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CLINICAL REVIEW
Pathophysiology

Inflamm-ageing: the role of inflammation in age-dependent cardiovascular disease

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The ongoing worldwide increase in life expectancy portends a rising prevalence of age-related cardiovascular (CV) diseases in the coming decades that demands a deeper understanding of their molecular mechanisms. Inflammation has recently emerged as an important contributor for CV disease development. Indeed, a state of chronic sterile low-grade inflammation characterizes older organisms (also known as inflamm-ageing) and participates pivotally in the development of frailty, disability, and most chronic degenerative diseases including age-related CV and cerebrovascular afflictions. Due to chronic activation of inflammasomes and to reduced endogenous anti-inflammatory mechanisms, inflamm-ageing contributes to the activation of leucocytes, endothelial, and vascular smooth muscle cells, thus accelerating vascular ageing and atherosclerosis. Furthermore, inflamm-ageing promotes the development of catastrophic athero-thrombotic complications by enhancing platelet reactivity and predisposing to plaque rupture and erosion. Thus, inflamm-ageing and its contributors or molecular mediators might furnish targets for novel therapeutic strategies that could promote healthy ageing and conserve resources for health care systems worldwide. Here, we discuss recent findings in the pathophysiology of inflamm-ageing, the impact of these processes on the development of age-related CV diseases, results from clinical trials targeting its components and the potential implementation of these advances into daily clinical practice.

Keywords Inflamm-ageing • Vascular ageing • Inflammation • Endothelial dysfunction • Cardiovascular disease

General introduction

A progressive decline in physiological processes characterizes ageing. Thus, the elderly comprise a highly vulnerable population burdened with morbidity and disability. In particular, ageing affects the cardiovascular (CV) system leading to increased incidence of CV disease including hypertension, heart failure, atherosclerosis, and its acute complications, i.e. myocardial infarction (MI) and stroke.[1–5]

An increased incidence of age-related CV disease accompanies the steady worldwide increase in life expectancy. This pandemic presents an urgent need to understand how age regulates CV pathophysiology to confront and control its impact.[6] Inflammation has emerged as an independent CV risk factor and pathogenic contributor to CV disease.[7–9] Accordingly, this article reviews the recent concept of inflamm-ageing and summarizes experimental and clinical evidence linking inflammation to age-dependent CV disease. Moreover, it discusses the potential role of inflamm-ageing as a target for future therapeutic interventions.

Inflamm-ageing

The term 'inflamm-ageing' emerged in the early 2000s to describe the state of chronic sterile low-grade inflammation observed in older organisms.[10] Since then, this concept has evolved to indicate a broader immune dysregulation in elderly people during which persistent increased levels of pro-inflammatory mediators accompany a blunted inflammatory response to immunogenic triggers (reference

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11 reviews these aspects in detail). Accordingly, hallmarks of inflamm-ageing include chronic activation of the innate immune system and increased circulating levels of pro-inflammatory mediators, such as interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF)-α, and of the biomarker C-reactive protein (CRP).[12–15] Curiously, in contrast, adaptive immunity wanes with age, as demonstrated by reduced responsiveness to influenza vaccines in the elderly.[16–18] Inflamm-ageing associates with frailty, motor, and cognitive disability and overall mortality as well as being a recognized risk factor for most chronic degenerative diseases including cancer, dementia, renal, and CV disease (*Figure 1*).[19–26] Yet, establishing the causative role of inflamm-ageing for many others age-related conditions will require further longitudinal studies. Various lines of evidence suggest that high-sensitivity C-reactive protein (hsCRP) assesses CV risk factor in both young and old individuals.[27,28] Indeed, hsCRP retains an independent association with CV events after adjusting for age as shown by stratification or multivariable statistical adjustment in the setting of CV risk prediction.[29] Also, results from the Cardiovascular Health Study and the Rural Health Promotion Project demonstrate the ability of hsCRP to predict coronary events in the elderly after extensive adjustment for known risk factors and measures of subclinical atherosclerosis.[30] A precise set of criteria for inflamm-ageing remains unsettled, nor do we advocate the use of a panel of biomarkers to identify inflamm-ageing. Rather, we wish to convey to practitioners a forward-looking view of the burgeoning basic science progress that provides novel mechanistic links between ageing, inflammation, and metabolic changes with ageing. We offer clinicians who confront an increasingly ageing population a perspective that the processes are not inevitable and unrelated degenerative processes. Rather, the emerging biological insights reviewed here promise to spur the development of novel strategies to combat the inflamm-ageing complex.

Although no universally accepted theory thus far explains the chronic inappropriate activation of inflammation during ageing, several cellular and molecular mechanisms may contribute to this process (*Figure 1*). These mechanisms include cellular senescence and particularly immune-senescence, telomere shortening, genomic instability, defects in protein catabolism, dysregulation of autophagy and mitophagy, alteration of the host microbiota (i.e. dysbiosis) and chronic infections (e.g. cytomegalovirus and periodontitis), mitochondrial dysfunction, and chronic exposure to toxins at low level (e.g. chronic low-grade endotoxaemia, perhaps derived from the intestinal microbiome and accentuated by impaired epithelial barrier function with age or illness) (*Figure 1*).[31] Production and accumulation of such danger signals as self damage-associated molecular patterns (DAMPs) or non-self pathogen-associated molecular patterns, may promote inflamm-ageing and mediate inflammation by acting on promiscuous sensors, such as Toll-like and NOD-like receptors (TLRs and NLRs, respectively).[32] Recent work has implicated other receptors involved in cellular signalling, such as the Notch and Klotho/FGF23 pathways, in inflamm-ageing and in the development of age-related CV disease although evidence remains rudimentary.[33,34] Although these pathways promote survival of younger organisms, with ageing, their pro-inflammatory activities—which includes NF-kB activation, production of ILs (mainly IL-1 and IL-18), Type 1 interferons, and other cytokines—increase chronically and can thus fuel inflamm-ageing (*Figure 1*).[32] Genetic variants that affect systemic levels of cytokines and other inflammatory mediators may shape individual responses to these age-related challenges.[35–38] Cellular changes that contribute to inflamm-ageing can also act at the epigenetic levels by regulating RNA transcription and translation through DNA methylation, histone modification, and by modulating non-coding RNAs.[39] *Figure 1* presents an overview of the different pathways involved in the development of inflamm-ageing.

Recently, Prof. Franceschi *et al.*[40]—who coined the term inflamm-ageing— proposed that age-related metabolic diseases result from an age-related increased inflammatory tone (inflamm-ageing) and nutrient excess (metaflammation). As immune and metabolic response co-evolved over millennia,[41] inflamm-ageing and metaflammation show similar molecular mechanisms with sterile inflammatory response as a critical determinant and macrophages as master cells.[40,42,43] Ageing heightens metaflammation which in turn supports inflamm-ageing that may reflect accelerated ageing in response to caloric excess.[44] At a crossroad between metabolism and inflammation, the gut microbiota may modulate both metaflammation and inflamm-ageing. Indeed, the myriad of bacteria that colonize our digestive tract transform dietary molecules into metabolites and regulate the functionality of different organs and tissues.[45,46] The gut microbiota undergoes profound shaping with ageing and these changes may promote physiological nutrition-related inflammation.[47,48] For example, transfer of gut microbiota from aged to young germ-free animals associates with increased intestinal permeability and accelerated inflamm-ageing (macrophage dysfunction, increased systemic TNF-α, and alteration of adaptive immunity).[49,50] In humans, an increase in gut Proteobacteria with age correlates with the systemic increase of pro-inflammatory cytokines, such as IL-6 and IL-8.[51] Also, with ageing the gut microbiota becomes more abundant but less diverse,[52] features considered harmful for the host.[52,53] In contrast, an increase in microbial diversity characterizes the composition of ultra-centenarian gut flora and accompanies healthy ageing in several populations.[53]

The impact of inflamm-ageing on age-related cardiovascular disease

Both low-grade systemic inflammation and ageing augment risk of CV morbidity and mortality.[2,54] Not only does inflamm-ageing promote atherosclerosis *per se* but it also interacts with traditional CV risk factors (e.g. overweight/obesity, hypertension, and Type 2 diabetes mellitus) to exacerbate their deleterious CV effects. This relationship is reciprocal as CV conditions can promote inflammation and fuel a vicious cycle linking inflamm-ageing to the development of CV disease.[55] Whether inflamm-ageing occurs in absence of metabolic diseases, such as obesity and diabetes remain uncertain and will require further study.

In overweight and obese patients, high-caloric intake associates with a chronic pro-inflammatory status (the above-mentioned metaflammation) as a result of sustained post-prandial inflammatory stimulation and activation of adipose tissue.[56,57] During the post-prandial period, circulating triglyceride-rich lipoprotein particles directly induce the expression of adhesion molecules, cytokines, and pro-oxidants in endothelial cells and leucocytes and thus favour vascular

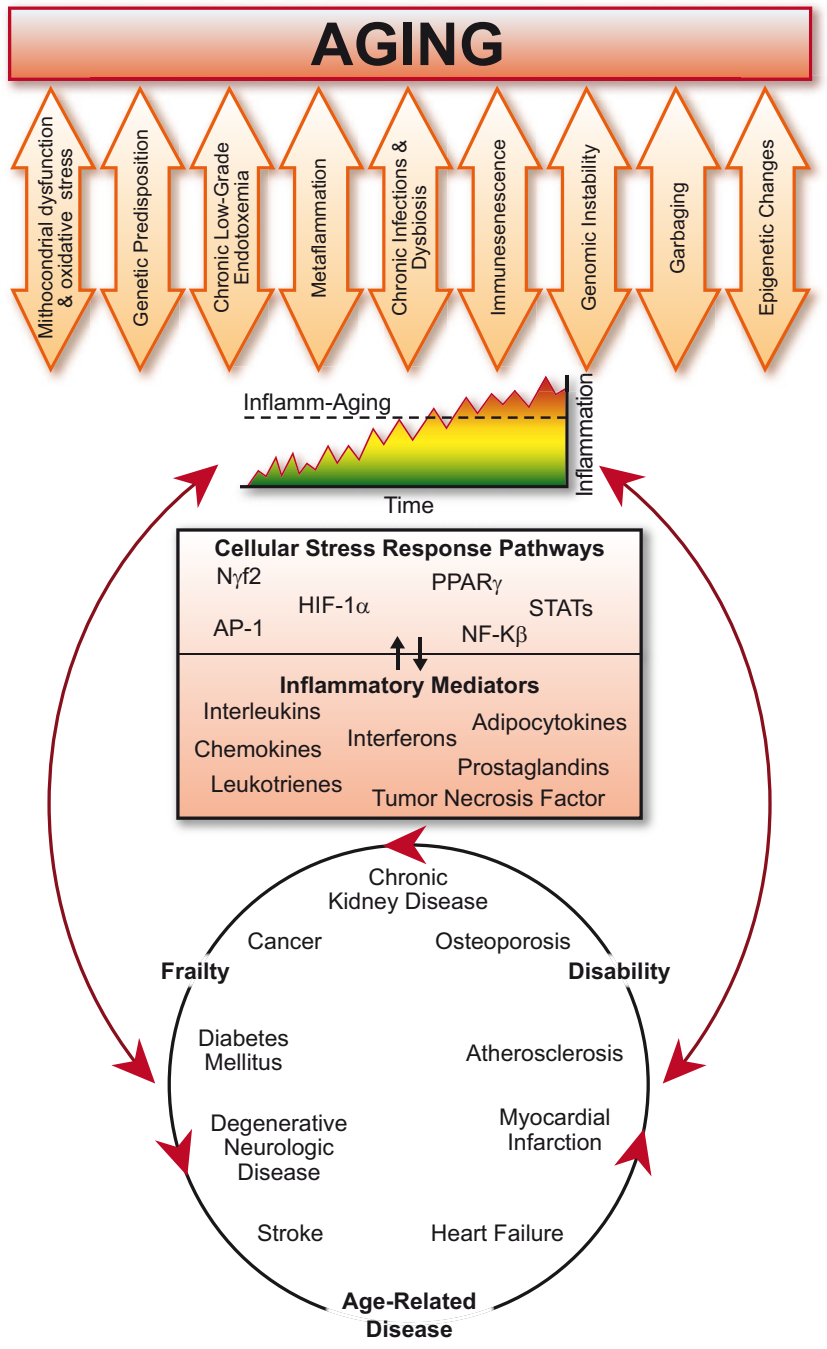

Figure 1 The deep interactions between ageing, inflamm-ageing, and age-related conditions/disease. With ageing, several cellular and molecular mechanisms contribute to the chronic inappropriate activation of the inflammatory system (orange arrows). The resulting complex interaction between genetic predisposition and chronic exposure to a broad spectrum of exogenous and endogenous danger stimuli causes the continuous activation of a limited range of promiscuous sensors triggering different cellular stress-response pathways (upper half of the box). The resulting synthesis and release of different inflammatory mediators (lower half of the box) can drive a progressive increase of the inflammatory burden and lead to inflamm-ageing. Inflamm-ageing is the common pathophysiological mechanisms of frailty and several age-related diseases (included in the lowest circle), which in turn increase the rate of ageing and thus feed a vicious circle (red arrows).

inflammation.[58–61] Moreover, adipocyte hypertrophy (i.e. increased cellular size) can lead to local hypoxia and endoplasmic reticulum stress. Those conditions can impair the protective AMPK and SIRT pathways and increase production of pro-inflammatory and chemotactic adipocytokines which promote inflammation and cooperate with inflamm-ageing to boost CV damage.[62,63] Ageing augments monocyte and lymphocyte accumulation in adipose tissue where these cells contribute to increased production of

inflammatory mediators.[64] In contrast, caloric restriction and weight loss associate with the activation of longevity-promoting pathways and reduction of inflammation.[1,57,62,65–69]

The pro-inflammatory milieu characterizing high-caloric intake also promotes insulin resistance, a well-recognized CV risk factor associated with ageing.[70,71] Indeed, the CV risk associated with diabetes increases strongly in elderly as compared with young individuals.[72–74] Here, inflamm-ageing broadens the vicious circle that connects obesity, insulin resistance, ageing, and age-related CV disease. Elevated levels of pro-inflammatory cytokines as seen in inflamm-ageing and metaflammation promote the infiltration of immune cells within insulin-responsive tissue (i.e. fat and muscles), thereby increasing local oxidative stress and inflammation and reducing the expression of the insulin receptor.[75–77] As a result, high circulating levels of glucose, lipids, free fatty acids, and reactive oxygen species (ROS) promote the transition from metabolically compensated obesity to acquisition of features of the metabolic syndrome cluster.[78] Also, pro-inflammatory cytokines, glucose, and modified lipids favour endothelial dysfunction thus accelerating CV ageing. Indeed, vascular cells from diabetic patients show shorter telomeres and increased senescence biomarkers (e.g. β-galactosidase) related to the accentuated atherosclerotic risk observed in the elderly with diabetes.[79,80]

Inflammatory mediators also exacerbate vascular endothelial and smooth muscle cell dysfunction that accompanies hypertension in the elderly.[7,81] Accumulation of inflammatory cells (i.e. dendritic cells, NK cells, macrophages) in arteries characterizes experimental hypertension.[82] Monocytes express receptors for angiotensin II (AngII) and for mineralocorticoids, pathways that drive hypertension, and promote inflammatory polarization and increase ROS production.[83] With inflamm-ageing, macrophages within the vessel wall produce higher amounts of ROS that reduce NO availability, boost adhesion molecule expression, stimulate VSMC hypertrophy and activate matrix metalloproteinases, processes implicated in vascular remodelling and dysfunction.[84] Accordingly, mice with functionally deficient macrophages show blunted vascular oxidative stress, endothelial dysfunction, and resist hypertension.[85,86] Mediators of inflamm-ageing, such as TNF-α, IL-1β, caspase 1, and other components of the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome can contribute to age-related progression of hypertension and thus represent potential therapeutic targets in this setting.[87] Indeed, the multimeric intracellular sensor, NLRP3 can detect different microbial peptides, endogenous danger signals, and non-self-irritants resulting in the assembly and activation of the NLRP3 inflammasome. Inflammasome activation requires two steps that tightly regulate its functions. A priming step augments the expression of individual inflammasome components and their assembly into the macromolecular multimer. A wide variety of potentially injurious stimuli then provide a second signal that confers activity on a proteolytic enzyme component of the inflammasome, caspase 1. This proteinase cleaves the inactive precursors of the pro-inflammatory cytokines IL-1β and IL-18, unleashing their biological actions.[88] In inflamm-ageing, the sustained increase in inflammatory mediators together with the reduction of circulating anti-inflammatory cytokines (e.g. IL-10 and adiponectin) might exacerbate vascular extracellular matrix remodelling and arterial stiffening, thereby widening pulse pressure, promoting systolic hypertension, and accelerating plaque formation.[89]

Atherosclerosis and its thrombotic complications account for most CV morbidity and mortality. Inflamm-ageing drives atherosclerosis, a recognized chronic inflammatory condition. Cells within atherosclerotic plaques often show features of senescence.[8,90] Cytokines and inflammatory cells participate in atherosclerosis at all stages, able to exert either pro- or anti-atherogenic effects.[91–94] Pro-inflammatory cytokines can impair endothelial cell barrier function, vasodilator properties, and induce adhesion molecules and chemokines that recruit leucocytes to lesions. Cholesterol crystals and other DAMPs within the atherosclerotic lesion co-activate the NLRP3 inflammasome in macrophages and augment the production of active forms of the pro-inflammatory cytokines IL-1β and IL-18.[83] The resulting inflammatory environment mediates the ongoing cellular recruitment with generation of foam cells and fatty streaks eventually leading to the development of complex plaques. NLRP3 activity also rises during endothelial cell senescence as a result of increased oxidative stress, and by defective autophagy in senescent cells.[2] Accordingly, the NLRP3 inflammasome has emerged as a therapeutic target for inflamm-ageing that might counteract age-related CV disease. In atherosclerotic arteries, some studies show decreases in anti-inflammatory factors, such as glucocorticoids, IL-10, IL-1 receptor antagonist, and NO limiting a return to homoeostasis and promoting chronic unresolved inflammation.[95]

Recently, a class of molecules referred to as specialized pro-resolving mediators have garnered increasing interest. SPMs derive from polyunsaturated fatty acids *via* lipoxygenase, cyclooxygenase, cytochrome P450, or their combination.[96] Levels of SPMs (such as resolvin D1) decrease significantly in vulnerable regions of atherosclerotic plaques where macrophages abundantly express nuclear 5-lipooxygenase. This enzyme converts arachidonic acid to pro-inflammatory leukotrienes potentially promoting plaque rupture.[96]

Senescent cells accumulate in advanced atherosclerotic plaques, as identified through specific markers, such as senescence-associated β galactosidase (SAβG), p16, and tumour suppressor alternative reading frame (ARF). Increased rate of SAβG-positive cells localizes in the intimal and medial layers from atherosclerotic arteries compared with age-matched healthy vessels.[97,98] Cellular senescence refers to the irreversible loss of cell's ability to divide[2]; furthermore, these cells acquire a defined senescence-associated secretory phenotype characterized by enhanced production of inflammatory cytokines, growth factors, and proteases.[99] The increased production of matrix metalloproteinases by senescent cells can enhance extracellular matrix breakdown and augment remodelling of the advanced atherosclerotic plaque.[100,101] Degradation of the extracellular matrix facilitates VSMC migration from the media, mediates arterial compensatory enlargement and can weaken the plaque's protective fibrous cap.[102] Indeed, under these conditions VSMC can sustain DNA damage and telomere shortening. These alterations promote stress-induced premature senescence (SIPS) with loss of proliferative abilities, defective autophagy, and induction of apoptosis.[98,103] Shortening of telomeres (i.e. repetitive nucleotide sequences at the ends of chromosomes) normally occurs at each cell division but inflammation and oxidative stress can enhance this process. Telomere shortening regulates cellular senescence and associates with atherosclerosis and major CV events.[104,105]

Oxidative stress as a result of ROS overproduction or defective scavenging critically contributes to SIPS and participates in ageing of

the CV system.[1] Indeed, several genes that modulate ageing and influence lifespan (i.e. ageing and longevity genes) contribute critically to atherosclerosis by augmenting cellular oxidative stress and levels of inflammatory mediators.[1,2] Also, the altered expression of those genes (e.g. *p66*^{Shc} and *JunD*) can impair age-related angiogenesis, essential for repairing damaged vessels and maintaining vascular homoeostasis.[106–108] Ageing, inflammation, and oxidative stress can alter the epigenetic regulation of such genes leading to defective response to hypoxia, blunted endothelial cell migration/proliferation and reduced number and functionality of stem and progenitor cells and thus favour premature CV ageing.[39,109]

The human microbiome may also influence age-related CV inflammation, and several studies have reported the presence of bacteria in atherosclerotic plaques.[110,111] Furthermore, bacterial DNA can circulate in patients with CV diseases and the personal microbiota fingerprint links to CV risk.[112–115] As previously mentioned, during ageing microbiota composition undergoes modifications associated with rise of local and systemic inflammatory markers, altered intestinal permeability and increased circulating bacterial DNA which may facilitate the development of atherosclerosis (*Figure 2*).[49,116] Although mechanistic studies implicated the ability of different microbial pathogens to directly invade vascular cells and leucocytes, thereby promoting inflammation, the intestinal microbiome also functions as an endocrine organ releasing different metabolites in response to dietary intake; in turn, such metabolites can enter the systemic circulation and act as hormones, signalling at a distance.[116] Although some metabolites may promote health, dysbiotic conditions may generate toxic metabolites that can accumulate with age. One such microbial product, trimethylamine N-oxide (TMAO), associates in some, but not all, studies with coronary artery disease (CAD) and with major adverse cardiac events in some cohorts.[117–119] Trimethylamine N-oxide links mechanistically to experimental CV disease. Indeed, several reports showed increased inflammation, foam cell formation, plaque burden, and thrombotic potential in animals fed with choline- or carnitine-rich diet (both substrates for TMAO synthesis) or in germ-free animals transplanted with synthetic microbial communities capable of producing TMAO.[119–121] Mechanistically, TMAO may modulate vascular dysfunction by acting the NLRP3 inflammasome pathway, increasing oxidative stress, promoting forward cholesterol transport, and directly enhancing platelet thrombogenicity via increased Ca^{2+}-signalling.[122] Accordingly, recent experimental data suggest targeting TMAO synthesis by TMA lyase inhibitor as a potential anti-atherosclerosis therapy.[123]

Inflammation as a target to blunt age-related cardiovascular disease: current evidence from clinical studies

Despite major remaining knowledge gaps, current evidence points towards inflamm-ageing as an important contributor of age-related CV disease; indeed, some of its molecular pathways have already proven effective as targets in the clinical setting. In keeping with the inflammatory nature of atherosclerosis and its acute thrombotic manifestations, patients with chronic extra-cardiac inflammatory diseases, such as rheumatoid arthritis or psoriatic arthritis have a higher risk of CV disease (adjusted relative risk of 2.0 for MI) and related mortality compared with the general population. Systemic inflammation may contribute independently to this increased CV risk and lead to MI, cerebrovascular disease, and heart failure.[124–129] Furthermore, prospective cohort studies have consistently shown that elevated plasma levels of hsCRP and several other biomarkers of inflammation associate independently with increasing risk of future CV events in different populations.[130–132] In a comprehensive meta-analysis of >50 prospective studies, the magnitude of risk associated with 1 SD elevation in hsCRP resembled that observed for a 1 SD elevation in total cholesterol or blood pressure.[133] Results from the JUPITER study of rosuvastatin in primary prevention patients with above median hsCRP, LDL <130 mg/dL, and without extra-cardiac inflammatory disease indicated that the statin-induced reduction in CV events derived from both the observed reductions in vascular inflammation, as measured by hsCRP, and in LDL levels.[134] Data from PROVE-IT also support the concept of targeting both LDL and inflammation for CV disease prevention.[135] As they impact both inflammation and lipids, statin trials could not test whether selectively reducing inflammation (without lowering LDL) would improve CV outcomes. Taken together, these data along with those of multiple animal experiments bolstered the hypothesis that targeting mediators of inflammation may improve clinical outcomes in patients with atherosclerosis and encourage further clinical trials testing anti-inflammatory treatments in this setting (*Table 1*).

Clinical trials have shown that the P-selectin antagonist inclacumab and an anti-inflammatory serpin both reduced myocardial damage after percutaneous coronary intervention performed for an acute MI.[142,148] A 5-lipoxygenase inhibitor has also appeared to reduce levels of leukotrienes and hsCRP and slow atherosclerosis progression in another relatively small clinical study.[149] Despite these results of intermediate endpoint studies, large-scale CV outcome studies have not shown benefit for these interventions.

Completed clinical trials of anti-inflammatory agents targeting cardiovascular clinical outcomes

Canakinumab anti-inflammatory thrombosis outcomes study (CANTOS) tested canakinumab, a monoclonal antibody that neutralizes the pro-inflammatory cytokine IL-1β, in a study of 10 061 patients with previous MI and an hsCRP concentration of 2 mg/L or above on full standard of care medications including effective statin treatment.[137] Canakinumab administered subcutaneously every 3 months dose-dependently reduced hsCRP in the treated groups compared with placebo, without changes in atherogenic lipoprotein levels. At a median follow-up of 3.7 years, the middle dose met the stringent criteria for statistical significance, both for the primary endpoint of non-fatal MI, non-fatal stroke, or CV death and the secondary endpoint that included hospitalization for unstable angina that led to urgent revascularization. All-cause mortality did not differ significantly in the canakinumab groups compared with placebo. Canakinumab did associate with a small but significant increase in the incidence of fatal infections compared with placebo.

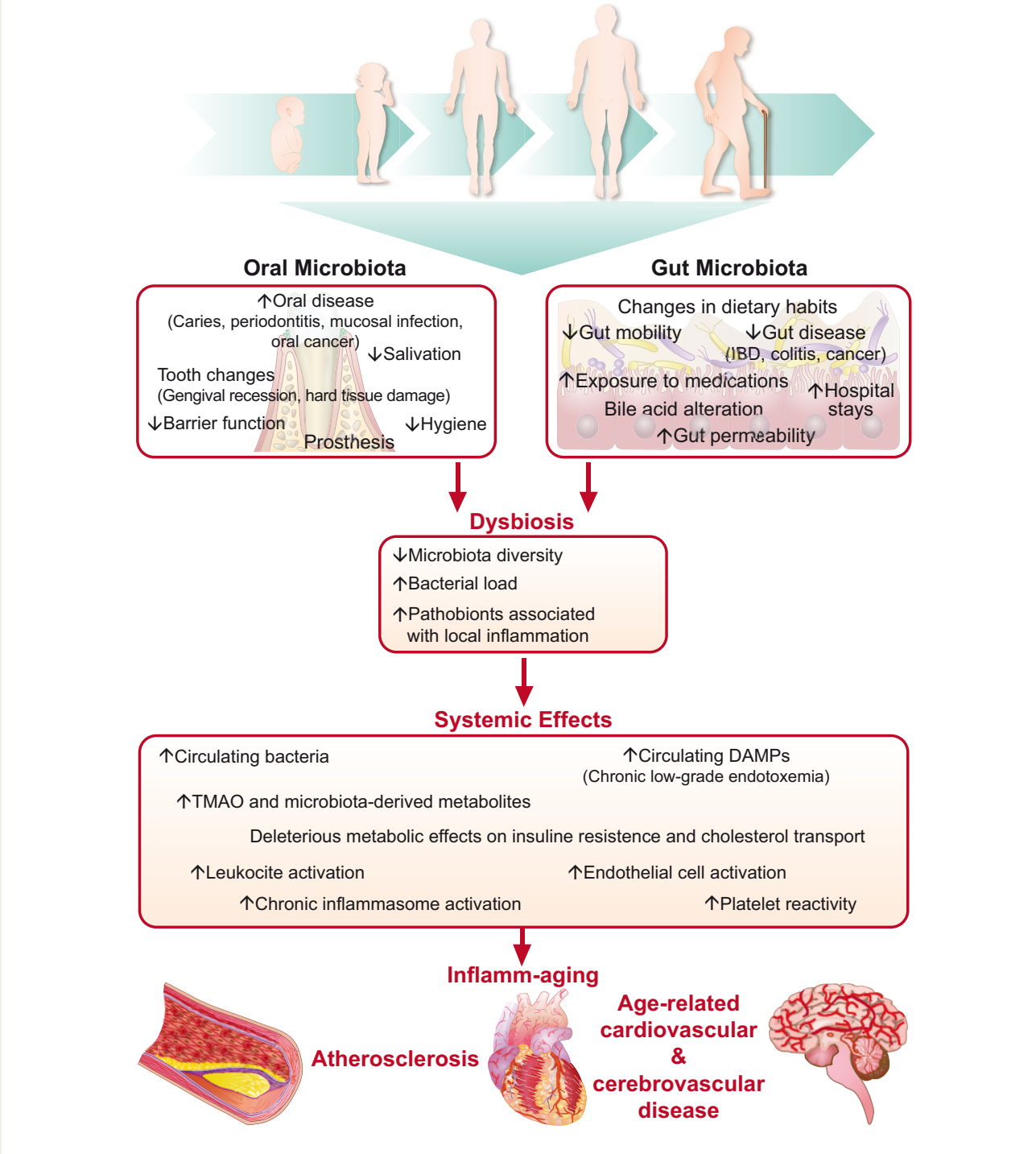

Figure 2 Age-related dysbiosis enhances inflamm-ageing eventually accelerating atherogenesis. Age-related dysbiosis enhances inflamm-ageing eventually accelerating atherogenesis. With ageing several endogenous and exogenous changes (included in the upper boxes), foster a less healthy oral and gut microbiota (left and right boxes, respectively). These alterations impair the homoeostatic symbiosis between the intestinal flora and the host. Total bacterial load increases, while microbiota diversity falls allowing pathobionts to establish a condition of chronic local inflammation known as dysbiosis (central box). Together with local consequences, dysbiosis also causes systemic effects (lower box) including increased circulating bacteria, danger-associated molecular pathways as well as trimethylamine N-oxide and other microbiota-derived metabolites eventually causing the systemic activation of inflammatory pathways, promoting age-related cardiovascular and cerebrovascular disease (bottom of the figure).

Table 1 Main recent clinical trials of anti-inflammatory therapy in cardiovascular disease

Trial, year	Drug	Target	Dosage	Size	Main result
ARISE[136]	Succinobucol	OxLDL	300 mg once daily	6144	Failure to reduce fatal and non-fatal cardiovascular events in patients with recent acute coronary syndromes
CANTOS[137]	Canakinumab	IL-1β	150 mg every 3 months	10 061	Reduction of recurrent fatal and non-fatal cardiovascular events
CIRT[138]	Methotrexate	Purinergic signalling	15–20 mg weekly	4786	Premature stop due to inefficacy in reduction of inflammatory mediators and cardiovascular events in patients with stable atherosclerosis
COLCOT[139]	Colchicine	Microtubule assembly	0.5 mg once daily	4745	Reduction of recurrent ischaemic cardiovascular events
LoDoCo[140]	Colchicine	Microtubule assembly	0.5 mg once daily	532	Reduction of cardiovascular events in patients with stable coronary artery disease
LoDoCo2 (EU Clinical Trial Regeister n 2015-005568-40)	Colchicine	Microtubule assembly	0.5 mg once daily	5000	Ongoing
MRC-ILA Heart[141]	Anakinra	IL-1R	100 mg once daily	182	Reduction of inflammatory markers 14 days after non-ST elevation acute coronary syndrome. Excess of MACE events 1 year after the treatment
SELECT-ACS[142]	Inclacumab	P-selectin	20 mg/kg single dose	544	Reduction of peak Troponin I and myocardial creatine kinase after non-ST-elevation acute coronary
SELECT-CABG[143]	Inclacumab	P-selectin	20 mg/kg every 4 weeks	384	Failure to reduce saphenous vein graft disease following coronary artery bypass graft
SOLID-TIMI-52[144]	Darapladib	Lp-PLA_2	160 mg once daily	13 026	Failure to reduce major coronary events in patients with recent acute coronary syndrome
STABILITY[145]	Darapladib	Lp-PLA_2	160 mg once daily	15 828	Failure to reduce fatal and non-fatal cardiovascular events in patients with stable coronary artery disease
VCU-ART 3[146]	Anakinra	IL-1	100 mg twice daily	99	Ongoing
VISTA-16[147]	Varespladib	$sPLA_2$	500 mg once daily	5145	Terminated for failure to reduce fatal and non-fatal cardiovascular events in patients with recent acute coronary syndrome. Warning for possible harm

IL, interleukin; IL-1R, interleukin 1 receptor; Lp-PLA_2, Lipoprotein-associated phospholipase A2; MACE, major adverse cardiovascular events; OxLDL, oxidized low-density lipoprotein cholesterol; $sPLA_2$, soluble phospholipase A2; TNFα, tumour necrosis factor α.

The Cardiovascular Inflammation Reduction trial (CIRT) tested the anti-inflammatory agent methotrexate in a randomized placebo-controlled study of 4786 patients with a previous MI or multivessel coronary disease, who also had Type 2 diabetes or the metabolic syndrome.[138] CIRT halted prematurely after a median follow-up of 2.3 years for futility. As opposed to the effect of canakinumab in CANTOS, low-dose methotrexate did not affect blood levels of IL-1β, IL-6, or hsCRP.

Clinical studies of the anti-inflammatory agent colchicine

Colchicine, a widely available, inexpensive, and orally administered anti-inflammatory drug, has approval for the management of patients with gout (microcrystalline inflammatory arthritis). Colchicine may exert its effects through the inhibition of tubulin polymerization,[150] interfere with the assembly of the multimeric NLRP3 inflammasome, and through effects on cellular adhesion molecules and inflammatory chemokines.[151–154] Thus, colchicine can interfere with many functions of white blood cells including migration and degranulation. Through the disruption of the cytoskeleton, colchicine can suppress secretion of cytokines and chemokines as well as platelet aggregation *in vitro*.[155,156] Considerable work has highlighted the potential of colchicine in the treatment of inflammatory CV diseases. Indeed, several clinical trials[157–161] have demonstrated the efficacy of colchicine for the treatment and prevention of recurrent pericarditis, a therapy that has become standard of care. In a retrospective cross-sectional study of 1288 low-risk patients with gout, those treated prophylactically with colchicine for 1 year had a significantly lower incidence of MI compared with patients who did not receive colchicine, with a trend towards reduced all-cause mortality.[162] Considerable confounding plagues such observational studies, emphasizing the necessity of rigorous randomized trials in this and in other domains.

Colchicine has also undergone study in patients with CAD. In the LoDoCo Trial, 532 patients with clinically stable coronary disease randomly received treatment with colchicine or no colchicine in addition to usual care for a minimum of 2 years in an unblinded PROBE design.[140] Following a mean follow-up of 36 months, colchicine-treated patients experienced significantly fewer CV events (composite incidence of acute coronary syndromes, out-of-hospital cardiac arrest, or non-cardioembolic ischaemic stroke) as compared with no colchicine. A reduction in acute coronary events unrelated to stent disease largely drove this effect. The results of this relatively small trial indicated that colchicine may be a safe and effective agent for the prevention of major CV events in this population and that its mechanism of action may be through inhibiting the inflammatory pathway identified in unstable atherosclerotic plaques. Although LoDoCo was small, included a control group without placebo, and focused on patients with stable CAD, it has stimulated larger and more rigorous clinical studies.

Indeed, a 2016 Cochrane systematic review concluded that 'colchicine may have substantial benefits in reducing MI in selected high-risk populations' but 'more evidence from large-scale randomized trials is needed'.[163] Accordingly, the recently published COLchicine Cardiovascular Outcomes Trial (COLCOT) evaluated the effects of colchicine on CV events as well as its safety and tolerability in ~4745 post-MI patients recruited within 30 days after the index event.[139] Daily administration of colchicine (0.5 mg daily) for a median follow-up time of 22.6 months significantly reduced the risk of primary composite endpoint (death from CV causes, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina leading to coronary revascularization) as compared with placebo.[139] This result derived mainly from lower risks of angina and stroke, while the treatment did not show effects on the risk of death from CV causes or MI. The gastrointestinal side effects of colchicine were somewhat more frequent in the treated group (i.e. nausea and flatulence). Pneumonia also increased in the colchicine-treated group, but infectious deaths and septic shock did not differ in the two groups.[139] Currently, the LoDoCo-2 study is assessing the value of colchicine on clinical outcomes in ~5500 patients with stable CAD and will provide an additional evaluation of anti-inflammatory therapy with colchicine in patients with coronary syndromes.

In sum, novel therapies that target inflammatory pathways can reduce atherosclerotic risk. Although not all inflammation blockers do so, neutralization of IL-1β and treatment with colchicine demonstrated effectiveness in secondary CV prevention. As expected, anti-inflammatory therapies can increase the risk of infections and future studies should strive to test interventions that interfere less with host defenses.

The strong age-dependency of clonal haematopoiesis and the consequent CV risk discussed below illustrates an example of accentuated inflammation directly related to ageing. Links between clonal hematopoiesis and telomere length provide another mechanism that links ageing to inflammation.[164] These observations should spur a quest to identify biomarkers that could distinguish inflamm-ageing from chronic inflammation of other causes. This distinction could inform the design of clinical trials that could enrol the frequently under-represented elderly population, and test directed anti-inflammatory therapies in a precision or personalized manner.

Future therapeutic perspectives

Recent findings have identified a novel connection between ageing, inflammation, and CV disease completely unsuspected until a few years ago: clonal haematopoiesis. With age, humans acquire somatic mutations in bone marrow stem cells that lead to accumulation in peripheral blood of clones of mutant leucocytes. This situation is common, and relates strongly to age. Seventy-year-old individuals have at least an 1 in 10 chance of harbouring >2% of the cells in their peripheral blood that bear a mutation associated with clonal haematopoiesis.[165,166] The genes mutated that give rise to these clones represent a small subset of the some 40 well-characterized driver genes for leukaemia. Leukaemic transformation generally requires the successive acquisition of three or more mutations in the same clone. Thus, only a few individuals with clones in peripheral blood bearing just one such mutation will develop acute leukaemia. Therefore, this condition has been dubbed clonal haematopoiesis of indeterminate potential (CHIP).[167] This situation is analogous to the development of monoclonal gammopathy of unknown significance, another age-related situation.

Although individuals with CHIP have only a 0.5–1% annual rate of conversion to acute leukaemia, they exhibit a 40% increase in CV risk, independent of traditional risk factors.[166,168] Experiments in mice genetically altered to have loss of function of Tet2 a commonly mutated gene that generates CHIP exhibit accelerated atherosclerosis.[169] Thus, CHIP does not merely accompany ageing, a strong risk factor for atherosclerosis, but appears causally related to aggravation of vascular disease. Analyses of leucocytes from these mice engineered to manifest CHIP indicate overexpression of pro-inflammatory genes, including the products of the NLRP3 inflammasome IL-1β and its downstream companion IL-6.[169] The presence of CHIP mutations, drives not only excess risk of MI and stroke but also of heart failure and death due to heart failure.[170] Thus, CHIP provides a newly recognized link between ageing, inflammation, major CV diseases, and cancer, another common affliction of ageing. The most common mutations associated with CHIP alter methylation of pyrimidines, strong evidence that epigenetic alterations contribute to the excess CV risk caused by CHIP.[167]

The advances in the understanding of the biology of ageing and inflammation provide a new perspective for potential therapies. One of the central hubs of inflammation signalling, the inflammasome, has received intense interest as a potential therapeutic target. This multimeric intracellular protein processes the precursors of the pro-inflammatory cytokines IL-1β and IL-18 to their active products.[81] Small molecule inhibitors of the inflammasome are currently in clinical development. The major product of the NLRP3 inflammasome, the active forms of IL-1β and IL-18 could be targets of therapy with receptor antagonists or neutralizing antibodies. The success of the CANTOS illustrates the efficacy of neutralizing IL-1β in individuals with already established coronary heart disease.[137] IL-1 and IL-18, in turn, trigger the expression of IL-6 in many cell types.[81] This cascade amplifies inflammasome signalling considerably. IL-6 can promote thrombotic events by boosting fibrinogen and plasminogen activator inhibitor-1 production by the liver, hence rendering blood more coagulable and impairing fibrinolysis.[171] Therapeutic antibodies can also target IL-6 and its receptor as well as IL-18. Thus, the inflammasome

pathway provides a rich palette of potential therapeutic targets for combatting this aspect of inflamm-ageing.

In contrast to the promise of the inflammasome pathway, other hubs of inflammatory signalling appear less attractive as therapeutic targets. The very centrality of the NF-κB pathway indicates that its inhibition might impair host defenses in a more global fashion than inhibition of the NLRP3 inflammasome. Indeed, the boost in inflammatory gene expression due to IL-1 isoforms and TNF depends largely on NF-κB.[172] Moreover, NF-κB activation can contribute to apoptosis, raising the possibility that its inhibition could promote tumour growth. Indeed, the increased lymphoma seen with anti-TNF therapies may reflect impaired tumour surveillance and/or decreased apoptosis.[173] Targeting of IL-17/IL-23 requires caution due to some signals of increased CV risk.[174]

Other anti-inflammatory therapies ranging from a variety of antioxidant interventions and inhibition of p38 mitogen-associated kinase (MAP kinase) did not prevent coronary events in a well-conducted clinical trial.[175] The recent demonstration that a high-dose pharmaceutical preparation of eicosapentaenoic acid can reduce CV events suggests another potential avenue for addressing inflamm-ageing therapeutically.[176] Three recent randomized clinical trials evaluated low-dose aspirin in primary CV prevention and led the American Heart Association and the American College of Cardiology (AHA/ACC) to issue updated aspirin recommendations in March.[177] Those trials compared low-dose aspirin (ca. 100 mg daily) with placebo over 5–7.5-year follow-up in diabetics (ASCEND), patients at moderate CV risk (ARRIVE) and elderly individuals (ASPREE).[178–180] None of the above-mentioned trials demonstrated CV benefit (reduction in MI, stroke, or CV mortality). Indeed, ASPREE found an increased risk of all-cause mortality and gastrointestinal malignancies for the aspirin arm.[178–180] As for secondary CV prevention, aspirin remains a cornerstone of therapy although some concerns pertain including (i) most studies were conducted decades ago and might not reflect current standard of care (e.g. statin treatment), (ii) most trials enrolled young and predominantly male patients, and (iii) the regimen used in those trials differs importantly from current recommendations.[181] As with aspirin, other non-steroidal anti-inflammatory agents also appear not to provide CV benefit, and particularly in the case of cyclooxygenase-2 selective inhibitors may even augment CV events.[182] Some of the benefit that accrued from the high-dose omega-3 fatty acid therapy could result from dampening of inflammation.[183]

Beyond pro-inflammatory interventions, the characterization of SPMs provides a way to enhance the resolution of inflammation without impairing host defenses, as mentioned above. The elegant chemistry and functional biology of SPMs provides an intriguing potential for further advances in the therapy of inflammation associated with ageing and other conditions.

The recognition of the nexus between ageing, inflammation, and CV disease has gained considerable new mechanistic understanding as summarized here. A number of novel potential therapeutic avenues have emerged from these advances in basic science. The use of biomarkers to assess over activity of specific pro-inflammatory pathways could help target therapies and enhance personalization of medical interventions in the growing population of elderly that will increasingly comprise a bulk of patients cared for cardiologists.

Conclusions

Older individuals commonly exhibit low-grade persistent inflammation, presenting a postulated mechanistic pillar of ageing biology. In the elderly, chronic inflammation predicts the risk of frailty, sarcopenia, disability, and age-related chronic disease, including CV conditions. Recent years have witnessed substantial progress in unveiling the mechanisms underlying inflamm-ageing. Clinical trials have validated the notion that the inhibition of selected inflammatory mediators can reduce CV events. However, studies focusing on anti-inflammatory agents and their effects on age-related CV conditions are scarce and controversial. This gap comprises an important area of unmet medical need that merits further focused clinical studies.

Novel pharmacological treatments that selectively target the pathways driving inflamm-ageing might prevent CV disease and retard the age-related decline in physiological processes. Translation of the basic findings into clinical tools able to challenge the burden of CV disease in an ageing population will require dedicated clinical trials designed to investigate the frequently underrepresented but growing segment of elderly individuals.

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