Ageing and atherosclerosis: vascular intrinsic and extrinsic factors and potential role of IL-6

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Abstract | The number of old people is rising worldwide, and advancing age is a major risk factor for atherosclerotic cardiovascular disease. However, the mechanisms underlying this phenomenon remain unclear. In this Review, we discuss vascular intrinsic and extrinsic mechanisms of how ageing influences the pathology of atherosclerosis. First, we focus on factors that are extrinsic to the vasculature. We discuss how ageing affects the development of myeloid cells leading to the expansion of certain myeloid cell clones and induces changes in myeloid cell functions that promote atherosclerosis via inflammation, including a potential role for IL-6. Next, we describe vascular intrinsic factors by which ageing promotes atherogenesis — in particular, the effects on mitochondrial function. Studies in mice and humans have shown that ageing leads to a decline in vascular mitochondrial function and impaired mitophagy. In mice, ageing is associated with an elevation in the levels of the inflammatory cytokine IL-6 in the aorta, which participates in a positive feedback loop with the impaired vascular mitochondrial function to accelerate atherogenesis. We speculate that vascular and myeloid cell ageing synergize, via IL-6 signalling, to accelerate atherosclerosis. Finally, we propose future avenues of clinical investigation and potential therapeutic approaches to reduce the burden of atherosclerosis in old people.

Clonal haematopoiesis of indeterminate potential
(CHIP). Clonal expansion of haematopoietic stem cells that carry certain somatic mutations that confer a cell proliferation advantage.

Mitophagy
Type of macroautophagy for the removal of damaged or dysfunctional mitochondria.

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The number of old people (aged >65 years) is rising worldwide, and cardiovascular diseases are the largest contributor to morbidity and mortality in this population[1,2]. Changes in diet and lifestyle contribute to the high cardiovascular morbidity and mortality in old individuals, but many biological processes that are altered with ageing also contribute to this increased cardiovascular risk. As a result, therapies for cardiovascular disease that are effective in young and middle-aged people might be less effective in older people. Additionally, novel therapies might be required to improve disease management specifically in old people. Deciphering the mechanisms by which ageing promotes atherosclerotic cardiovascular disease will be fundamental for the development of novel therapies to reduce the burden of atherosclerosis with ageing. The development of new therapies is especially relevant with the coronavirus disease 2019 (COVID-19) pandemic, because old people and particularly those with cardiovascular diseases are at a substantially higher risk of morbidity and death[3,4].

In this Review, we describe two major areas by which ageing promotes atherosclerosis. First, we discuss age-related factors that are extrinsic to the vasculature, focusing on the effects of ageing on myeloid cells of the immune system. Age-related effects in the bone marrow skew the differentiation of haematopoietic cells towards the myeloid cell lineage. Ageing also promotes the generation of clones of haematopoietic cells without clear development of haematopoietic malignancy or other known clonal disorder, a phenomenon known as clonal haematopoiesis of indeterminate potential (CHIP)[5–8]. Clinical studies from the past decade have revealed that the presence of CHIP increases the risk of cardiovascular diseases[6,7]. Intriguingly, the increased risk of cardiovascular disease associated with the presence of CHIP is abrogated in patients with a loss-of-function mutation in *IL6* (REF.[9]). However, the precise mechanisms by which CHIP promotes the development of cardiovascular diseases are yet to be fully clarified.

We next address vascular intrinsic factors, discussing how ageing impairs vascular bioenergetics by compromising mitochondrial function and how this alteration connects with inflammatory pathways within the vasculature to promote atherosclerosis[10,11]. We describe studies in mice and humans showing that, in the aorta, ageing impairs both mitochondrial function and the removal of damaged mitochondria (mitophagy)[10,11]. We describe experimental evidence reported in 2020 demonstrating that the age-mediated increase in the levels of the pleiotropic cytokine IL-6 in the aorta occurs in a positive

Key points

- Ageing-related alterations in the bone marrow increase the phenomenon of clonal haematopoiesis of indeterminate potential (CHIP) and promote a skewing towards myeloid cell differentiation, both of which can accelerate atherosclerosis.
- The increased risk of atherosclerotic cardiovascular diseases associated with the presence of CHIP might be mediated by IL-6 signalling and/or inflammasome activation.
- Ageing is associated with a decline in mitochondrial function and an increase in IL-6 levels in the vasculature, and both effects probably accelerate atherosclerosis independently of chronic hyperlipidaemia.
- The role of the vasculature and myeloid cells of the immune system in promoting age-related atherosclerosis might be mediated by shared inflammatory pathways, in particular IL-6 signalling.

feedback loop with vascular mitochondrial dysfunction and that these alterations promote atherosclerosis[11].

The CANTOS study[12] demonstrated that IL-1β blockade reduces the risk of recurrent cardiovascular events in patients aged >60 years. Importantly, the greatest benefit of IL-1β blockade was seen in patients who had low plasma IL-6 levels[13]. This pivotal clinical study indicates that chronic inflammation, potentially via IL-6 signalling, is a major contributor to age-related atherosclerosis. Given this observation, we speculate that increased atherosclerosis with ageing could result from a synergy between myeloid cells of the immune system and the vasculature via IL-6 signalling (FIG. 1). This mechanism is especially important because clinically-approved agents targeting this pathway (such as anti-IL-6 therapies) are already available and could reduce the risk of cardiovascular disease in old people. Finally, we propose that future experimental and clinical investigation will be required to determine the contribution of this inflammatory pathway in age-related atherosclerosis. We acknowledge that other inflammatory pathways and cytokines could contribute to age-related atherosclerosis, and the source of these cytokines (including IL-6) could be senescent adipocytes. A detailed discussion of the contribution of ageing to senescence and atherosclerosis has been published previously[14].

Ageing affects the immune system in complex ways (as reviewed previously[15–17]), and various components of the immune system contribute to atherosclerosis[18,19]. This Review focuses on clones of myeloid cells that increase with ageing and how these clones contribute to atherosclerosis. We do not describe how ageing affects other cells of the immune system, which has been reviewed previously (for example, B cells[20], T cells[21], eosinophils or dendritic cells[22]). In addition, we focus on vascular mitochondrial function and how mitochondrial dysfunction could influence inflammatory pathways within the vasculature. However, given that most of the available evidence indicates that oxidative stress is not a major driver of biological ageing[23–25], and given the complex roles that oxidative stress has in atherosclerosis[26], we do not describe in detail the contributions of oxidative stress in age-related atherosclerosis. Neither do we describe in detail how ageing affects other processes within the arterial wall, such as extracellular matrix remodelling or production of pro-fibrotic and pro-calcific factors, which can promote atherosclerosis indirectly via increasing arterial stiffness and hypertension[27,28], and which have been previously reviewed[29,30].

Senescence
A state of permanent replicative arrest in normally proliferative cells.

Vascular extrinsic mechanisms

Effects of ageing on myeloid cell production. Numerous subpopulations of immune cells of various lineages have been implicated in atherosclerosis, including macrophages[31], dendritic cells[32], T helper 1 (T_H1) cells[33] and B cells[34] (reviewed previously[18,35]), all of which are affected by ageing. Immune cells are generated in the bone marrow via haematopoiesis from regenerative haematopoietic stem cells (HSCs)[36,37]. Monocytes, macrophages and neutrophils are derived from myeloid-biased HSCs. With ageing, although the absolute number of HSCs increases[38], HSCs lose their regenerative capacity[39,40]. This loss of regenerative potential is accompanied by an expansion of the number of HSCs that are committed to the platelet (megakaryocytes) and myeloid lineages[38,41]. Competitive bone marrow transplantation studies in mice have demonstrated that aged HSCs have a reduced repopulation capacity, with an imbalance towards myeloid cell differentiation, compared with young HSCs[38,42]. Several major biological pathways contribute to ageing, including DNA damage, mitochondrial dysfunction, cell senescence, impaired autophagy, epigenetic alterations and gene transcription dysregulation[25]. Transcriptomic studies in mice have shown that with ageing, HSCs upregulate stress responses and inflammatory pathways and downregulate the expression of genes related to genetic stability[43]. With ageing, HSCs exhibit an increase in epigenetic dysregulation, specifically downregulation in chromatin remodelling and transcriptional silencing[43], and increases in DNA methylation (as reviewed previously[44]). These epigenetic alterations are accompanied by functional defects in HSCs, including a reduction in HSC homing to the bone marrow and HSC proliferation[43,45]. Importantly, mutations in genes such as *IDH2*, which alter epigenetic regulation, lead to impairments in haematopoietic progenitors in mice[46] and are associated with T cell lymphomas in humans[47], a malignancy that increases with ageing. Although whether HSCs, or stem cells in general, undergo senescence is questionable[48], the clearance of senescent cells improves HSC engraftment in bone marrow transplantation mouse models and reduces myeloid skewing[49]. Autophagy-deficient young mice have increased mitochondrial content and metabolism that lead to mitochondrial stress in HSCs compared with wild-type young mice[50]. These features are also observed in aged wild-type mice and are associated with a skewing towards the myeloid lineage and a reduced proliferative capacity of HSCs[50,51]. Loss of microRNA-146a (miR-146a) in HSCs with ageing also promotes a myeloid bias[52]. Furthermore, myeloid cells derived from miR-146a-deficient HSCs have elevated levels of both IL-6 and tumour necrosis factor (TNF)[52], which connects altered regulation of transcription in HSCs to inflammation. Overall, these studies indicate that ageing has effects on HSCs via several complex pathways that lead to reduced HSC function.

Ageing also alters haematopoiesis by influencing the bone marrow niche independently of the direct effects

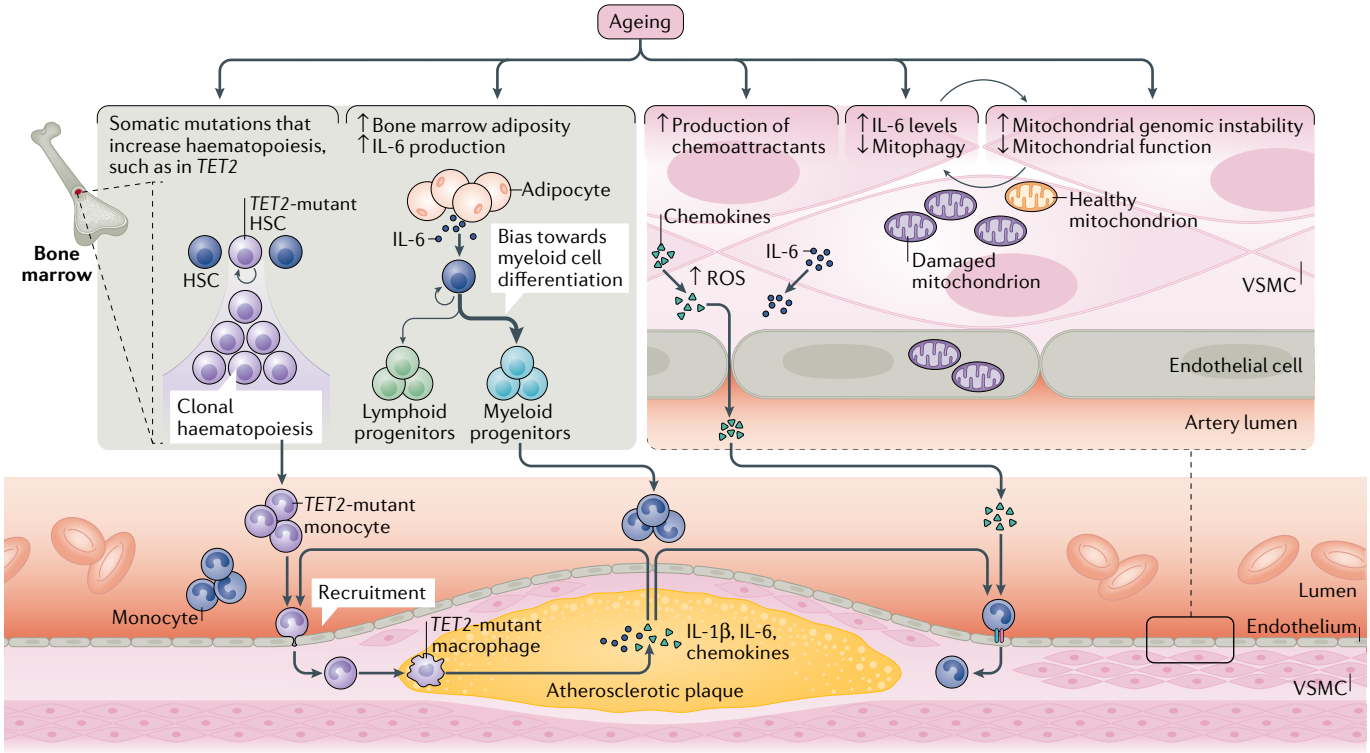


Fig. 1 | **Ageing-related processes that promote atherogenesis: IL-6 as a potential shared pathway.** Ageing promotes the development and progression of atherosclerosis through different mechanisms, which might be related to age-induced elevations in circulating and intracellular IL-6 levels. During ageing, IL-6 signalling in bone marrow adipocytes increases. This increased IL-6 signalling might skew haematopoietic stem cells towards myeloid cell differentiation and increase the risk of mutations in genes encoding transcriptional regulators, such as *TET2*, that can result in the positive selection and expansion of clones of haematopoietic cells without clear development of malignancy or other known clonal disorder, a phenomenon known as clonal haematopoiesis of indeterminate potential (CHIP). Ageing can also have direct effects on haematopoietic stem cells (HSCs) that lead to CHIP. Clones of myeloid cells with a *TET2* mutation show an increased production of IL-6 and IL-1β, which can contribute to accelerated atherosclerosis. Ageing can also have pro-atherogenic effects directly on the vasculature. Ageing is associated with an increase in the levels of IL-6, possibly mediated by increased production by vascular smooth muscle cells (VSMCs), and mitochondrial genomic instability and with a decline in mitochondrial function in the vasculature. The reduced mitochondrial function alters mitophagy and increases IL-6 levels, creating a positive feedback loop that accelerates atherogenesis. Vascular ageing also leads to the production of chemoattractants that increase myeloid cell recruitment into the arterial wall, further promoting atherosclerosis. Impaired mitochondrial function combined with reduced mitophagy might lead to increased levels of reactive oxygen species (ROS).

on HSCs. The bone marrow niche provides a supporting environment for HSC function and includes mesenchymal cells and endothelial cells[53]. How ageing affects the bone marrow niche is not clear, but the presence of chronic systemic inflammation might contribute. Ageing leads to a chronic systemic low-grade inflammatory state[54,55], which might be mediated by cellular senescence that leads to the production of inflammatory mediators (termed the senescence-associated secretory phenotype (SASP))[56,57]. One source of senescent inflammatory cells is the adipose tissue, which typically increases in size with ageing[58,59]. The number of adipocytes also increases in the bone marrow with ageing, accompanied by an elevation in the levels of pro-inflammatory cytokines, including IL-6 (REFS[60,61]). These cytokines promote a skewing towards myeloid cell differentiation and an increase in platelet production, the latter of which could contribute to thrombosis[60,61]. Importantly, adipocytes arising from leptin-receptor-positive progenitors in the bone marrow, but not within other fat depots, synthesize stem cell factor, which promotes HSC regeneration[62,63]. Senescent stromal cells in the bone marrow are another potential source of inflammation[64]. These cells can differentiate into adipocytes in the ageing bone marrow[65] and further promote an inflammatory environment. The function of bone marrow endothelial cells also declines with ageing[66]. Furthermore, the number of vascular niches in the bone marrow that support HSC regeneration decreases with ageing, but can be restored in aged mice by activating Notch signalling in endothelial cells[53].

Activation of the innate immune receptor Toll-like receptor 4 (TLR4) induces myeloid differentiation in HSCs in mice[67]. Ageing is associated with alterations in gut microbiota[68], which could act as a microbial source for TLR4 stimulation (for example, lipopolysaccharide (LPS) from Gram-negative bacteria activates TLR4). TLR4 activation could then increase the imbalance of HSC differentiation towards the myeloid lineage. In a 2019 study in mice, β_2-adrenergic receptor signalling in the bone marrow niche was found to increase with ageing in association with increased generation of myeloid cells and platelets through an IL-6-dependent mechanism[60]. This study also demonstrated that the bone marrow niche switches from an endosteal to a non-endosteal

Senescence-associated secretory phenotype (SASP). Secretion of cytokines, chemokines, growth factors and proteases by senescent cells.

niche with ageing, indicating that ageing shifts myeloid cell production away from the bone tissue to further within the bone marrow[60]. This study also found that a mouse model of Hutchinson–Gilford progeria syndrome, which is associated with accelerated ageing[69], had an imbalance favouring myeloid cells over lymphoid cells in the peripheral blood[60]. This effect was mitigated by administration of a β_3-adrenergic receptor agonist[60]. Overall, clear evidence indicates that ageing alters the bone marrow niche via multiple mechanisms to impair HSC function and promote myeloid cell differentiation.

Clonal haematopoiesis and cardiovascular disease: clinical correlation. The positive selection and expansion of clones of HSCs carrying certain somatic mutations, known as clonal haematopoiesis, occurs commonly with ageing. Approximately 10% of individuals aged >70 years carry mutations associated with clonal haematopoiesis, whereas these mutations are rare in individuals aged <40 years[7,70,71]. These clones of haematopoietic cells harbour single somatic mutations most commonly in genes associated with haematological malignancies, such as *DNMT3A*, *TET2* and *ASXL1*. Individuals with mutations in these genes have an increased risk of developing haematological malignancies (HR 11–12, depending on the study)[7,70,71]. All-cause mortality is increased in individuals with any somatic mutation associated with clonal haematopoiesis (HR 1–2) compared with those with no mutations[7]. Interestingly, the cause of the increased mortality in these individuals is not only the higher rate of haematological malignancies but also a higher rate of adverse cardiovascular events[7,70–72]. The association between clonal haematopoiesis and the risk of adverse cardiovascular events remained even after adjustment for traditional cardiovascular risk factors, such as diabetes mellitus, hypertension, smoking and BMI, in multivariate analyses[6]. As a result of these studies, the term CHIP was coined to distinguish the phenomenon of clonal haematopoiesis without clear development of haematopoietic malignancy or other known clonal disorder from the pre-malignant clonal haematopoiesis of clinical importance[73].

A follow-up clinical study provided further evidence of the association between cardiovascular disease and CHIP[6]. In particular, old individuals (aged 60–70 years) with CHIP had an approximately twofold higher risk of incident coronary artery disease, a fourfold higher risk of early-onset myocardial infarction and a threefold higher coronary artery calcium score than similarly aged individuals without CHIP[6]. Importantly, the size of the CHIP clone, defined as the variant allele frequency (VAF), correlates with the risk of cardiovascular disease. Specifically, individuals with a CHIP clone with a VAF of >10% have a 12-fold increased risk of cardiovascular disease compared with individuals with no mutations, whereas the risk of cardiovascular disease is not significantly increased in CHIP carriers with a VAF of <10%[6]. This study has established that CHIP is associated with the risk of cardiovascular diseases and has developed a potential new paradigm that certain clones of haematopoietic cells accelerate atherogenesis[6]. However, to date, the presence of CHIP can be used only as a biomarker of atherosclerosis and is not therapeutically actionable.

Variant allele frequency (VAF). The proportion of sequences that match a gene mutation divided by the overall coverage at that gene locus.

CHIP and ageing increase atherogenesis via myeloid cells. Mutations in *TET2* are the second most prevalent somatic mutations associated with CHIP after those in *DNMT3A*. Mouse models have been used to elucidate the mechanistic contributions of *TET2* mutations to atherogenesis. In irradiated, atheroprone *Ldlr*$^{-/-}$ mice, those reconstituted with either *Tet2*$^{-/-}$ or *Tet2*$^{+/-}$ bone marrow had increased atherosclerotic lesion size compared with mice receiving wild-type bone marrow[6,74]. These data imply that deletion of one copy of the *Tet2* gene is sufficient to increase atherosclerosis in mice. Further studies in mice showed that myeloid-cell-specific TET2 deficiency increases atherosclerotic plaque size[74]. Interestingly, TET2 deficiency in bone marrow-derived macrophages leads to an elevated secretion of IL-6 and IL-1β (a signature cytokine produced by inflammasome activation)[75] in response to various stimuli (such as LDL, LPS and IFNγ) in vitro[74]. Furthermore, the increased atherogenic potential of *Tet2*$^{-/-}$ bone marrow cells is reduced when bone marrow transplantation recipients are treated with a small-molecule inhibitor of the NLRP3 inflammasome[74]. The effect of TET2 deficiency might not be limited to vascular diseases, because experimental studies have demonstrated that transfer of *Tet2*$^{-/-}$ bone marrow cells into non-irradiated mice accelerates the development of age-related cardiac hypertrophy and fibrosis[76], and TET2 deficiency in myeloid cells worsens the development of heart failure in mice after acute injury[77].

The contribution of IL-6 to the cardiovascular risk in individuals with large CHIP clones (VAF >10%) was evaluated in 35,416 individuals without prevalent cardiovascular disease enrolled in the UK Biobank registry[9]. The investigators examined whether an *IL6R* coding mutation that leads to reduced IL-6 signalling alters the association between CHIP and the risk of adverse cardiovascular events (myocardial infarction, coronary artery disease revascularization, stroke or death). The study revealed that the presence of the *IL6R* mutation mitigated the increased risk of adverse cardiovascular events in individuals with large CHIP clones but not in individuals without CHIP[9]. These data indicate that IL-6 signalling is causally linked to the increased risk of cardiovascular disease associated with CHIP.

IL-6 is released following inflammasome activation[78]; therefore, the observed link between IL-6 and CHIP suggests that inflammasome activation is a mechanism by which CHIP promotes the development of cardiovascular diseases. This concept is compatible with the study in mice discussed above, showing that the NLRP3 inflammasome contributes to the increased atherosclerotic burden induced by *Tet2*$^{-/-}$ bone marrow transplantation[74]. The contribution of IL-1β to cardiovascular disease in humans was demonstrated in the CANTOS study[12,13], which showed that a monoclonal antibody against IL-1β reduces the risk of recurrent cardiovascular events in patients with previous myocardial infarction. The effects of IL-1β blockade in the CANTOS trial were greater in patients who had lower circulating

IL-6 levels after IL-1β blockade than in those with higher circulating IL-6 levels[13]. However, the role of IL-1β and IL-6 in experimental models of atherosclerosis is not completely clear because data indicating that each of these cytokines has atheroprotective effects have been reported[79,80]. However, these studies were performed in young mice, so these cytokines might have increasingly pathogenic roles with ageing.

Monocytes and macrophages contribute to both the initiation of the chronic inflammatory process of atherosclerosis and the resolution of the chronic vascular inflammation[81]. Ageing directly influences the function of monocytes and macrophages[16]. Human monocytes have lower levels of TLRs and a reduction in TLR-dependent pro-inflammatory cytokine production with ageing[16]. A study comparing bone marrow-derived monocytes from young and aged atheroprone *Ldlr*$^{-/-}$ mice showed that ageing leads to a downregulation in the expression of *Tnf* and *Il1b* but monocyte chemotaxis is preserved[82]. Aged (6-month-old) atherosclerotic *Apoe*$^{-/-}$ mice have a reduction in the number of vascular progenitor cells in the bone marrow compared with 1-month-old atherosclerotic *Apoe*$^{-/-}$ mice[83]. Furthermore, administration of bone marrow-derived HSCs from young non-atherosclerotic mice to non-irradiated 6-month-old *Apoe*$^{-/-}$ mice reduced atherogenesis after feeding a high-fat diet[83]. This finding suggests that ageing is accompanied by a reduction in the number of atheroprotective progenitor cells in the bone marrow. Aged (18–21-month-old) mice with chronic or induced acute hyperlipidaemia have more macrophage infiltration into atherosclerotic lesions than young mice[11,82]. Furthermore, the aortas of aged atherosclerotic mice (12-months-old) and rats (30-months-old) have higher levels of macrophage-attracting chemokines and IL-6 than the aortas of young atherosclerotic mice (2-months-old) and rats(10-months-old)[82,84]. Although macrophages and monocytes can have an increased basal secretion of inflammatory cytokines, such as IL-1β, IL-6 and IL-8, with ageing[85] (possibly owing to senescence)[57], whether these cells are the major contributors to the increased vascular production of IL-6 with ageing during atherogenesis is unclear. Vascular cells such as vascular smooth muscle cells (VSMCs) have been shown in animal models to have an elevated IL-6 production with ageing before any signs of atherosclerosis development[86,87].

Efferocytosis is a crucial mechanism for resolving plaque inflammation and reducing atherosclerosis progression[88]. In vivo and in vitro assays have indicated that the phagocytic function of tissue alveolar macrophages to take up apoptotic neutrophils declines with ageing[89] and is associated with reduced expression of scavenger receptor CD204 (REF.[90]). In a mouse model of peritonitis, ageing led to reduced resolution of acute inflammation and was associated with reduced levels of pro-resolution lipid mediators, specifically resolvins[91]. Resolution of inflammation was also delayed with ageing in a human model of skin blistering[92]. This phenotype is related to reduced expression of the efferocytotic receptor TIM4 in macrophages. Reduced TIM4 expression with ageing was caused by elevations in p38 mitogen-activated protein kinase activity in macrophages, and treatment with an oral p38 inhibitor increased the resolution of blister inflammation in old individuals[92]. Overall, macrophages show impaired inflammation resolution properties with ageing; however, whether this impaired macrophage function contributes to increased atherosclerosis is not yet clear.

Efferocytosis
Phagocytosis of apoptotic cells by phagocytic cells.

Vascular intrinsic mechanisms

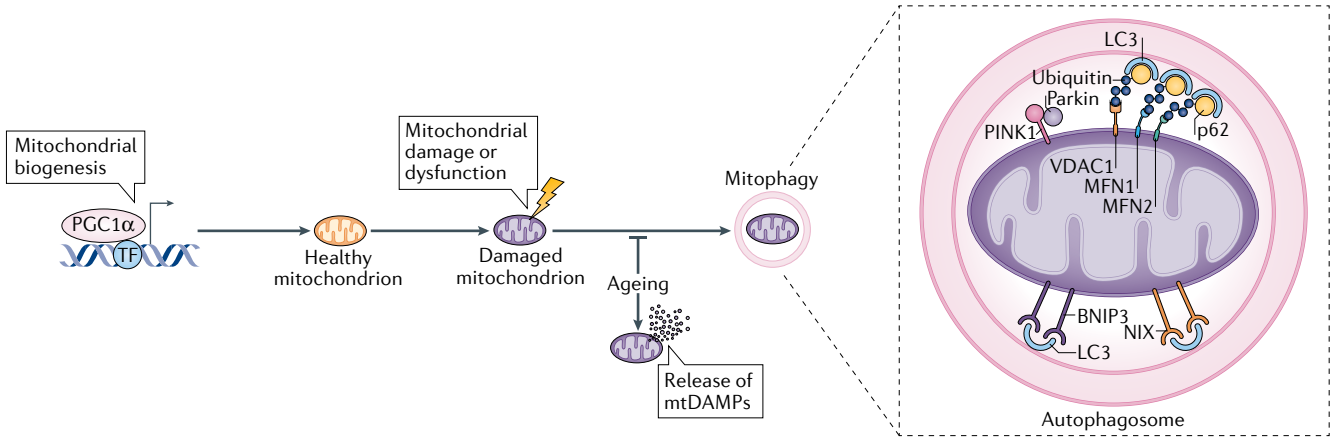
Vascular mitochondrial dysfunction with ageing before atherogenesis initiation. Ageing affects the vasculature before the development of atherosclerosis. Generally, ageing is associated with remodelling of the arterial wall, with evidence of reduced endothelial cell function, increased collagen deposition, fibrosis and functionally stiffer vessels[28,93,94]. In addition, VSMCs acquire a more proliferative and synthetic function with ageing[86]. VSMCs also show an increased generation of reactive oxygen species (ROS) and high oxidative damage[95]. Endothelial cells also have a dysregulated antioxidant capacity with ageing (mediated by the disruption of nuclear factor erythroid 2-related factor 2 signalling), thereby contributing to vascular ageing[96,97]. All these effects of ageing can contribute to the development of hypertension, a major risk factor for cardiovascular disease.

Most studies on vascular ageing in rodent models have been performed in normolipidaemic animals. These studies provide evidence that mitochondrial dysfunction, a known hallmark of ageing[25], contributes to vascular ageing before the initiation of atherogenesis. Disease-free, normolipidaemic mice develop mitochondrial dysfunction in the aorta as they age, first detected at 11 months of age (measured as a decline in oxygen consumption rate (OCR)) and becoming more evident as the mice reach 18 months of age[98]. The reduction in OCR is accompanied by an increase in mitochondrial DNA (mtDNA) damage[98], a sign of mitochondrial genomic instability, which is another hallmark of ageing[25]. Furthermore, reduced vascular mitochondrial function with ageing is accompanied by a decrease in the expression of the mtDNA helicase Twinkle[98], an enzyme involved in preserving mtDNA integrity. Aged transgenic mice expressing high levels of Twinkle show delayed vascular ageing; in particular, the decrease in aortic compliance and the increase in aortic stiffness are delayed in these mice compared with aged wild-type mice[98]. Overall, experimental evidence indicates that mitochondrial dysfunction and mitochondrial genomic instability contribute to vascular ageing.

Vascular mitochondrial dysfunction during atherogenesis. In humans, atherosclerotic plaques show evidence of damage to mtDNA, which is associated with reduced mitochondrial function, specifically lower OCR in the fibrous cap and core regions of the atherosclerotic plaque than in the shoulder region of the plaque or in non-overtly diseased regions of the aorta[10]. These findings are compatible with those of previous experimental work indicating that *Apoe*$^{-/-}$ mice fed a low-fat, standard chow diet have increased vascular mtDNA damage but not nuclear DNA damage as the mice age[99,100]. Furthermore, human atherosclerotic plaques have lower levels of mitochondrial complex I and complex II than non-diseased aortic regions[10]. Similar findings are noted in

Box 1 | **Mitophagy**

During homeostasis, damaged mitochondria are recycled via mitophagy, which is a specialized subset of macroautophagy (see the figure). Mitophagy reduces the production of mitochondrial damage-associated molecular patterns (mtDAMPs) and limits inflammation. Mitochondrial depolarization results in the accumulation of the serine/threonine protein kinase PINK1 at the outer mitochondrial membrane, leading to the recruitment of Parkin, an E3 ubiquitin ligase that ubiquitylates mitochondrial membrane proteins including mitofusin 1 (MFN1), MFN2 and voltage-dependent anion-selective channel protein 1 (VDAC1). This ubiquitylation primes the mitochondria for targeting by the autophagy machinery, including sequestosome 1 (p62) and microtubule-associated protein 1 light chain 3 (LC3), to package mitochondria in autophagosomes and deliver them to lysosomes for degradation. Other mitophagy mechanisms involve the apoptotic BCL-2 family proteins BCL-2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and NIP3-like protein (NIX; also known as BNIP3L), which dimerize and bind directly to LC3 and function as adaptors between mitochondria and autophagosomes. BNIP3 and NIX can also facilitate apoptosis and cell death by participating in the release of mitochondrial cytochrome *c* and opening of the mitochondrial permeability transition pore.



PGC1α, proliferator-activated receptor-γ co-activator 1α; TF, transcription factor.

atherosclerotic *Apoe*$^{-/-}$ mice fed a high-fat diet[10]. *Apoe*$^{-/-}$ mice overexpressing Twinkle have a reduced necrotic core area in atherosclerotic plaques compared with control *Apoe*$^{-/-}$ mice[10]. Mitochondrial dysfunction probably has a central role in ROS generation but the interaction between these two factors is complex. For example, low levels of ROS might improve cell fitness and promote survival, a concept known as mitohormesis[25,101]. However, higher levels of ROS might contribute to age-related chronic vascular diseases. The complex interaction between mitochondrial dysfunction and ROS might explain why disruption of some mitochondrial enzymatic pathways (such as NADPH oxidase 1 (NOX1) and NOX2 signalling) in atherosclerotic mice has no effect on age-related atherosclerosis[102], whereas partial deficiency of ROS-scavenging enzymes (such as superoxide dismutase)[103] in atherosclerotic mice contributes to atherosclerosis[99]. However, one study found that mtDNA damage occurs in both VSMCs and monocytes and correlates with atherosclerotic burden in humans but without evidence of alterations in ROS levels[100]. Furthermore, clinical trials on antioxidants have yet to reveal a beneficial effect in patients with atherosclerotic cardiovascular disease[104,105]. Overall, mitochondrial dysfunction occurs during chronic hyperlipidaemia and atherogenesis, and this mitochondrial dysfunction promotes atherosclerosis. However, the precise role of ROS in this context is complex and requires further investigation.

Dissecting the role of vascular ageing and chronic hyperlipidaemia. Part of the challenge of using standard mouse models of atherosclerosis (such as *Ldlr*$^{-/-}$ or *Apoe*$^{-/-}$ mice) to understand the role of ageing on atherogenesis is that even when fed a standard low-fat diet, these mice age with chronic hyperlipidaemia. Therefore, the effects of ageing cannot be dissected from the effects of chronic hyperlipidaemia. A study in mice published in 2020 circumvented this issue by first examining mitochondrial function in the aortas of young and aged wild-type mice without hyperlipidaemia or vascular diseases[11]. Consistent with previous studies, aged mice had evidence of reduced OCR in the aortas compared with young mice[11]. This OCR reduction in the vasculature from aged mice was accompanied by increased expression of the mitophagy protein Parkin and increased basal mitophagy (BOX 1), a macroautophagy process to remove damaged mitochondria. Altered mitochondrial quality control in the ageing vasculature without hyperlipidaemia is linked to arterial stiffening in mice[106]. The mitochondrial dysfunction and elevated Parkin levels with ageing in the mouse aorta are accompanied by an increase in TLR9, MYD88 and IL-6 levels[11]. Importantly, blocking IL-6 in aged mouse aortas in vitro increased the OCR and reduced Parkin levels. This study identified a positive feedback loop in which mitochondrial dysfunction and elevated IL-6 levels coexist and positively influence each other[11]. However, the exact identity of the IL-6-producing and IL-6-responsive cell(s) has yet to be identified, although evidence suggests that VSMCs secrete more IL-6 with ageing[87].

To study the link between the changes occurring with normolipidaemia in the aged aorta and atherogenesis, young and aged wild-type mice were made acutely hyperlipidaemic by inducing a decrease in LDL receptor levels with adeno-associated virus vector-mediated

delivery of *Pcsk9* and by feeding the mice a high-fat diet for 10 weeks[11], which is an established technique[107]. With this protocol, young and aged mice had similar and durable levels of hyperlipidaemia; however, aged hyperlipidaemic mice had larger atherosclerotic lesions with larger necrotic cores than young hyperlipidaemic mice[11]. Importantly, administering spermidine, an agent that increases macroautophagy and mitophagy, to aged hyperlipidaemic mice reduced the levels of both IL-6 and Parkin in the aorta and reduced the size of atherosclerotic plaques[11]. This finding is consistent with previous studies showing that treatment with spermidine or trehalose, an agent that increases mitophagy, reduces stiffness in the aged vasculature in normolipidaemic, non-atherosclerotic aged mice[106,108].

Dysfunctional mitochondria activate inflammation. The CANTOS study[12] showed that in old patients (aged >60 years) with cardiovascular disease, IL-1β blockade reduces the risk of recurrent cardiovascular events, indicating that chronic inflammation is a major contributor to age-related atherosclerosis. As described above, mitochondrial dysfunction might coexist in a positive feedback loop with IL-6 signalling[11] to increase chronic inflammation in vascular ageing. Furthermore, mitochondrial components that are released to the cytosol after mitochondrial damage can stimulate innate immune responses[109]. Mitochondrial injury in turn can be induced by TLR stimulation leading to the activation of caspase 4 and caspase 5 in humans or caspase 11 in mice[110]. These inflammatory caspases cleave gasdermin D, which enables gasdermin D to form pores in the outer mitochondrial membrane, leading to impaired mitochondrial membrane potential and further increasing mitochondrial injury[110]. Whether this pathway is activated during ageing and in particular vascular ageing is unclear. Nevertheless, the involvement of such a pathway could explain why chronic TLR activation, via either microbial products or sterile inflammatory mediators, could lead to a chronic basal inflammatory state and mitochondrial dysfunction in the vasculature with ageing.

Mitochondrial injury leads to the release of mitochondrial components, known as mitochondrial damage-associated molecular patterns (mtDAMPs), including mtDNA, that when in the cytosol, can activate intracellular innate immune signalling pathways, such as the DNA-sensing receptor cyclic GMP–AMP synthase and the inflammasome[75,110–112]. Transfer of mitochondrial components into endosomes also activates the TLR9 inflammatory pathway[111], but the detailed mechanisms are not fully elucidated. Mitochondria also contain *N*-formylated peptides that induce inflammation via engaging the *N*-formyl peptide receptor 1 to increase neutrophil chemoattraction[113], arterial injury and ROS release[114]. Cardiolipin, a component of mitochondrial membranes, can directly bind to NLRP3 and activate the NLRP3 inflammasome[115]. If chronically activated, all these pathways could promote vascular ageing and also diminish mitochondrial function, although ascertaining the definitive contributions of each pathway requires future investigation.

Mitochondrial damage-associated molecular patterns (mtDAMPs). Pro-inflammatory components of mitochondria that are released as a result of mitochondrial dysfunction or damage.

Synergistic mechanisms

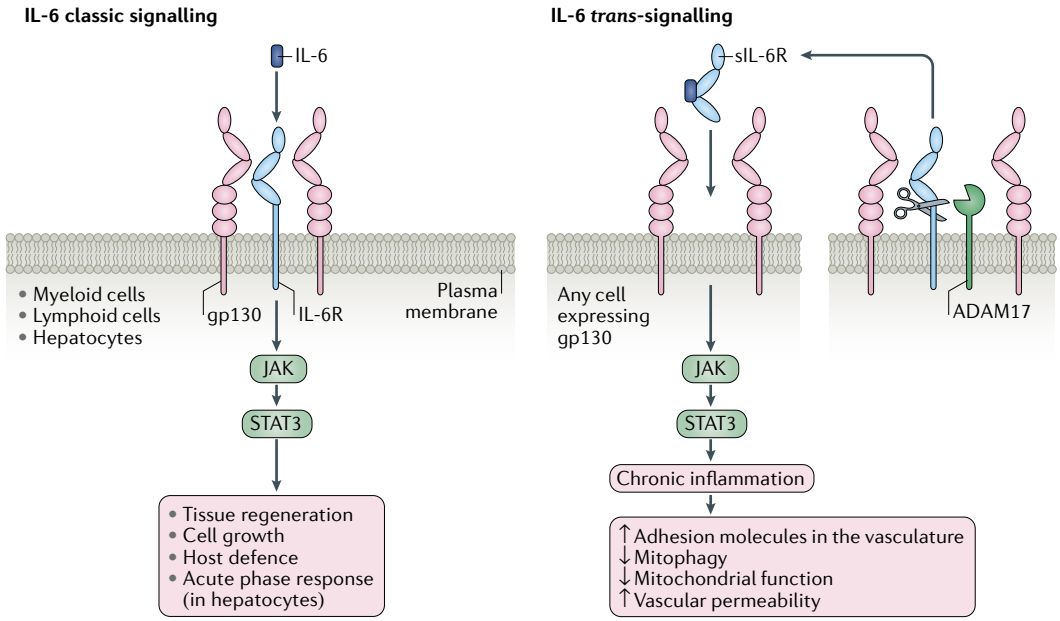
Shared inflammatory pathways between myeloid cells and the vasculature. Age-related atherosclerosis might be mediated by alterations in the vasculature and myeloid cells via a shared inflammatory pathway. A potential candidate pathway is IL-6 signalling because available evidence indicates that the level of IL-6 is elevated with ageing in both the immune system and the vasculature. In the bone marrow niche in mice, IL-6 levels increase with ageing, which is probably mediated by increased β_2-adrenergic receptor signalling and increased numbers of adipocytes[60] (FIG. 1). IL-6 directly acts on HSCs to promote a bias towards myeloid cell differentiation[60]. In mouse macrophages, TET2 deficiency, which is one of the most common genetic alterations found in the age-related condition CHIP, increases IL-6 secretion in vitro[74]. Importantly, the atherosclerosis-promoting effects of CHIP seem to be abrogated in individuals with a loss-of-function *IL6* genetic polymorphism[9]. In the vasculature, the level of IL-6 increases with ageing, which is at least in part mediated by IL-6 production by VSMCs[87].

IL-6 is associated with ageing in general and is part of the 'inflammageing' phenotype[15,16,116]. Why ageing leads to elevated basal secretion of inflammatory cytokines (not solely IL-6 but also other inflammatory mediators such as TNF) is not clear but might be caused by alterations in the microbiota[117], increased adiposity[118], and changes in the immune system[17] and the vasculature[29,30]. Elevated cytokine levels with ageing could also be a manifestation of chronic, latent infections such as with herpesviruses[119], of cellular senescence[57,86,87] or, potentially, of mitochondrial dysfunction.

The role of IL-6 in young animal models of atherosclerosis remains unclear and might relate to the complexities of IL-6 signalling (BOX 2). Specifically, signalling via the classic IL-6 pathway occurs in a restricted number of cells (such as hepatocytes and some immune cells) and involves IL-6 binding to the membrane-bound IL-6 receptor (IL-6R), with subsequent association with the signal-transducing IL-6R subunit β (also known as gp130). Evidence indicates that classic IL-6 signalling is important for tissue homeostasis, regeneration and host defence (as reviewed previously[120]). Soluble IL-6R can also engage IL-6 in the circulation and activate a broader range of cells than the classic pathway, via membrane activation of gp130. This pathway is termed IL-6 *trans*-signalling (BOX 2) and can result in chronic inflammation[120]. These different IL-6 signalling pathways might explain the pleiotropic effects of IL-6 in different tissues and cellular compartments and also the divergent role of IL-6 in experimental atherosclerotic models. For instance, one study in *Apoe*$^{-/-}$ mice showed that administration of exogenous IL-6 worsens atherosclerosis[121]. By contrast, another study in *Apoe*$^{+/-}$ mice showed that IL-6 deficiency worsens atherosclerosis[80], indicating that IL-6 might have atheroprotective effects. Neither of these studies distinguished between the classic and *trans*-signalling pathways of IL-6. However, a third study specifically inhibited the IL-6 *trans*-signalling with a fusion protein that blocks the soluble form of gp130 in

Box 2 | **IL-6 signalling**

IL-6 can signal via a classic signalling pathway and a *trans*-signalling pathway (see the figure). In the classic IL-6 signalling pathway, IL-6 engages the membrane-bound IL-6 receptor (IL-6R) and subsequently interacts with the IL-6R subunit-β (also known as gp130). Intracellular signalling mainly involves activation of the Janus kinase (JAK) and the signal transducer and activator of transcription 3 (STAT3). The classic pathway is generally restricted to hepatocytes and immune cells such as myeloid cells and lymphocytes. The *trans*-signalling pathway is activated by IL-6 binding to soluble IL-6R (sIL-6R) in the circulation and then binding of the IL-6–sIL-6R complex to membrane-bound gp130 on a broad range of cells. sIL-6R is released by enzymatic cleavage of membrane-bound IL-6R by disintegrin and metalloproteinase domain-containing protein 17 (ADAM17). Activation of the IL-6 *trans*-signalling pathway generally leads to chronic inflammation, whereas the IL-6 classic signalling pathway is involved in cell growth, regeneration and host defence.



atherosclerotic *Ldlr*$^{-/-}$ mice[122]. The study found that inhibiting IL-6 *trans*-signalling reduced atherosclerosis[122], indicating that IL-6 *trans*-signalling might have a pathogenic role in atherosclerosis. Therefore, clinical therapeutics to reduce atherosclerosis should focus on this IL-6 pathway.

Whether IL-6 has a causal role in age-related atherosclerosis is not known yet. The contribution of IL-6 to age-related atherosclerosis should be investigated in the future and should determine the main IL-6-producing and IL-6-responding cells. Furthermore, the identification of the major IL-6-producing cells (FIG. 2) and whether IL-6 activation occurs via the classic or *trans*-signalling pathway with ageing could lead to more targeted therapeutics for atherosclerosis, especially given the availability of clinically-approved agents to target IL-6 (REFS[123,124]). Importantly, the risk–benefit balance of targeting IL-6 in atherosclerosis will need to be determined, given that anti-IL-6 therapies in human studies increased the risk of infections[120], similar to other biological agents (such as anti-IL-1β antibodies) that have been used to reduce atherosclerosis[12,13]. However, other biological agents such as TNF inhibitors[125] might be beneficial for the treatment of atherosclerotic cardiovascular disease and should be investigated in age-related atherosclerosis. Finally, other inflammatory cytokines (such as TNF, C-C motif chemokine 2 and IL-18, which are all part of the SASP)[56] might have a pathogenic role in age-related atherosclerosis and should be assessed in future studies.

Therapies that can mitigate some of the detrimental biological effects of ageing, such as removing senescent cells (including senescent adipocytes)[126,127], improving mitochondrial function (for example, with metformin therapy)[128], or augmenting macroautophagy (for example, with rapamycin therapy)[129] or mitophagy, might reduce the burden of atherosclerosis in old people and should be investigated in future clinical studies. Agents that increase mitophagy, such as spermidine, have been shown in experimental studies to reduce atherosclerosis in both young[130] and aged[11] mice. Some or all these agents might have pleiotropic effects, which could reduce inflammation. Furthermore, these agents might synergize with specific anti-inflammatory therapies to reduce atherosclerosis with ageing, which will require future clinical investigation.

Conclusions

Ageing influences atherogenesis via multiple complex pathways, and one sole factor is unlikely to be a dominant pathophysiological mechanism. In this Review, we provide an overview of how ageing affects two systems, myeloid cell haematopoiesis and the vasculature, to promote atherosclerosis. We lay a framework of a potential shared inflammatory pathway, mediated by IL-6 signalling, that connects the role of the two systems in

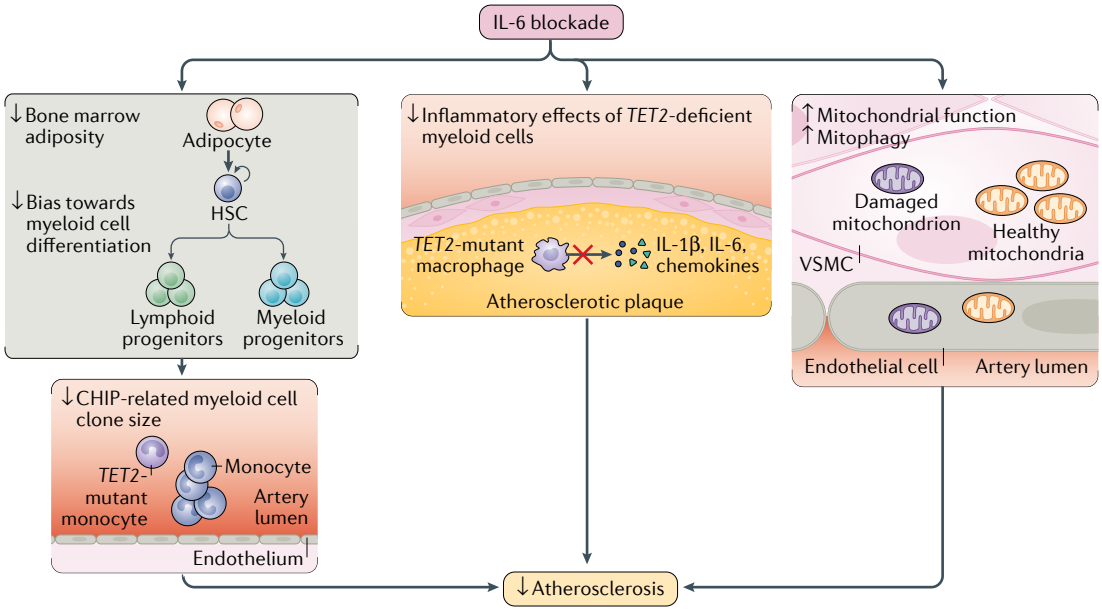


Fig. 2 | **IL-6 as a potential therapeutic target in age-related atherosclerosis.** IL-6 is upregulated in multiple tissues that have important roles in the increase in atherogenesis with ageing. Therefore, blockade of IL-6 might be an effective therapeutic strategy to reduce atherosclerosis development and progression during ageing. Blocking IL-6 might interfere with the increased IL-6 signalling in bone marrow adipocytes that occurs with ageing (which promotes a skewing towards myeloid cell differentiation), thereby reducing the risk of clonal haematopoiesis of indeterminate potential (CHIP). IL-6 blockade might also reduce the inflammatory potential of clones of myeloid cells associated with CHIP. IL-6 blockade might reduce atherosclerosis burden, although direct comparisons of efficacy and safety with IL-1β inhibition requires future investigation. IL-6 blockade might increase mitochondrial function and reduce the expression of Parkin, a mitochondrial stress protein, which might also contribute to reducing atherogenesis during ageing. HSC, haematopoietic stem cell; VSMC, vascular smooth muscle cell.

age-related atherosclerosis and propose future avenues of investigation to determine whether IL-6 and/or other inflammatory pathways are feasible and effective therapeutic targets to reduce the burden of atherosclerosis in old people. Anti-inflammatory strategies should be considered in the context of other therapies that aim to reduce many of the detrimental biological effects of ageing. Overall, we hope that with the pursuit of further clinical investigation and trials, therapeutic options will be available in the future to reduce the burden of atherosclerosis in the increasing number of old people in our society.

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