalez, O. A., Kirakodu, S., Gudhimella, S., Stromberg, A. J., Ebersole, J. L., Orraca, L., Gonzalez-Martinez, J., Novak, M. J. & Huja, S. S. (2016) Bone biology-related gingival transcriptome in ageing and periodontitis in non-human primates. *Journal of Clinical Periodontology* **43**, 408–417.

Parish, S. T., Kim, S., Sekhon, R. K., Wu, J. E., Kawakatsu, Y. & Effros, R. B. (2010) Adenosine deaminase modulation of telomerase activity and replicative senescence in human CD8 T lymphocytes. *Journal of Immunology* **184**, 2847–2854.

Peltola, P., Vehkalahti, M. M. & Wuolijoki-Saaristo, K. (2004) Oral health and treatment needs of the long-term hospitalised elderly. *Gerodontology* **21**, 93–99.

Power, S. E., Jeffery, I. B., Ross, R. P., Stanton, C., O'Toole, P. W., O'Connor, E. M. & Fitzgerald, G. F. (2014) Food and nutrient intake of Irish community-dwelling elderly subjects: who is at nutritional risk? *Journal of Nutrition, Health & Aging* **18**, 561–572.

Rink, L. & Haase, H. (2007) Zinc homeostasis and immunity. *Trends in Immunology* **28**, 1–4.

Rose, P. T., Gregory, R. L., Gfell, L. E. & Hughes, C. V. (1994) IgA antibodies to *Streptococcus mutans* in caries-resistant and -susceptible children. *Pediatric Dentistry* **16**, 272–275.

Sanders, A. E., Divaris, K., Naorungroj, S., Heiss, G. & Risques, R. A. (2015) Telomere length attrition and chronic periodontitis: an ARIC Study nested case-control study. *Journal of Clinical Periodontology* **42**, 12–20.

Shaik-Dasthagirisaheb, Y. B., Huang, N., Weinberg, E. O., Shen, S. S., Genco, C. A. & Gibson, F. C. 3rd (2015) Aging and contribution of MyD88 and TRIF to expression of TLR pathway-associated genes following stimulation with *Porphyromonas gingivalis*. *Journal of Periodontal Research* **50**, 89–102.

Shaik-Dasthagirisaheb, Y. B., Kantarci, A. & Gibson, F. C. 3rd (2010) Immune response of macrophages from young and aged mice to the oral pathogenic bacterium *Porphyromonas gingivalis*. *Immunity & Ageing* **7**, 15.

Sharma, R., Kapila, R., Haq, M. R., Salingati, V., Kapasiya, M. & Kapila, S. (2014) Age-associated aberrations in mouse cellular and humoral immune responses. *Aging Clinical and Experimental Research* **26**, 353–362.

Sheiham, A. & James, W. P. (2015) Diet and dental caries: the pivotal role of free sugars reemphasized. *Journal of Dental Research* **94**, 1341–1347.

Shimizu, N., Goseki, T., Yamaguchi, M., Iwasawa, T., Takiguchi, H. & Abiko, Y. (1997) In vitro cellular aging stimulates interleukin-1 beta production in stretched human periodontal-ligament-derived cells. *Journal of Dental Research* **76**, 1367–1375.

Shimizu, N., Yamaguchi, M., Uesu, K., Goseki, T. & Abiko, Y. (2000) Stimulation of prostaglandin E2 and interleukin-1beta production from old rat periodontal ligament cells subjected to mechanical stress. *Journals of Gerontology: Biological Sciences and Medical Sciences* **55**, B489–B495.

Solana, R., Tarazona, R., Gayoso, I., Lesur, O., Dupuis, G. & Fulop, T. (2012) Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans. *Seminars in Immunology* **24**, 331–341.

Steffens, J. P., Masi, S., D'Aiuto, F. & Spolidorio, L. C. (2013) Telomere length and its relationship with chronic diseases – new perspectives for periodontal research. *Archives of Oral Biology* **58**, 111–117.

Sztajer, H., Szafranski, S. P., Tomasch, J., Reck, M., Nimtz, M., Rohde, M. & Wagner-Döbler, I. (2014) Cross-feeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*. *ISME Journal* **8**, 2256–2271.

Takahashi, K., Nishida, H., Takeda, H. & Shin, K. (2004) Telomere length in leukocytes and cultured gingival fibroblasts from patients with aggressive periodontitis. *Journal of Periodontology* **75**, 84–90.

Takiguchi, H., Yamaguchi, M., Mochizuki, K. & Abiko, Y. (1996) Effect of in vitro aging on *Campylobacter rectus* lipopolysaccharide-stimulated PGE2 release from human gingival fibroblasts. *Oral Diseases* **2**, 202–209.

Takiguchi, H., Yamaguchi, M., Okamura, H. & Abiko, Y. (1997) Contribution of IL-1 beta to the enhancement of *Campylobacter rectus* lipopolysaccharide-stimulated PGE2 production in old gingival fibroblasts in vitro. *Mechanisms of Ageing and Development* **98**, 75–90.

Tanida, T., Ueta, E., Tobiume, A., Hamada, T., Rao, F. & Osaki, T. (2001) Influence of aging on candidal growth and adhesion regulatory agents in saliva. *Journal of Oral Pathology & Medicine* **30**, 328–335.

Taubman, M. A., Han, X., Larosa, K. B., Socransky, S. S. & Smith, D. J. (2007) Periodontal bacterial DNA suppresses the immune response to mutans streptococcal glucosyltransferase. *Infection and Immunity* **75**, 4088–4096.

Tenovuo, J. (1992) Oral defense factors in the elderly. *Endodontics & Dental Traumatology* **8**, 93–98.

Toffanello, E. D., Inelmen, E. M., Minicuci, N., Campigotto, F., Sergi, G., Coin, A., Miotto, F., Enzi, G. & Manzato, E. (2010) Ten-year trends in dietary intake, health status and mortality rates in free-living elderly people. *Journal of Nutrition, Health & Aging* **14**, 259–264.

Tsalikis, L., Parapanisiou, E., Bata-Kyrkou, A., Polymenides, Z. & Konstantinidis, A. (2002) Crevicular fluid levels of interleukin-1alpha and interleukin-1beta during experimental gingivitis in young and old adults. *Journal of the International Academy of Periodontology* **4**, 5–11.

Vincent-Bugnas, S., Vitale, S., Mouline, C. C., Khaali, W., Charbit, Y., Mahler, P., Precheur, I., Hofman, P., Maryanski, J. L. & Doglio, A. (2013) EBV infection is common in gingival epithelial cells of the periodontium and worsens during chronic periodontitis. *PLoS One* **8**, e80336.

Vukmanovic-Stejic, M., Agius, E., Booth, N., Dunne, P. J., Lacy, K. E., Reed, J. R., Sobande, T. O., Kissane, S., Salmon, M., Rustin, M. H. & Akbar, A. N. (2008) The kinetics of CD4+Foxp3+ T cell accumulation during a human cutaneous antigen-specific memory response in vivo. *Journal of Clinical Investigation* **118**, 3639–3650.

Weiskopf, D., Weinberger, B. & Grubeck-Loebenstein, B. (2009) The aging of the immune system. *Transplant International* **22**, 1041–1050.

Weng, N. P. (2006) Aging of the immune system: how much can the adaptive immune system adapt? *Immunity* **24**, 495–499.

Yakovlev, E., Kalichman, I., Pisanti, S., Shoshan, S. & Barak, V. (1996) Levels of cytokines and collagen type I and type III as a function of age in human gingivitis. *Journal of Periodontology* **67**, 788–793.

Yoneda, T., Tomofuji, T., Ekuni, D., Azuma, T., Endo, Y., Kasuyama, K., Machida, T. & Morita, M. (2013) Anti-aging effects of co-enzyme Q10 on periodontal tissues. *Journal of Dental Research* **92**, 735–739.

Yu, M., Li, G., Lee, W. W., Yuan, M., Cui, D., Weyand, C. M. & Goronzy, J. J. (2012) Signal inhibition by the dual-specific phosphatase 4 impairs T cell-dependent B-cell responses with age. *Proceedings of The National Academy of Sciences of The United States of America* **109**, E879–E888.

Zhao, A., Blackburn, C., Chin, J. & Srinivasan, M. (2014) Soluble toll like receptor 2 (TLR-2) is increased in saliva of children with dental caries. *BMC Oral Health* **14**, 108.

Address:
Philip M. Preshaw
School of Dental Sciences and Institute of Cellular Medicine
Newcastle University
Framlington Place
Newcastle upon Tyne
NE2 4BW
UK
E-mail: philip.preshaw@newcastle.ac.uk
and
Georg Conrads
Division of Oral Microbiology and Immunology
Department for Operative Dentistry, Periodontology and Preventive Dentistry
RWTH Aachen University Hospital
Pauwelsstrasse 30
D-52074 Aachen
Germany
E-mail: gconrads@ukaachen.de

Clinical Relevance

Scientific rationale for the study: Immune functioning is known to alter with increasing age, and this is likely to be relevant for conditions such as periodontitis and dental caries.
Principal findings: There is evidence that immune function related to periodontitis and (less investigated) dental caries alters with increasing age. In both disorders, the patterns of changes in immune functioning with age are complex and incompletely understood.
Practical implications: Clinicians should be aware of altered immune functioning with increasing age, which may lead to changed susceptibility to periodontitis and dental caries. Clinicians should remain vigilant to signs of disease progression in ageing patients and implement appropriate preventive strategies.