

Review

# Interconnections between inflammageing and immunosenescence during ageing

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**Abstract:** Acute inflammation is a physiological response to injury or infection, with a cascade of steps that ultimately leads to recruitment of immune cells to clear invading pathogens and heal wounds. However, chronic inflammation arising from continued presence of the initial trigger, or dysfunction of signalling and/or effector pathways, is harmful to health. While successful ageing in older adults including centenarians is associated with low levels of inflammation, elevated inflammation increases the risk of poor health and death [1–3]. Hence inflammation has been described as one of seven pillars of ageing. Age-associated sterile, chronic, and low-grade inflammation is commonly termed inflammageing - it is not simply a consequence of increasing chronological age, but is also a marker of biological ageing, multimorbidity and mortality risk. While inflammageing was initially thought to be caused by “continuous antigenic load and stress”, reports from the last two decades describe a much more complex phenomenon also involving cellular senescence and ageing of the immune system. In this review, we explore the sources and consequences of inflammageing and highlight potential interventions. In particular, we assess the contribution of cellular senescence to age-associated inflammation, identify patterns of pro- and anti-inflammatory markers characteristic of inflammageing, describe alterations in the ageing immune system that lead to elevated inflammation, and finally assess the ways that diet, exercise and pharmacological interventions can reduce inflammageing and thus improve later life health.

**Keywords:** ageing; inflammation, cytokines, inflammageing; inflammaging; immunosenescence; immunosurveillance; senescence; SASP

## 1. Introduction

### 1.1 Senescent cells contribute to a state of chronic low-grade inflammation

A variety of cellular stresses can lead both proliferating and long-lived cells to enter a state of cell cycle arrest with accompanying phenotypic and functional alterations known as cell senescence [4–11]. Many of these changes are dependent upon constitutively active mTOR (mechanistic target of rapamycin) signalling [12–15], which promotes protein synthesis and an increase in biomass with resulting hypertrophy; senescent cells are also resistant to apoptosis. Senescent cells also show local DNA methylation changes [16,17] and global chromatin rearrangements [18], leading to altered gene expression patterns and secretion of a

panoply of chemokines, cytokines and tissue remodelling enzymes. This 'senescence-associated secretory phenotype' or SASP [19,20], is thought to have evolved to signal to the immune system for clearance [21,22]. Different immune cell subtypes, such as NK cells, macrophages and T cells have been reported to participate in immunosurveillance for removal of senescent cells [21,23–26]. Indeed, senescence is important in tissue remodelling during development, regeneration and tissue remodelling [27–32], as well as serving an important tumour suppressor role [33]. However, age-related failure of immunosurveillance, coupled with immune evasion by senescent cells (through SASP-dependent cleavage of intrinsic surface markers), leads to increasing numbers of senescent cells with age [21,34,35]. Moreover, immune cell senescence can drive organismal ageing, as shown in a mouse study where accelerated ageing of immune cells through loss of a DNA repair factor led to increased SASP, premature senescence of multiple organs, and reduced lifespan, while transplantation of splenocytes from young healthy animals to aged mice significantly reduced senescence [36].

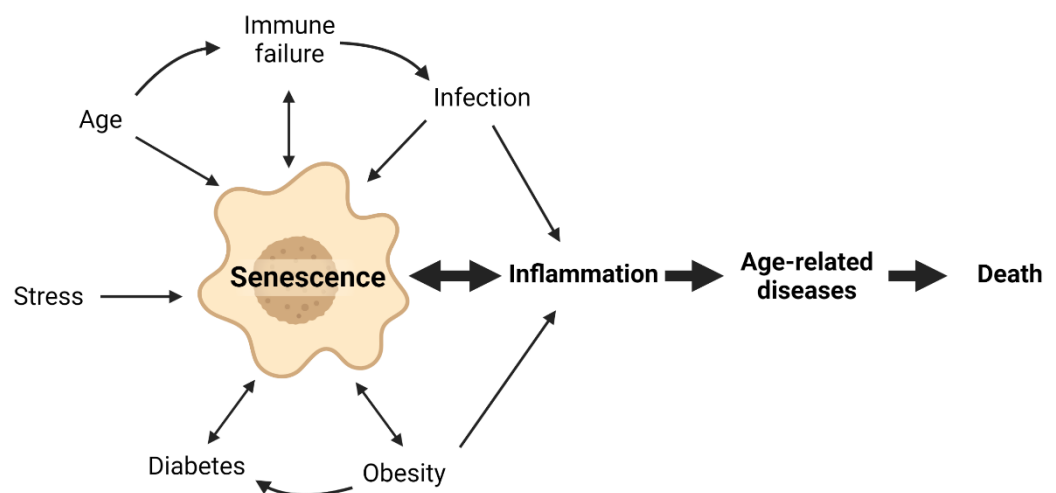
This accumulation of senescent cells leads to a pro-inflammatory state, since the SASP includes pro-inflammatory cytokines (*e.g.* IL-6, IL-1, HMGB1, S100), chemokines (*e.g.* IL-8, MCP-1), soluble receptors (*e.g.* sTNFRs), metalloproteases (*e.g.* collagenase, PAI-1) and growth factors [20,37,38]. A major regulator of inflammation, NF- $\kappa$ B, plays a critical role in the onset of the SASP [39–41]. The SASP is both autocrine and paracrine in nature, triggering secondary senescence in neighbouring cells [42–44]. For example, transplantation of senescent cells into the knee joint caused an osteoarthritis-like phenotype in mice, suggestive of accelerated tissue ageing [43,44], while local clearance of senescent cells diminished osteoarthritis, at least in mice [45]. It has been postulated that circulating senescence-derived extracellular vesicles that carry SASP factors may be responsible for development of systemic age-related diseases, even in response to initially localised senescence [44,46].

A causative relationship between the presence of senescent cells and elevated inflammation has been demonstrated both *in vitro* [47] and *in vivo* [48], reinforcing the idea of a role for senescence in inflammageing. Chronic inflammation can also induce telomere dysfunction and promote senescence, suggesting that inflammageing and senescence are mutually reinforcing [49]. Mechanistically, this reinforcement may be mediated, at least in part, through inflammatory signalling initiated by RAGE (receptor for advanced glycation end-products). Notably, adipose tissue is pro-inflammatory, and obesity and diseases such as diabetes that are associated with early onset of ageing pathologies, involve activation of RAGE signalling, which contributes to inflammation.

RAGE is a multiligand receptor, initially described as a receptor for advanced glycation end-products (AGEs) but which is now well described to interact with multiples ligands, including a number of pathogen-associated molecular patterns (PAMPS) and damage-associated molecular patterns (DAMPS), while mainly promoting a self-sustaining pro-inflammatory cascade, similar to other pattern recognition receptors such as TLRs [50]. RAGE expression has also been linked to physiological ageing of the kidney and blood vessels in mice, possibly by enhancing sustained low-grade inflammation and mTOR signalling [51], although to date there is no report regarding the influence of RAGE expression on lifespan. Interestingly, HMGB1, a major nuclear protein released from senescent cells [52], is a component of the SASP likely to promoting inflammageing through very high binding affinity to RAGE [50]. Not only does the SASP itself contains a wide variety of inflammatory cytokines and chemokines, but other SASP factors further amplify inflammatory signals through RAGE, NF- $\kappa$ B and inflammasome stimulation; the SASP is therefore a strong candidate for driving age-

related increases in inflammatory markers and is likely to contribute significantly to inflammageing.

Additional triggers of senescence include viral infection [53–56] and bacterial infection [57] leading to prolonged and harmful inflammation. In the context of an already elevated inflammatory state due to inflammageing, any additional infection-associated inflammation may cross a tolerable threshold, leading to significant tissue damage, disease and even death. It is of particular note that COVID-19 deaths are highest in adults over the age of 65 and those with pre-existing inflammatory conditions such as diabetes and obesity [58–60], suggesting that viral infection exacerbates an already high inflammatory state as a result of elevated senescent cell burden with increasing age [55,56]. Chronic non-infectious diseases associated with ageing, as well as infectious pathogens and age-related inappropriate responses to infection are therefore all likely to contribute to chronic inflammation, at least in part through cellular senescence; this vicious cycle means that inflammation worsens with age and more so with accompanying disease (Figure 1).



**Figure 1.** Relationship between cell senescence, age-related diseases and inflammation.

## 1.2 Inflammageing depends on a complex interplay of pro- and anti-inflammatory mediators

The overall inflammatory status in mammals is dependent not only on increases in individual markers of inflammation [61,62], but also on complex interactions between the various mediators of inflammation. Thus, it is important to investigate the association between pro- and anti-inflammatory markers and other parameters of ageing such as frailty, comorbidities and lifespan, to determine whether inflammatory changes arise from increasing age *per se*, or from other confounding factors such as underlying age-related diseases.

### 1.2.1 Elevation of clusters of pro-inflammatory markers with age

Studies of circulating inflammation modulators have identified several that increase with age and may participate in the overall inflammatory burden. Individual factors include IL-8, IL-15, soluble glycoprotein 130 (SGP130, involved in IL-6 signalling), soluble cluster of differentiation 30 (sCD30) and monocyte chemoattractant protein-1 (MCP-1), all of which increase with age in peripheral blood mononuclear cells (PBMCs) or serum [63–67]. Of the common clinical serum biomarkers used to assess inflammation, C-reactive protein (CRP) and IL-6 are by far the most closely linked in pro-inflammatory clusters [68–71] (see Figure 2 and Table 1). They correlate well with age (especially IL-6) [67,72], poor physical performance [69,73,74] and increased death rate in longitudinal studies [71,75]. The strong association between IL-6 and CRP is not surprising as IL-6 is known to induce the production of acute phase proteins such as CRP [61,76]. Interestingly, IL-6 on its own can drive cell senescence [77], leading to further inflammatory signalling through the SASP; hence high local levels of pro-inflammatory cytokines are self-reinforcing, potentially preventing resolution of inflammation in the context of ageing. Plasminogen activator inhibitor-1 (PAI-1) belongs to the same pro-inflammatory cluster as IL-6 and CRP (Table 1). Levels of PAI-1 correlate with other biomarkers of ageing, including reduced walking speed and lower grip strength [69]. While it has been suggested as a marker of frailty [78], the physiological role of PAI-1, at least in cardiac function, has been debated [79,80]. Consistent with a detrimental role of PAI-1 in ageing, inhibition or deletion of PAI-1 limited senescence in models of accelerated ageing, both *in vitro* and *in vivo* [81–83].

Higher levels of IL-6 and CRP are usually interpreted as indicating a pro-inflammatory state, and in older adults are used as a readout of inflammageing, but this association is not linear with age. CRP, routinely used as the best biomarker of inflammation in the clinic, is also identified to be a good target for frailty diagnosis [84]. The effect of a high inflammatory score on mortality was observed to be greatest at age 65–70, while the link between inflammation and age diminished in subjects  $\geq 80$  years old, in studies of two independent cohorts [75]. Furthermore, increased plasma levels of both IL-6 and CRP in adults over 90 years of age were not associated with increased mortality, when fully adjusted for covariates [85]. It is possible that the relative importance of inflammation might decrease at very old ages when other biological processes, such as stem cell exhaustion, kidney dysfunction, cardiovascular disease, cancer or dementia, may play a greater role in age-related morbidity and mortality. Alternatively, people who have survived beyond 90 years may represent a relatively unusual subset of individuals who are physiologically more robust and less prone to age-related decline – indeed, studies of supercentenarians and their offspring strongly support this view of centenarian genetic exceptionalism [86–89].

In defining a role for inflammatory mediators in age-related morbidity and mortality, it is important to remember that IL-6 can also act in an anti-inflammatory capacity. Thus, an increase in IL-6 does not necessarily mean that there is an increase in pro-inflammatory processes. It has been suggested that levels of both IL-6 and CRP can be moderately increased without being associated with elevated inflammation [90]. Indeed, studies on muscle physiology have shown that on exercise, muscles release into the circulation hormone-like molecules with anti-inflammatory properties called myokines, which include IL-6 [91]. In this context, IL-6 production is independent of NF- $\kappa$ B or TNF- $\alpha$  activation [92] and exerts an anti-inflammatory effect by stimulating the production of IL-1RA and IL-10 [91,93], while production of TNF- $\alpha$  by macrophages is diminished [94]. However, increased NF- $\kappa$ B signalling and down-regulation of NF- $\kappa$ B attenuators is reported in ageing muscle [95],

suggestive of chronic inflammatory signalling. Therefore, a high serum level of IL-6 in healthy young people or in frail older adults may indicate opposite situations of anti- and pro-inflammatory activity, so that IL-6 concentration per se is not informative of inflammatory status without additional contextual information including age, disease and exercise status.

**Table 1. Examples of pro-inflammatory factors**

IL-1 $\beta$	IL-6	CRP	SAA	ICAM	PAI-1	Fibrinogen	Population characteristics	Data processing	Reference
No cluster reported	PCA1 - Correlated with age, mortality, morbidity and age-related diseases						1010 participants (428 men, 582 women), aged 21-96 y	Cluster analysis	
	Positively correlated with	Positively correlated with	---	---	---	---			----
Pro- and anti-inflammatory factors				Evidence					
IL-18		TNF- $\alpha$		Population characteristics			Data processing	Reference	
PCA1 - Correlated with age, mortality, morbidity and age-related diseases				1010 participants (428 men, 582 women), aged 21-96 y			Cluster analysis	[67]	
Positively correlated with age		Absence of correlation with age, mortality and morbidity. Positively correlated with some age-related diseases		General population - InCHIANTI cohort			Individual associations		
Significant independent predictor of 5- and 10-year mortality		Non-predictive of 5-year mortality		7043 participants (2995 men, 4048 women), aged 65-102 y			Fully adjusted correlation with 10-year mortality	[75]	
				General population - Includes CHS and InCHIANTI cohorts					
No cluster reported		"Down"-regulation - Correlated with frailty outcomes		967 participants (416 men, 551 women), aged 65 y and older			Cluster analysis	[68]	
Individually correlated with worsened mobility				General population - InCHIANTI cohort					
NI		TNF- $\alpha$ related - Negatively correlated with knee strength, HPPB score and grip strength; Positively correlated with 400-m walk time		1269 participants, aged 70-79 y			Cluster analysis	[69]	
		Highest $R^2$ of the TNF- $\alpha$ -related component for the HPPB score and knee strength		General non-frail population			Individual associations		
NI		NI		285 participants (67 men, 218 women), aged 90 y and older with a 4-year follow-up			Correlation with 4-year mortality	[85]	
				General population			Fully adjusted		
Local inflammation-endothelial dysfunction		NI		320 participants (236 men, 84 women), aged 58-74 y with a 1-year follow-up			Cluster analysis	[70]	
Independent predictor of 1-year recurrent coronary events				Acute coronary syndrome patients			Individual associations		
Positively correlated with age		Absence of correlation with age		1327 participants (586 men, 741 women), aged 20-102 y			Correlation with age	[72]	
Reduced the size of the regression coefficient for age		NR		General population - Includes InCHIANTI cohort			Adjustment for cardiovascular risk factors and morbidities		
Proinflammation and anti-inflammation - rate		Absence of association with 4-year death rate		580 women aged 31-85 y with a 4.7-year follow-up			Cluster analysis	[71]	
				Clinically referred for coronary angiography					
NI		Negatively correlated with walking and standing balance performance		1020 participants (483 men, 537 women), aged 65-102 y			Correlation with physical performance	[73]	
				SAA		angiography			
No significant association with physical performance	Negatively correlated with global physical performance and hand-grip strength	Negatively correlated with global physical performance and hand-grip strength	NI	NI	NI	NI	1020 participants (483 men, 537 women), aged 65-102 y	Correlation with physical performance	[73]

Other inflammatory mediators also appear to have both pro- and anti-inflammatory activity in different biological contexts. For instance, IL-18 is usually considered to be a pro-inflammatory factor [96,97], but its role in inflammation is not one-sided, and it clusters between pro- and anti-inflammation groups [70,71] (Figure 2, Table 2). IL-18 acts as an anti-inflammatory cytokine in early stage gut inflammation, with pro-resolving properties, while it appears to be pro-inflammatory at later stages [98,99]. Consistent with pro-inflammatory action, IL-18 levels positively correlate with age [67,72] and are a significant independent predictor of 5-year mortality in a longitudinal study [75]. Tumour necrosis factor alpha (TNF- $\alpha$ ) is often considered as a pro-inflammatory marker similar to other 'strong' markers of inflammation such as IL-6. However, it has repeatedly been included in different clusters than IL-6 [68,69,71], indicating a very different pattern of induction. Moreover, while TNF $\alpha$  is associated with frailty [74], its levels do not to correlate with age [67,72].

**Table 2. Examples of factors with both pro- and anti-inflammatory activity**



### 1.2.2 Do anti-inflammatory factors contribute to inflammageing?

Anti-inflammatory factors, by their nature, would be expected to reduce inflammation including inflammageing. This is indeed the case with the potent anti-inflammatory cytokine IL-37 [100,101], which has an unequivocal effect in damping down harmful inflammation (Table 3). For example, transgenic mice expressing human IL-37 showed little age-related increase in pro-inflammatory cytokines, and maintained B cell progenitor function with age [102]. Moreover, IL-37 administration significantly reduced inflammation, restored T cell function and ameliorated vascular, metabolic and motor function in old mice [103–105]. Similarly, levels of the anti-inflammatory cytokine IL-10 [106] negatively correlate with age [67], and high IL-10 was not associated with any detrimental outcomes in physical performance [73]. In fact, IL-10 was included in an anti-inflammation cluster together with HDL cholesterol, and is associated with good prognosis in acute coronary syndrome patients (~67 years old) [70].

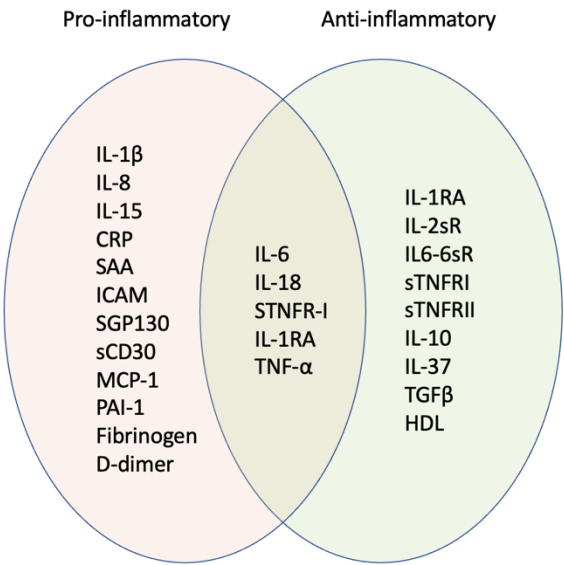
Counterintuitively, however, a number of factors classified as anti-inflammatory appear to correlate with poor physiological function when they increase with age. For instance, soluble TNF receptor I (sTNFR-I) and II act as inhibitors of TNF- $\alpha$  pro-inflammatory signalling by preventing its interaction with membrane TNFR. Hence they would be anticipated to be anti-inflammatory, yet they have a strong positive correlation with increasing age [67], and are associated with a lower physical performance [69]. An index that combined IL-6 and sTNFR-I was the best predictor of 10-year mortality in a longitudinal study of two large cohorts of older adults [75], suggesting that sTNFR-I acts with IL-6 to promote inflammation.

The interleukin-1 receptor antagonist (IL-1RA) blocks IL-1 signalling and has been described as anti-inflammatory. However, levels of IL-1RA positively correlate with age [67,72] and it clusters with IL-6 and CRP [68,73]. In fact, IL-1RA is a significant predictor of mortality in older adults independent of inflammatory exposure or genotype [75,85]. Two other soluble interleukin receptors, IL-2 soluble receptor (IL-2sR) and IL-6sR, have been included in the same cluster as TNF- $\alpha$  and sTNFRs, and are associated with poor physical performance [69]. However, in another study, IL-6sR was not associated with negative performance [73] and was not correlated with age in men [67], though this may not be the case in women [72]. Significant sex-specific differences in inflammatory cytokine levels in response to infection have been described in patients with COVID-19 [107], highlighting the importance of accounting for biological sex when interpreting cohort findings on inflammatory markers and inflammageing. The influence of biological sex on inflammatory responses may in part be due to selective X inactivation in women (the single X chromosome in men is constitutively active). De-repression of previously silenced immune loci on the X chromosome with age may account for the increase in pro-inflammatory autoimmune conditions and X-skewing in older women [108].

The association between multiple anti-inflammatory factors and age-related disease does not necessarily mean that these molecules are detrimental and cause inflammageing. Instead, their elevation may reflect an adaptive physiological response to pro-inflammatory stimuli, to try to damp-down the inflammation. Where high levels of anti-inflammatory factors are

associated with poor outcomes, it could be that the adaptation failed to reduce inflammation below a damaging threshold. Thus, it has been suggested that the balance between IL-1RA and IL-1 may be more important than IL-1RA levels alone in determining inflammatory outcomes [85,109,110]. Despite this, some studies have shown that plasma IL-1 $\beta$  was not associated with poor outcomes [68,73] and did not correlate with age [67,72], making IL-1RA a better marker of inflammation than IL-1 $\beta$ . Transforming growth factor  $\beta$  (TGF- $\beta$ ) [106] shows similar regulation to IL-1 $\beta$  in older adults [68] and levels did not correlate with age [67,72].

Despite the lack of consistency in the markers analysed between different studies, a conserved pattern can be identified in which a few inflammatory mediators appear key to an ageing signature of inflammation *i.e.* inflammageing. Based on this, we suggest that determination of inflammageing needs more than simple measures of the level of individual cytokines, and that instead a full range of inflammatory mediators (pro- and anti-inflammatory) should be analysed, as well as the context in which high levels are seen, such as tissue, disease state, biological sex and chronological age. Drawing together the studies discussed above, we identify a possible ‘ageing signature’ of inflammatory mediators (Figure 2, Table 1).



**Figure 2. Pro-inflammatory signature of inflammageing.** Orange oval shows factors most strongly associated with age-associated inflammation. Note that factors that overlap between pro- (orange) and anti-inflammatory activity (green) may be useful factors in that signature. Some cytokines usually considered as pro-inflammatory can have anti-inflammatory activities *e.g.* IL-6 produced as a cytokine through NF $\kappa$ B signalling pathways is pro-inflammatory, while IL-6 produced as myokine following exercise is anti-inflammatory. Some factors may be anti-inflammatory in children and young adults but pro-inflammatory in later life; biological sex is also important in determining whether the factors act in a pro- or anti-inflammatory manner (see text for details).

IL-1RA	IL-2sR	IL-6sR	sTNFRI	sTNFRII	IL-10	TGF- $\beta$	HDL cholesterol	Population characteristics	Data processing	Reference
PCA1 - Correlated with age, mortality, morbidity and age-related diseases	NI	No cluster reported	PCA1 - Correlated with age, mortality, morbidity and age-related diseases		No cluster reported	No cluster reported	NI	1010 participants (428 men, 582 women), aged 21-96 y	Cluster analysis	[67]
Positively correlated with age		Absence of correlation with age	Positively correlated with age, mortality, morbidity and some age-related disease	Positively correlated with age	Negatively correlated with age	Absence of correlation with age		General population - InCHIANTI cohort	Individual associations	
Significant independent predictor of 5- and 10-year mortality	NI	Non-predictive of 5-year mortality	Significant independent predictor of 5- and 10-year mortality	Non-predictive of 5-year mortality	Non-predictive of 5-year mortality	NI	NI	7043 participants (2995 men, 4048 women), aged 65-102 y	Fully adjusted correlation with 10-year mortality	[75]
			Best predictor of 10-year mortality in combination with IL-6					General population - Includes CHS and InCHIANTI cohorts		
"Up"-regulation - Positively correlated with worsened mobility and frailty risk	NI	NI	NI	NI	NI	IL-1/TGF - No adverse outcome reported	NI	967 participants (416 men, 551 women), aged 65 y and older	Cluster analysis	[68]
								General population - InCHIANTI cohort		
NI	TNF- $\alpha$ related - Negatively correlated with knee strength, HPPB score and grip strength; Positively correlated with 400-m walk time					NI	NI	1269 participants, aged 70-79 y	Cluster analysis	[69]
	Highest $R^2$ of the TNF- $\alpha$ related component for walking speed	Highest $R^2$ of the TNF- $\alpha$ related component for grip strength	Highest $R^2$ of the TNF- $\alpha$ related component for the HPPB score and 400-m walk time					General non-frail population	Individual associations	
Positively associated with 4-year mortality	NI	NI	NI	NI	NI	NI	NI	285 participants (67 men, 218 women), aged 90 y and older with a 4-year follow-up	Correlation with 4-year mortality	[85]
Strong independent predictor of 4-year mortality								General population	Fully adjusted	
alone or in combination with CRP										
NI	NI	NI	NI	NI	Protective or anti-inflammation - Negative predictor of adverse cardiac events	NI	Protective or anti-inflammation - Negative predictor of adverse cardiac events	320 participants (236 men, 84 women), aged 58-74 y with a 1-year follow-up	Cluster analysis	[70]
					Significant negative predictor of 1-year adverse cardiac events		Significant negative predictor of 1-year adverse cardiac events	Acute coronary syndrome patients	Individual associations	
Positively correlated with age	NI	Positively correlated with age in women only	NI	NI	NI	Absence of correlation with age	NI	1327 participants (586 men, 741 women), aged 20-102 y	Correlation with age	[72]
Removed the effect of age		Increased the size of the regression coefficient for age						General population - Includes InCHIANTI cohort	Adjustment for cardiovascular risk factors and morbidities	
NI	NI	NI	NI	NI	NI	Immunosuppressive - Absence of association with 4-year death rate	NI	580 women aged 31-85 y with a 4.7-year follow-up	Cluster analysis	[71]
								Clinically referred for coronary angiography		
Negatively correlated with global physical performance but not with hand-grip strength	NI	No significant association with physical performance	NI	NI	No significant association with physical performance	NI	NI	1020 participants (483 men, 537 women), aged 65-102 y	Correlation with physical performance	[73]

**Table 3. Anti-inflammatory factors.** Note some of these are elevated with age and poor outcomes which may reflect acquisition of pro-inflammatory characteristics or an unsuccessful adaptive response in an attempt to quell elevated inflammation driven by pro-inflammatory factors (Tables 1 and 2).



### **1.2.3 Inflammageing is linked to blood clotting – an example of inflammation driving morbidity in older adults**

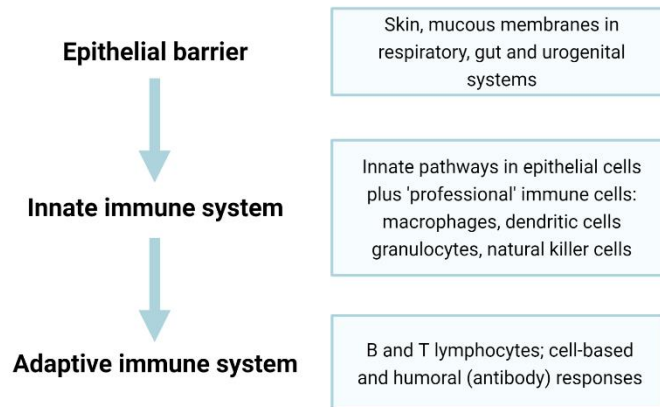
While acute inflammation is an evolved protective response, chronic inflammation, as seen in inflammageing, has detrimental consequences across multiple tissue and organ systems. In this context, thromboembolic events provide an excellent example of how inflammation can cause age-related disease and increase the risk of death. Changes in blood viscosity and elevated clotting risk are closely related to inflammation in older age [111], with direct mechanistic links suggesting that inflammation may trigger clotting [112]. Venous thrombosis is rare in young adults (1 in 10,000 per year) rising to an incidence of 1 in 100 in the elderly. While major risk factors include malignancy and other comorbidities [113], age-related inflammation may also play a part. In one study, fibrinogen clustered with CRP in the “systemic inflammation” group [70]; it has also been shown to correlate with age [72]. High serum levels of coagulation factors such as D-dimer (a fibrin degradation product) are associated with functional decline and increased mortality in older persons [114,115]. This is relevant not only in ‘normal’ ageing, but also in infectious diseases in the elderly – increased plasma viscosity resulting from high fibrin levels with extremely elevated D-dimer measurements is associated with markedly increased inflammation and risk of cardiovascular disease and thrombotic events in older adults with COVID-19 [58,116]. As well as fibrinogen, other coagulation factors such as von Willebrand factor, prothrombin activation peptides, factor VII, VIII, IX or X and thrombin-antithrombin complexes are modulated with age and associated with a state of hypercoagulability in elderly subjects [117–119]. It is possible that inflammageing and hypercoagulability share a common pathway with senescence that involves insulin-like growth factor binding protein-5 (IGFBP-5) [120].

## **2. Immunosenescence affects many components of the immune system**

Immunosenescence, the ageing of the immune system, is closely related to inflammageing. With time, the immune system becomes less responsive to pathogens, and vaccine efficacy is also often reduced with age [121]. The ability to initiate acute inflammation has survival benefit, and a lack of responsiveness to acute inflammation is associated with higher blood CRP levels and increased mortality risk [122]. This failure results in increased susceptibility to various infectious diseases, including common infections that are relatively harmless in healthy adults (for example, influenza infections do not markedly differ between old and young people, but deaths from influenza are greatly elevated in the elderly [123–125]). Immunosenescence and failure of protective innate responses may also contribute to the high mortality of older adults on SARS-CoV2 infection [126]. Below, we discuss dysfunction of

various components of the immune system, including barriers, innate immune cells and the adaptive immune system (Figure 3) in terms of ageing and senescence.

**Figure 3:** Schematic overview of the three major components of the immune system – barriers, innate and adaptive immunity (see text for further details).



The epithelium forms the primary barrier against pathogen infection. Secretions such as mucus, saliva, or tears present a second barrier that remove pathogens *via* mechanical and enzymatic actions [127–129]. Epithelial barriers gradually lose integrity with age, directly through senescence of the epithelial cells themselves, and possibly also through disorganised tight junction proteins leading to gaps between cells through which pathogens can pass. This is particularly relevant in the gastrointestinal (GI) tract, where gut leakiness can result in exposure of tissues to bacteria bearing the potent PAMP, lipopolysaccharide (LPS) [130,131]. In addition, changes in the integrity of the blood-brain barrier may permit entry either of pathogens (*e.g.* gum disease bacteria have been mooted as candidates in inflammation and dementia [132–134]) or of immune cells [135–138]. Age-associated decline in secretion of mucous and tears through changes to aquaporins [139], as well as decreased cilia function [140], may lead to increased pathogen dwell times in target tissues, increasing the risk of infection. It is of note here that older adults are at much higher risk of community-acquired pneumonia than younger adults, potentially through diminished capacity to capture and expel pathogen from the respiratory epithelium [141,142].

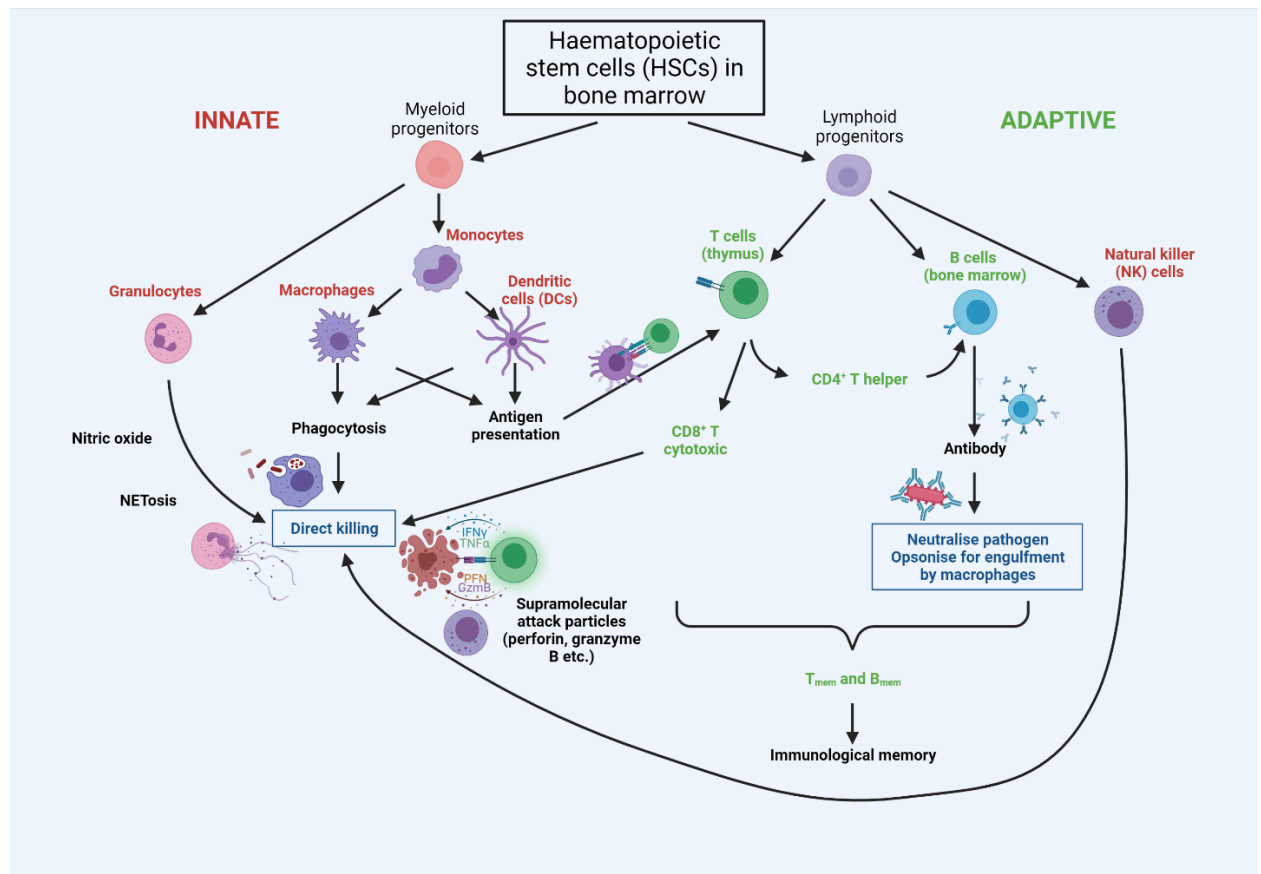
Innate immunity is the oldest and most conserved aspect of immunity in animals, although its complexity varies greatly between species [143]. The innate response represents a rapid but relatively non-specific reaction to infection or injury. Innate immune signalling is first triggered by binding of conserved PAMPs or DAMPs [144,145] to pattern-recognition receptors (PRRs) of 'professional' innate immune cells [144,145]. Many other cell types, including epithelial cells and fibroblasts, also possess PRRs and can initiate innate immune responses.

The PRRs fall in to several distinct classes including: toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), C-type lectin receptors (CLRs), as well as cytosolic DNA sensors such as cyclic GMP-AMP synthase (cGAS) or absent in melanoma 2 (AIM2) [146–150]. In addition,

RAGE may act as a PRR with a specific and important role in inflammageing [50,151]. Signalling through these pathways activates a cascade of pro-inflammatory responses mediated through key nodes, including the inflammasome and transcription factor NF $\kappa$ B (see below). Accumulation of DAMPs and PAMPs with age will therefore induce a systemic pro-inflammatory response. Notably, senescent cells produce a number of DAMPS including HMGB1 and cytosolic DNA from damaged mitochondria DNA, cytoplasmic chromatin fragments [18,152], and LINE1 transposon reactivation [153], suggesting that accumulation of senescent cells with age may stimulate an ongoing non-resolved innate inflammatory response. Indeed, lack of clearance or excess production of molecular “garbage” has led to the “Garb-aging” theory [154].

Following breaching of epithelial barriers by a pathogen, in mammals, the complement system is activated and can provoke the lysis of targeted pathogens. Complement activation is accompanied by cellular responses involving tissue-resident immature dendritic cells (DCs), mast cells, granulocytes (encompassing basophils, eosinophils and neutrophils), natural killer cells (NKs) and macrophages [155]. Some cells have a role in both innate and adaptive immunity, including cytotoxic  $\gamma\delta$  T lymphocytes and NKT cells [155] (see Figure 4). The contribution of specific innate immune cell types and their progenitors to inflammageing is discussed further below.

A failure of any component of these early innate defences with age is likely to both increase susceptibility to infectious disease, as well as elevate the risk of chronic inflammation and subsequent morbidity. Furthermore, failure of resolution of the initial inflammatory trigger leads to chronic inflammation with tissue injury (reviewed in [156]).



**Figure 4: Cross-talk between cells of the innate and adaptive immune systems.** Innate cells are labelled in red, with adaptive in green. Both innate and adaptive arms arise from haematopoietic stem cells (HSCs) in the bone marrow. The first line of defence is provided by the rapidly-acting PAMP/DAMP-sensing innate system, with innate cells presenting antigen to adaptive cells; this stimulates rapid proliferation of adaptive cells bearing receptors cognate for the antigen. T cells are 'educated' in the thymus to eliminate cells that recognise 'self' so that the immune system is self-tolerant. Breakage of this tolerance leads to autoimmunity, where the immune system attacks specific molecules or cells of the body as if they were infectious pathogens. Notably autoimmunity rises significantly with increasing age.

### 3. Dysregulation of innate immunity on ageing

#### 3.1 Ageing of haematopoietic stem cells leads to myeloid skewing

All professional innate and adaptive immune cells originally arise from haematopoietic stem cells (HSCs) in the bone marrow (Figure 4). With increasing age, HSCs become less quiescent [157] and even though they increase in number [158,159], they exhibit a functional decline in regenerative potential [158,160,161], possibly through telomere-driven replicative senescence. The capacity of aged transplanted HSCs to home to the bone marrow is also greatly decreased compared with young HSCs [160,162], suggesting intrinsic impairment of aged HSCs. By contrast, mobilization of hematopoietic stem and progenitor cells from the bone marrow to peripheral blood was greater in old versus young mice, possibly as a consequence of reduced adhesion to stroma [163]. These defects might arise from accumulation of DNA damage, epigenetic changes, increase ROS production, altered proteostasis (all features of cell senescence) and/or changes in cell polarization [164–166].

The impact of extrinsic factors on HSCs is still not well understood [164], although increased inflammation may negatively impact on HSC function [158,167,168]. Age-related accumulation of senescent cells in the bone marrow (including mesenchymal stem cells as well as bone macrophages and long-lived osteocytes) may contribute, through the SASP, to a pro-inflammatory microenvironment which favours differentiation of HSCs towards myeloid progenitors [169] at the expense of lymphoid progenitors (adaptive immune B, T cells and NK cells) [157,158] (see Figure 3). Chronic exposure of HSCs to other inflammation-promoting molecules may be important in driving this bias, as elevated chemokine (C-C motif) ligand 5 (CCL5) is thought to promote myeloid skewing [167,170]. Inflammation can arise through many routes, but one driver which is of particular interest in ageing is the presence of bacterial LPS in tissues through loss of barrier function (see above); whether this has a direct impact on HSC differentiation is not yet clear. Overall, myeloid skewing predisposes immune responses towards innate processes, which are generally pro-inflammatory, and is likely to reduce adaptive immune clearance of infection, leading to prolonged inflammation.

### 3.2 Ageing in innate immune effector cells

#### 3.2.1 Dendritic cells

Dendritic cells are major antigen presenting cells (APCs) that display processed antigen in conjunction with MHCII to activate helper T cells [171,172]. In older adults, DCs show diminished function, including impaired migration and phagocytosis, increased production of TNF- $\alpha$  and IL-6, and decreased production of IL-10 upon stimulation [173–175]. (Note however, that assessment of cytokine release may depend on whether or not DCs are expanded *in vitro* prior to activation [176]). Decreased phagocytosis by ageing DCs leads to decreased antigen presentation and hence reduced T and B cell priming [176–178]. This is relevant both in the context of age-associated autoimmunity (since self-tolerance relies on elimination of self-reactive T cells) [179], and in age-related susceptibility to infectious diseases. Whether the numbers of plasmacytoid and myeloid DC are altered with ageing is less clear, as some reports find a decrease in both cell types [180], others in only one cell type [181,182], while still others report no decrease at all [173]. Overall, however, it is clear that DC function decreases with age and this contributes to poor immunity in the elderly.

#### 3.2.2 Granulocytes

Neutrophils serve important roles in direct pathogen killing, and migrate rapidly to sites of infection in response to inflammatory cytokines. The numbers of neutrophils appears to stay constant over the life course [183,184]. While their chemokinesis (*i.e.* speed of movement) appears to be unaltered with ageing, importantly, their phagocytic capacity and directional movement towards a chemical stimulus (*i.e.* chemotaxis) both diminish with age [184–186]. This poor directionality results in additional damage to tissues through the action of tissue-degrading neutrophil elastases as well as local neutrophil-mediated inflammation [187–190]. Neutrophils produce bursts of reactive oxygen species (ROS) which can directly kill pathogens, but this intense acute ROS production is significantly lower in neutrophils from older adults compared with those from young donors [191–193]. However, neutrophils from older adults instead show higher basal ROS levels, which could indirectly contribute to inflammageing and reduce effective response to pathogens [192,194]. Moreover, the



propensity of neutrophils to extrude their genomic DNA to trap pathogens in a process known as NETosis – another pathogen killing strategy – also shows an age-related decline [195]. Hence age-related alterations in neutrophil function delay pathogen clearance and wound healing [196], and increase local inflammation.

Signalling pathways by which neutrophils respond to cytokines appear to be altered with ageing. For example, responses to granulocyte–macrophage colony-stimulating factor (GM-CSF) diminish with age [197,198]. Whether aged neutrophils exist in a state of hyperactivation is more moot – reports of decreased expression of CD50 and CD62L (L-selectin), increased spontaneous ROS production and upregulation of pro-inflammatory pathways all suggest hyperactivity of old neutrophils [192,199,200], in contrast with other studies reporting little difference in hyperactivity markers between neutrophils from young and old donors [191].

Since aged neutrophils can still sense inflammatory cytokines, but fail to deal appropriately with pathogens, it is likely they are defective in signal transduction pathways downstream of ligand binding to receptor [195,201]. Consistent with this, mobilization of the second messenger calcium [184,202], as well as polymerization of actin are reduced in neutrophils from older adults [203,204]. Cell motility and phagocytosis both require a dynamic actin cytoskeleton, so signalling *via* the small G-protein Cdc42 through to actin is likely to be altered in old neutrophils. Indeed, treatment of aged neutrophils with statins, which act on Cdc42 (in addition to their role in cholesterol regulation), improves neutrophil chemotaxis and reduces NETosis [142,195]. Cdc42 inhibition may also be beneficial in reducing inflammation from other sources such as senescent vascular endothelium [205].

Compared with the wealth of data on neutrophils, the effect of ageing on eosinophils and basophils has been much less studied [206]. The data for basophils appear contradictory according to the species studied. For example, in mice, spleen basophils, but not bone marrow basophils, increase in number during ageing [207], whereas in macaques, daily basophil production decreases with age but their half-life remains stable [208]. At least one study has suggested a reduction in basophil numbers in the blood from healthy older humans [209], but basophil degranulation in response to allergens does not appear to change with age [210]. By contrast, eosinophil degranulation upon stimulation, and possibly superoxide anion production, were negatively affected by age but adhesion and chemotaxis were conserved [211]. Some reports indicated a deficit of eosinophils at infection sites with age, suggesting a potential alteration in chemokinesis [212,213]. It is important that this gap in knowledge is addressed – basophils may be important in Th2-mediated responses to allergens and multicellular pathogens [214], as well as regulating autoimmunity, yet whether diminished basophil function is involved in age-related autoimmunity is not currently known.

### 3.2.3 Natural killer cells

Natural killer (NK) cells bridge the innate and adaptive immune system – they derive from lymphoid lineages but act as innate effector cells (Figure 4), directly killing infected host cells as well as tumour cells expressing neoantigens. There is some conflict in the literature concerning how NKs change with age that may reflect species-specific differences - levels and inflammatory responses of classical NK T cells have been reported to increase with age in mice [215,216] though decrease in older humans [217–221]. When considering functional NK cells, levels of immature NKs, which express high levels of surface CD56 (CD56<sup>bright</sup>) diminish with age [222,223], while mature NK cells, with low CD56 (CD56<sup>dim</sup>), increase [222–224]. Even

in cases where overall NK levels increase with age, there is likely to be reduced functionality since cytotoxicity per cell (e.g. lower perforin release) decreases [225–229].

NKs are activated by IL-2, which is produced by helper T cells (Th1). Interestingly, IL-2 levels decrease with age or with increasing frailty [230–232], which is likely to have knock-on effects on NK activation (as well as on CD8<sup>+</sup> T cell function – see later). Furthermore, the response of NKs to IL-2 is diminished with age, as observed by reduced proliferation, Ca<sup>2+</sup> mobilization and expression of CD69 (an early activation marker) [217,233]. However, non-stimulated NK cells show elevated CD69 expression with age, suggesting higher basal activation levels but decreased responsiveness to activation signals [226]. Whether production of immune modulators by NK cells changes with age is more contradictory, with some reports demonstrating stable TNF- $\alpha$  and IFN- $\gamma$  induction [217,226] whereas others report age-associated decreases in production of IFN- $\gamma$ , IL-8 and macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ) by NKs [125]. NK cells play an important role in clearing senescent cells [21,25,34], especially senescent tumour cells (reviewed in [23]) so reduced NK activity with age may contribute to the accumulation of senescent cells.

### 3.2.4 Macrophages

Macrophages are at the centre of both the initiation and resolution of inflammatory events. Initiation is characterized by the secretion of pro-inflammatory cytokines (IL-6, IL-1 $\beta$ , TNF- $\alpha$ ), reactive oxygen species (ROS), nitric oxide, and neutrophil chemokines. Resolution involves pathogen removal, downregulation of neutrophil chemokines and the removal of apoptotic neutrophils (efferocytosis) which tilts equilibria towards an anti-inflammatory state [234,235]. Macrophages also produce specialized pro-resolving mediators (SPMs), a class of molecules including resolvins, maresins, protectins and lipoxins derived from some poly-unsaturated fatty acids (such as eicosapentaenoic acid or docosahexaenoic acid), and have recently been described as potent factors able to resolve both innate and adaptive pro-inflammatory events [236–240]. By contrast, molecules derived from arachidonic acid (apart from lipoxins) are predominantly pro-inflammatory [240]. A correct balance of these modulators is likely to be important in ensuring appropriate activation and resolution of inflammation [241–244], so their dysregulation could lead to inflammageing and tissue ageing. While one small study showed a profound reduction of lipoxin A<sub>4</sub> levels in urine samples from elderly people [245], to date, there has been little research on the role of SPMs in human ageing *per se*, though they have been linked to age-related diseases [246]. Aged mice have been reported to show delayed resolution of acute inflammation [247] which may be linked to lower levels of SPMs [248–250]. Low SPM levels in older mice can be overcome by exogenous SPM delivery, which reduced inflammation and accelerated the resolution of peritonitis [247]. Mechanistically, it appears that decreased SPM levels may result from age-associated promoter methylation which reduces gene expression. For example, *Elovl2*, a gene involved in SPM precursor synthesis, is progressively hypermethylated with age, while reversal of *Elovl2* promoter hypermethylation in the retina of ageing mice rescued age-related decline in visual function [251]. It has been suggested that levels of ELOVL2 may therefore serve as a sensitive, non-tissue-specific biomarker of ageing [252–254].

With a half-life of several weeks [255], macrophages are much longer lived than the majority of innate immune cells, whose half-life rarely exceeds a few days *in vivo* [208,256,257]. Macrophages therefore survive long enough to accumulate molecular damage associated with senescence. This has the potential for significant pathological effects, since senescence-

associated changes in lysosomal pH [258] may prevent macrophages from breaking down engulfed pathogens or cell debris. For example, high lysosomal pH in microglial cells, which serve as central nervous system macrophages, makes them unable to degrade A $\beta$  amyloid peptides [259], leading to amyloid deposition and CNS inflammation associated with Alzheimer's disease [260].

As well as serving key roles in inflammation and stress responses, macrophages can also be considered to be at the centre of inflammageing, since multiple stressors increasingly stimulate macrophages over time [261]. While overall numbers of monocytes and macrophages do not change with age [262], the most pro-inflammatory subsets of monocytes increase with age thereby contributing to inflammageing [263], and show dysregulation of pro-inflammatory and phagocytic functions [64,263,264]. Macrophage phagocytosis appears to be maintained with age, except in some tissues such as the peritoneum, suggesting that the microenvironment can influence macrophage function [265–267]. Macrophages from old mice show diminished cytokine release, which may be due in part to negative feedback responses to an already elevated inflammatory environment in aged mice [268–273], since reduction in global inflammation (in an *Il-6*<sup>-/-</sup> genetic background), resulted in macrophages from old mice exhibiting a stronger inflammatory response than those from younger mice [268]. This suggests that the high senescent cell burden in old age, with associated SASP and chronic inflammageing, may block appropriate activation of professional innate immune cells following exposure to pathogens, which could account for the increased risk from infectious diseases with increasing age.

### 3.3 The inflammasome drives pyroptosis and further inflammation

Innate responses to infection, tissue damage or certain environmental triggers (*e.g.* asbestos) can lead to a specialised form of lytic cell death that is highly inflammatory, termed pyroptosis [274,275]. It is triggered through activation of the inflammasome, a cytosolic multiprotein complex which responds *via* Toll-like, Nod-like and AIM-like receptors (TLRs, NLRs and ALRs) to microbial and cellular damage (PAMPs and DAMPs) [276], as well as extracellular ATP and potassium efflux from the cell. The inflammasome forms a scaffold which activates caspase-1 (caspase-4, 5 or 11 can also be involved) [277,278], leading to cleavage of gasdermin D and precursors such as pro-IL-1 $\beta$ , pro-IL-18 [146] into active IL-1 $\beta$  and IL-18 [275,278,279], as well as formation of pore-like components that are responsible for membrane destruction and pyroptotic death. The inflammasome has been implicated in a number of metabolic and age-related diseases, including diabetes, autoimmunity, Parkinson's and Alzheimer's disease (reviewed in [280]).

The NLR, pyrin domain containing 3 (NLRP3) inflammasome is the most studied inflammasome and is particularly important in the link between inflammation and ageing. NLRP3 has been implicated in the systemic inflammation that characterizes metabolic diseases, while its deletion significantly limits this inflammatory phenotype [281,282]. In the context of ageing and inflammageing, NLRP3 is activated by age-related DAMPs, such as extracellular ATP, urate, ceramides or palmitate. Cytokines such as TNF- $\alpha$ , levels of which increase with age in mice, are also able to prime the NLRP3 inflammasome [283]. Importantly, mitochondrial dysfunction can trigger NLRP3 inflammation, since damaged mitochondria release cardiolipin and oxidized mitochondrial DNA (mtDNA) that are recognized as DAMPs and promote NLRP3 priming [284,285]. Notably, NLRP3 deletion reduced inflammatory

innate immune activation in the ageing central nervous system, and ameliorated age-related decline in cognitive function, strongly suggesting that excess CNS inflammation can promote cognitive decline, a common feature of ageing [286]. Such effects were not confined to the CNS as metabolic function, motor function and bone structure were also better preserved upon NLRP3 deletion [286], and age-related thymic involution and reduction in naïve T cells pools were significantly prevented [286,287]. Moreover, in a mouse model of age-related clonal haematopoiesis of indeterminate potential (CHIP), detrimental phenotypes of increased clonality and atherosclerosis, together with overexpression of NLRP3 and IL-1 $\beta$ , were largely prevented by the use of an NLRP3 inhibitor [288]. Importantly, inflammasome components caspase-1 and ASC were found to increase with age in mice and in humans, including in a longitudinal study of cells from the same human donor, with susceptibility to oxidative stress being ameliorated by treatment with an inflammasome inhibitor [289]. This finding of increased ASC is critical for propagation of inflammageing, as ASC-mediated specks can be phagocytosed from the extracellular space by macrophages, triggering lysosomal damage and further inflammation [290]. While direct studies on ageing are limited, it is becoming increasingly clear that the inflammasome is intrinsically linked to inflammageing and age-related diseases.

### 3.4 Epigenetic changes provide innate immune cells with memory

Although innate immunity has long been considered as non-specific and to lack memory, it now appears that it is able to adapt and retain some form of memory, termed “trained immunity”, which appears to rely upon epigenetic changes [291–294] including patterns of CpG methylation, post-translational modification of histone tails (the ‘histone code’) and longer-range chromatin interactions involving looping into transcriptional activation domains [295]. Such epigenetic remodelling is thought to lead to a quicker and stronger immune response on re-exposure to the same PAMP or DAMP. Trained monocytes, key contributors to innate immunity, show increased H3K4me3 [291], which has been associated with upregulated production of pro-inflammatory cytokines [296]. It is therefore tempting to suggest that trained immunity, while beneficial in the short term, may also produce long-term effects favouring a sustained increase in inflammatory status. Indeed, innate immune microglial training increased Alzheimer disease (AD) features including neuronal loss and cognitive decline in a streptozoin-induced AD model in mice, which was further exacerbated by peripheral LPS-induced inflammation. Notably, inhibition of the NLRP3 inflammasome protected against microglial training in this model and reduced neurological pathologies [297].

Epigenetic information is plastic, reflecting environmental and endogenous events over the cellular and organismal lifespan (the ‘exposome’) [298–300], though it can also be inherited trans-generationally [301]. Major alterations in epigenetic information occur during cell senescence, including global loss of heterochromatin [302,303], reduced levels of histone trimethylation H3K9me3 [304,305] and hypomethylation at most CpG sites in DNA [152,306], with hypermethylation on a few CpGs. The methylation status of just 353 CpG sites can be used as an ageing ‘clock’ to determine both chronological and physiological age [16,17]. Such methylation patterns can be used to identify individuals at risk of poor ageing outcomes and even predict all-cause mortality [307]. By contrast, people who show ‘successful ageing’ *i.e.* without overt morbidity at late chronological age, such as super-centenarians and their offspring, show a lower epigenetic age than expected [87].

Innate immune cells are not exempt from these senescence- and age-related epigenetic alterations, including shifts in patterns of methylation that lead to expression of genes that should be silenced, and silencing of genes that should be expressed. For example, age-related demethylation of the *TNF- $\alpha$*  promoter is associated with higher expression of *TNF- $\alpha$*  in human leucocytes, monocyte-derived macrophages and PBMCs [308,309]. Similarly demethylation of the *CXCL10* (C-X-C motif chemokine 10) promoter in lymphocytes and neutrophils also leads to its upregulation [310]. By contrast, age-related hypermethylation of *ATG5* and *LC3B* promoters in macrophages is associated with decreased autophagy, contributing to chronic inflammation [311,312]. Taken together, these studies suggest that epigenetic changes associated with ageing and with senescence may contribute to inflammageing through increasing pro-inflammatory gene expression and repressing anti-inflammatory factors.

#### 4. Dysregulation of adaptive immunity during ageing

Adaptive immunity is activated subsequent to innate immunity and when the innate response to the pathogen is insufficient to clear infection. It is a slower process, taking days or even weeks compared with the very rapid minutes to hours for the innate response, but is much more specific to particular antigens. The key players are the B and T lymphocytes, which are activated upon MHCII-mediated antigen presentation by professional APCs (usually dendritic cells) through the immunological synapse, together with type I interferon and cytokine signalling. Common lymphoid progenitors (CLPs) in the bone marrow produce small lymphocytes that will become B cells if processed in the bone marrow [313] or T cells if processed in the thymus [314]. T cells carry out cell-mediated responses [315–317], while B cells predominantly produce antibodies in the humoral response. Lymphocytes can differentiate into multiple different types, designated by the presence of surface markers. In this discussion of immunosenescence, we consider the major classes of CD4<sup>+</sup> helpers and CD8<sup>+</sup> cytotoxic, with regulator, effector and memory sub-classes, together with antibody-secreting plasma B cells and memory B cells.

##### 4.1 Decreased B and T cell responses with ageing

###### 4.1.1 B cells

B cells express unique immunoglobulin B-cell receptors (BCRs) on their surface, derived through V(D)J recombination and somatic hypermutation of the immunoglobulin Ig genes. Upon binding to antigen, presented by DCs or other professional APCs, and stimulation by T helper cells, they undergo clonal proliferation resulting in millions of identical plasma cell clones [318,319]; following Ig class switching, they secrete antibody specific to the target antigen [318]. Circulating Ig can neutralize pathogens displaying the cognate antigen, which can then be lysed by the complement system. Antibodies can also opsonise the pathogen to enhance phagocytosis by macrophages [320–322]. Some long-lived B cells will remain as quiescent memory cells after the initial response has been completed; these memory cells can rapidly respond on subsequent presentation of the same antigen [318].

An overall decrease in naïve B cells, including pro-, pre- and immature B cells, is seen in older adults [323]. This is a consequence both of B cell-intrinsic ageing [324,325], as well as age-



related defects in the bone marrow and lymph nodes [326]. Existing memory B cells persist but further  $B_{\text{mem}}$  differentiation is impaired in older adults [327], because of low expression of Blimp-1, a master regulator of plasma cell differentiation. An atypical subset of non-dividing B cells, termed age-associated B cells, accumulates with age [328,329]. They are able to present antigens, to secrete cytokines and antibodies and are responsive to innate receptor stimulation but are unresponsive to BCR stimulation [328,330]. Their presence is highly dependent on inflammatory processes, notably the stimulation of TLRs [329] which favours pro-inflammatory innate immune responses [330]. In addition, they are implicated in production of self-reactive antibodies important in the emergence of autoimmunity [329,330]. Indeed, the selective destruction of these cells in a murine model significantly diminished autoantibody production [329]. B cell ageing therefore not only accounts for decreased antibody responses to new pathogens but also contributes to increasing prevalence of autoimmunity with age.

#### 4.1.2 T cells

T cells express the T-cell receptor (TCR) which, like the BCR, has high specificity for individual antigens.  $CD4^+$  T cells can differentiate into helper (Th1 and Th2) and regulatory cells which control the activity of both B and T cells through secretion of pro- or anti-inflammatory cytokines [331]. Upon presentation of cognate antigen by mature DCs or B cells,  $CD8^+$  T cells will differentiate into cytotoxic cells capable of destroying infected or tumour cells expressing that antigen. Some T cells can end up as memory T cells by becoming quiescent [332]. The switch between effector ( $T_{\text{eff}}$ ) and memory ( $T_{\text{mem}}$ ) cells involves metabolic changes mediated in part by the kinase mTOR [333]. This is of significant interest given the finding of improved T and B cell responses to influenza vaccination and reduced incidence of respiratory tract infections in older adults treated with mTOR inhibitors [334–336]. While such drugs are immune suppressive at high doses and used clinically to prevent rejection of transplanted organs, at low doses, they are immune protective [337].

Ageing of T cell populations starts with thymic involution in early adolescence, with increasing adiposity leading to loss of most thymus function by adulthood; it has been suggested that involution may result from either reduced production of haematopoietic stem cell progenitors by the bone marrow [338,339] or defects of the stromal niches of the thymus or bone marrow [340–342]. Thymic involution is not simply an adaptive feature but instead it contributes to both immunosenescence and inflammageing [343]. The resulting reduction in thymic output of functional naïve T cells with age greatly reduces the TCR repertoire [344–346]. Indeed, some studies suggest that over two thirds of all T cells in older adults are CMV-reactive, greatly reducing the antigenic repertoire (reviewed in [347]). This narrowing of the repertoire diminishes the ability to respond to new pathogens [348], leading to poor vaccine responses [121] and elevated cancer risk [349]. Unlike mice, in human adults, the thymus does not make a significant contribution to naïve T cell production [350]. With age, naïve regulatory T cells ( $T_{\text{reg}}$ ) also decrease, while memory regulatory T cells increase, Th1 and Th2 are maintained, and the  $T_{\text{reg}}/T_{\text{eff}}$  ratio is increased [351]. Even though total numbers of pro-inflammatory Th17 decrease in ageing mice, active Th17 cells can accumulate locally in the ageing prostate and spleen, resulting in an increased Th17/ $T_{\text{reg}}$  ratio [352]. This increase in Th17 activity was also found in older humans and was the greatest contributor to their inflammatory status [353,354]. Failure to eliminate self-reactive T cells, potentially because of

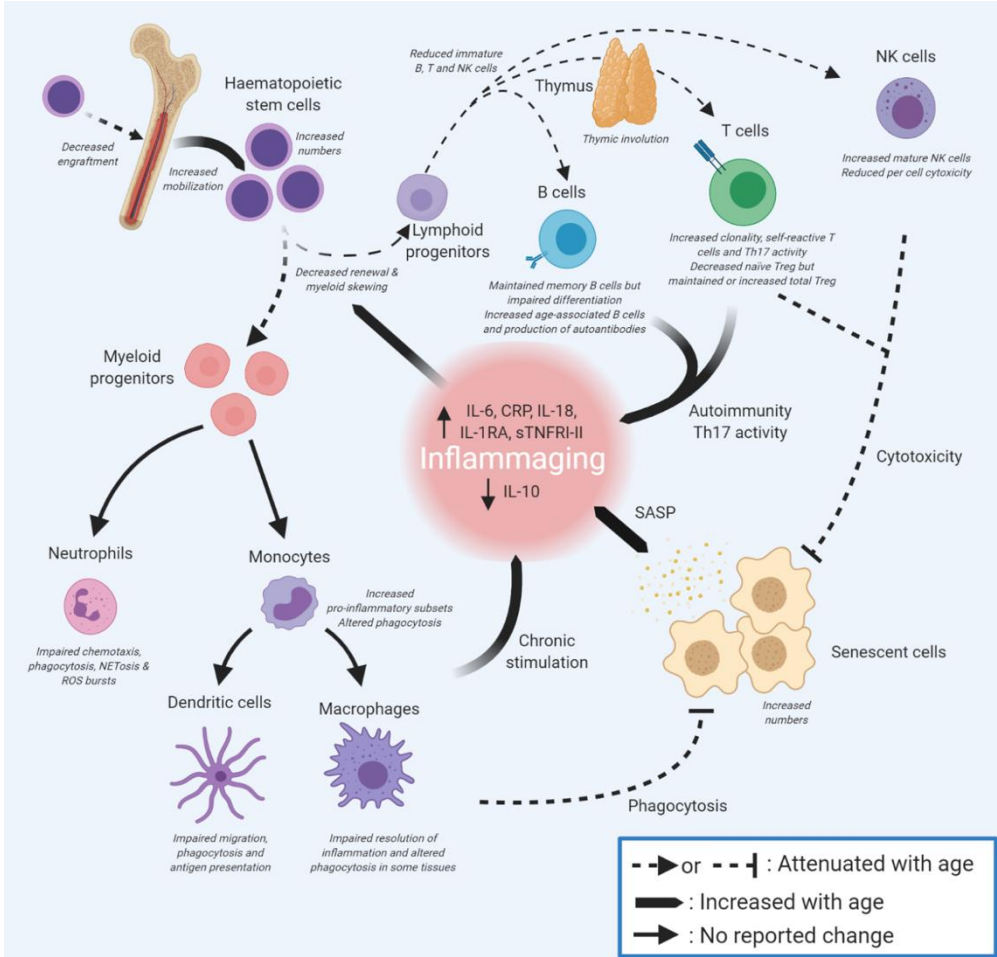
ageing defects in medullary epithelial cells of the thymus, contributes to peripheral damage and inflammation [355–357]. Furthermore, the decrease in T<sub>reg</sub> diversity in older adults may limit their ability to manage self-reactivity [343,358,359], leading to increasing inflammation and, together with B cell defects (see above), elevated risk of autoimmunity with age. Hence thymic involution is likely to be an important contributor to inflammageing.

Cytotoxic T cells, which express the CD8 surface marker, are important in killing of infected cells and cancer cells expressing neoantigens. IL-2 signalling by Th1 helper cells drives various CD8<sup>+</sup> pathways, leading either to proliferation, differentiation or establishment of a memory state [360]. With age, the compartment size and clonality of CD8<sup>+</sup> T cells is significantly decreased [361]. Recent results have demonstrated that almost two thirds of CD8<sup>+</sup> T cells from donors aged 60 or more were senescent [362]. Additionally, aged CD8<sup>+</sup> show markedly reduced cytotoxicity compared with young CD8<sup>+</sup> cells, which may account for accumulation of senescent cells in older adults as well as elevated cancer risk, through failure of immunosurveillance. Indeed, genetic deletion of perforin (which ablates CD8<sup>+</sup> cytotoxicity) led to increased accumulation of senescent cells and elevated chronic inflammation [34], together with tissue fibrosis and multiple age-related disorders. Notably, removal of senescent cells alleviated many of these phenotypes [34,363], strongly supporting the idea that senescent cells drive inflammation and tissue damage.

T cell ageing and in particular senescence leads to significant changes in function [370]. While memory CD8<sup>+</sup> T cells increase in number with ageing, they undergo cell cycle arrest and express the senescence marker KLRG1 (killer cell lectin-like receptor G1), and are considered non-functional [364,365]. Notably, mitochondrial dysfunction, a canonical hallmark of ageing and senescence, appears to have an important role in T cell ageing [353,366]. In addition, age-related stiffening of the extracellular matrix has been shown to reduce T cell mobility and might lead to cell death [367]. Sestrin-driven senescence of CD8<sup>+</sup> T cells has recently been reported to lead to loss of TCR expression together with acquisition of NK features [368]. It has been suggested (but not yet tested *in vivo*) that this may be a positive adaptation to prevent proliferation of T cells bearing potentially oncogenic changes, coupled with improved cell killing against chronic infections and cancers [369], but this plasticity does deplete important adaptive cells and concomitantly reinforce the pro-inflammatory innate immune system.

Overall, immunosenescence is intricately linked with inflammageing, through interactions between all components of the innate and adaptive immune systems, as well as inflammatory contributions through the SAP from senescent cells throughout the body. This complex interplay is summarised in Figure 5.

Figure 5. Inflammageing arises from increased cell senescence across many tissues as well as age-related changes to the bone marrow niche and individual innate and adaptive immune cells. Together this leads



to inappropriately high levels of sterile inflammation, with a number of secreted factors that may serve as a signature of inflammageing.

5. Strategies to attenuate inflammageing, immunosenescence and their effects

5.1 Physical exercise

Physical exercise has long been known to extend healthy lifespan. It is now becoming apparent that at least some of the health benefits arise from damping down inflammation. Lifelong endurance training is associated with reduced levels of pro-inflammatory markers [371–373] while interventions introducing regular endurance training for elderly people led to significant drops in inflammatory markers [374–376]. While intensive resistance training was associated with a brief spike in inflammatory markers such as IL-6 (potentially related to myokine function), this was then followed by an overall decrease in baseline pro-inflammatory markers in older adults [377]. This relationship goes both ways – inflammageing promotes muscle weakness and age-related sarcopenia [378,379], while

physical activity was able both to substantially prevent sarcopenia and also decrease age-related inflammation [374].

Overall, long-term exercise is associated with reduced immunosenescence with improved function of NK cells and neutrophils, reduced levels of the most pro-inflammatory monocytes subsets, increased percentage of naïve T and B cells, decreased Th17 cell polarization and reduced markers of senescence in T cells [91,373,380]. Interestingly,  $VO_{2max}$  (a marker of healthy lung function) was strongly negatively associated with senescent T cells and adjusting for  $VO_{2max}$  removed any effect of age on the proportion of senescent and naïve T-cells [381].

## 5.2 Diet

Diet may also play a role in age-related inflammation. In a large cohort from Hong Kong, in old men (but not in women), higher CRP levels were associated with lower quality diets [382]. Similarly, a study in Spain showed a strong correlation between self-reported intake of highly processed foods and short telomeres, which associate with senescence [383,384]. Restriction of dietary intake without malnutrition (often known as dietary restriction or DR) increases lifespan and healthspan in many different animal models [385], and has been reported to reduce age-related inflammation [386,387]; it is also likely to trigger autophagy. In non-human primates, the effect of DR varied according to previous dietary exposures and health status, though the overall finding was improved health and reduced inflammation [388–390]. While there are few research studies of DR in humans, at least one study showed a decrease in inflammation in young non-obese humans after long-term calorie restriction [391]. The mechanistic basis for DR improvements in health has not been formalised, but it is of note that the outcomes from drug inhibition of biochemical nutrient sensing pathways (*e.g.* mTOR inhibitors) are very similar to the DR studies in terms of better health, decreased inflammation and extended lifespan [392]. In addition, DR may impact on the gut microbiome; the microbial composition of the GI tract in young mammals includes bacterial species important in production of anti-inflammatory short chain fatty acids [393–397], while microbial diversity shrinks with ageing with a shift towards more pro-inflammatory species. Pre- and pro-biotic studies have not given consistent result in terms of impact on inflammation. However, the very recent PREDICT1 study has greatly advanced understanding of the relationship between the gut microbiome and health, highlighting microbial biomarkers of obesity and cardiovascular disease as well as inflammation. This study also showed that post-prandial blood glucose levels depend as much on an individual's microbiome as on sugar intake [398]; this is important in the context of inflammation since high circulating sugar levels contribute to chronic inflammation through production of glycation products (AGEs) which trigger proinflammatory RAGE signalling [50], also involved in physiological ageing potentially through inflammageing promotion [51]. Hence dietary interventions may need to be personalised to take into account microbiome as well as food intake in order to restrain age-related inflammation.

## 5.3 Immune rejuvenation

Direct rejuvenation of the immune system is proving a fruitful avenue of research. Restoration of thymic function through use of growth hormone (GH) in combination with metformin and DHEA (to counteract the diabetogenic action of GH) was shown in the TRIMM trial to

improve overall health and reduce biological ageing, as assessed through methylation clocks [399]. Stimulating haematopoietic stem cells to proliferate by depleting aged immune cells also appears promising, since rejuvenation of the B cell lineage has been achieved by complete B cell depletion (*e.g.* treatment with monoclonal anti-B cell antibody rituximab), which triggers expansion of progenitors; such B lineage rejuvenation was sufficient to enable treated individuals to mount a response to hepatitis B vaccination, but it did not fully restore immune system function [400,401]. The rejuvenation is thought to be a consequence of the elimination of old long-lived B cells [400], as similarly suggested in a genetic model of age-associated B cell removal [329]. Induced pluripotent stem cells derived from old HSCs were also able to restore haematopoiesis to levels seen in young mice and also avoided skewing towards myeloid lineage [402], suggesting that the epigenetic reprogramming to return to iPSC state was sufficient to remove age-related epigenetic information. Multiple other strategies to counteract immunosenescence have been investigated, such as the growth factor IL-7 and checkpoint inhibitors, and have been reviewed elsewhere [403].

#### 5.4. Interventions to remove senescent cells

Treating immunosenescence by targeting drugs to the underlying ageing biology provides an important new route to ameliorate immunosenescence and inflammageing [404]. One major biological contributor to ageing and inflammageing is the accumulation of senescent cells which secrete the pro-inflammatory SASP that contributes to chronic inflammation with increasing age. Thus, removal of senescent cells may prove useful in mitigating inflammageing by reducing the source of the SASP. Several strategies have been shown effective in selectively killing senescent cells, as outlined below.

Chimeric antigen receptor (CAR)-T cells can be designed to target specific antigen-expressing cellular targets. In a recent study, CAR-T cells were engineered to recognise the urokinase-type plasminogen activator receptor (uPAR) expressed on senescent cells; treatment with these CAR-T cells killed senescent cells *in vitro* and successfully restored tissue integrity and removed pro-inflammatory hepatic stellate senescent cells *in vivo* [405]. Therapeutic use of NK cells or macrophages to remove senescent cells has also been suggested [26,406].

Targeting of senescent cells with FOXO4 peptides that promote senolysis leads to better tissue integrity and apparent rejuvenation in prematurely aged mice [407]. Interestingly such peptides were found to have the greatest effect against senescent cells with high SASP expression, and treatment led to a local normalization of IL-6 levels in kidney tubular region of old mice [407]. Similar findings have been made with nanoparticles targeting senescent cells where senescent cell removal was possibly associated with reduced SASP [408]. Moreover, removal of senescent HSCs using the senolytic ABT263 permitted rejuvenation of aged but non-senescent HSCs and also reduced the expression of SASP factors [409]. Clinical trials in humans with diabetic kidney disease treated with senolytic combination dasatinib with quercetin through the Translational Geroscience Network (ClinicalTrials.gov identifier: NCT02848131) have shown decreased indicators of senescence (p16 and senescence-associated  $\beta$ -galactosidase) in conjunction with decreased macrophages in patients; most notably, plasma SASP factors were significantly decreased. Hence removing senescent cells to ablate the SASP is likely to be beneficial in reducing inflammageing.



Senolytic agents also appear valuable in improving immune function in ageing against infectious agents. As discussed above, viral infection in older adults exacerbates an already elevated inflammatory state. It is therefore extremely important in the context of the COVID-19 pandemic that various senolytic treatments were found to reduce inflammatory markers and greatly improve survival of mice infected with a  $\beta$ -coronavirus (mouse hepatitis virus, related to SARS-CoV-2) [55,56]. The senolytic agent fisetin is now being tested in human clinical trials for prophylaxis and treatment of mild, moderate or severe COVID-19 in older adults (ClinicalTrials.gov identifiers: NCT04537299, NCT04476953). Whether such therapies exert their beneficial effects thorough innate or adaptive immune cells is less clear, though antibody (*i.e.* B-cell mediated) responses to mouse hepatitis virus were elevated on senolytic treatment of aged animals [55].

### 5. 5 Molecular modifiers of senescence and inflammation

Senescent cell removal has shown significant promise in improving health and reducing inflammageing, as outlined above. However, removal of cells, albeit senescent, has the potential to result in tissue disruption, particularly in the oldest old, and to induce functional deficits. Various approaches have been tested to suppress the damaging features of senescent cells – particularly the SASP – without eliminating them. Many of these strategies target mTOR. mTOR inhibition improves later life health and increases lifespan in mice and other experimental animals [392,410]; notably mTOR inhibitors also suppress pro-inflammatory SASP factors [12,411,412]. In human clinical studies, inhibition of mTOR successfully improved B and T cell immune function, increased vaccine response and decreased overall infections in older adults [334,335], with stimulation of innate antiviral genes together with down-regulation of pro-inflammatory factors [336], while MAPK kinase inhibitors, that overcome senescence in progeroid Werner syndrome patient cells [413], also suppress inflammation in skin fibroblasts from normally aged donors [414]. Inhibition of PI3-kinase, an upstream activator of mTOR, corrected the aberrant migration seen in old neutrophils [190]. However, a recent study reported that rapamycin improved healthspan in a model of increased systemic inflammation but did not affect inflammageing [415]. It has been suggested differences in drug dosage may account for these inconsistencies [337]. Interestingly, statins also act on pathways regulated by mTOR including CDC42, with the effect of improving neutrophil function and overall immune outcomes, reducing deaths from community acquired pneumonia and even COVID-19 [404,416].

It is possible that at least some of the beneficial effects of mTOR inhibitors is exerted through activation of autophagy, since mTOR inhibition relieves mTOR-mediated suppression of ULK-1. Indeed, spermidine, which also activates autophagy, was able to restore age-related decline in autophagy and response to vaccination and infection of CD8<sup>+</sup> T cells in old mice [417], a finding that has now been verified in human studies examining vaccination responses in older adults [418], with human trials to improve COVID-19 vaccine responses now underway. TFEB is translated more efficiently in cells treated with spermidine [419]; since TFEB acts as a transcription factor for essential autophagy genes, translational and autophagy effects of spermidine appear tightly linked. mTOR inhibitors similarly act on translation as

well as autophagy, relieving inhibition on eIF4 and allowing a shift from cap-dependent to cap-independent protein translation, as well as promoting translation of oligopyrimidine tract-containing mRNAs, while spermidine improves translation of polypeptides with polyprolines (including TFEB) through eIF5 [419]. This alters the profile of proteins synthesised by treated aged cells back to those observed in young proliferating cells.

A further pharmacological intervention to decrease inflammageing uses the very well tolerated and commonly prescribed antidiabetic drug, metformin. Metformin blocks NF- $\kappa$ B signalling and the downstream transcription of pro-inflammatory cytokines [420], as well as activating autophagy [353]. Epidemiological studies suggest improved health in human subjects taking metformin, even above health baselines of age-matched non-diabetic controls [421], although effects were found to be dose-dependent in ITP trials in mice [422,423]. A recent study demonstrated that treatment of CD4<sup>+</sup> T cells from old donors with metformin improved autophagy and mitochondrial function, as well as limiting Th17 prevalence and its age-associated pro-inflammatory profile [353]. Metformin has also been reported to reduce autoimmunity, cytokine secretion and NETosis [404,424,425].

Inflammasome modulators are looking promising in suppressing inflammation and improving health outcomes. A recent study showed that deacetylation of NLRP3 by SIRT2 limited age-related inflammation caused by NLRP3 activation in macrophages of old mice [426], while age-related reduction in SIRT2 expression allows NLRP3 inflammasome priming induced by mitochondrial dysfunction, impairing the regenerative capacity and maintenance of aged HSCs [427]. Interestingly, administration of MCC950, a NLRP3 inhibitor, to aged mice improved age-related metabolic changes, including glucose tolerance, and hepatic function while inhibiting the mTOR pathway, increasing autophagy and reducing the levels of inflammation markers (active IL-1 $\beta$  and IL-18) [428]. NLRP3 is looking to be a very interesting target for drug inhibition to reduce inflammation without unwanted off-target effects on the wider immune system (reviewed in [429]). Sirtuin activators have also been widely tested in ageing, though at present they may be limited to diabetes [430–433]. Sirtuins require NAD<sup>+</sup> to function, but NAD<sup>+</sup> levels diminish markedly with age. Consistent with an important role for sirtuins in blocking biological ageing processes, supplementation with NAD<sup>+</sup> (or precursors such as NMN) also appears to improve health and potentially decreases mitochondrial ROS and hence senescence and inflammation [430,434,435]. However, wide availability of NAD<sup>+</sup> as a nutraceutical dietary supplement makes placebo-controlled blinded clinical trials in humans challenging.

## 6. Conclusion

The relationship between immunosenescence and inflammageing is complex, involving interplay between innate and adaptive arms of the immune system, together with senescent cells from non-immune system lineages, in a potentially vicious cycle. Inflammageing promotes senescence and impedes adaptive immune responses, while this impairment may lead to a greater mobilization of innate immune cells, thereby favouring inflammageing. With the ageing of the immune system, immunosurveillance becomes less efficient, leading to failure to remove senescent cells [436]. These in turn contribute locally and systemically to

inflammation. The evidence discussed above demonstrates an overall imbalance in inflammatory processes, tilting progressively towards increasing inflammation with age, with failure of anti-inflammatory molecules to counteract this. The important interconnections between inflammation, immunosenescence, frailty and age-related disease have been highlighted further by the development of an accurate ageing clock (iAge) based on inflammatory signatures [437].

The need to improve immune health and resistance to infectious diseases in older adults has been brought into sharp focus by the COVID-19 pandemic. Here we have outlined several strategies to improve elderly immune function and decrease inflammation. Of these, spermidine treatment, mTOR inhibition and selective removal of senescent cells are most strongly supported by model organism studies and human clinical trial data, with significant scope for immune benefit even against severe infections such as that caused by SARS-CoV-2. It is possible that combinations of these therapies may be needed to address immunosenescence and inflammageing in the context of an ageing body with accumulated senescent cells. We recommend that clinical trials of drugs for age-related diseases routinely include analysis of inflammatory mediators in order to determine whether the treatment has the added benefit of ameliorating inflammageing. We conclude that it is essential to investigate a diverse set of inflammation-related molecules to properly analyse the development of chronic inflammation with ageing; new proteomics platforms that permit simultaneous measurement of thousands of factors from very small blood or buccal swab samples should greatly facilitate such analysis and enable personalised interventions to reduce inflammation and support healthy immune function even in old age.

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## 7. References

1. Krabbe, K.S.; Pedersen, M.; Bruunsgaard, H. Inflammatory Mediators in the Elderly. *Exp. Gerontol.* **2004**, *39*, 687–699, doi:10.1016/j.exger.2004.01.009.

2. Arai, Y.; Martin-Ruiz, C.M.; Takayama, M.; Abe, Y.; Takebayashi, T.; Koyasu, S.; Suematsu, M.; Hirose, N.; von Zglinicki, T. Inflammation, But Not Telomere Length, Predicts Successful Ageing at Extreme Old Age: A Longitudinal Study of Semi-Supercentenarians. *EBioMedicine* **2015**, *2*, 1549–1558, doi:10.1016/j.ebiom.2015.07.029.
3. Bruunsgaard, H. The Clinical Impact of Systemic Low-Level Inflammation in Elderly Populations. With Special Reference to Cardiovascular Disease, Dementia and Mortality. *Dan. Med. Bull.* **2006**, *53*, 285–309.
4. Arnoult, N.; Karlseder, J. Complex Interactions between the DNA-Damage Response and Mammalian Telomeres. *Nat. Struct. Mol. Biol.* **2015**, *22*, 859–866, doi:10.1038/nsmb.3092.
5. d'Adda di Fagagna, F. Living on a Break: Cellular Senescence as a DNA-Damage Response. *Nat. Rev. Cancer* **2008**, *8*, 512–522, doi:10.1038/nrc2440.
6. Hayflick, L. The limited in vitro lifetime of human diploid cell strains. *Exp. Cell Res.* **1965**, *37*, 614–636.
7. Brandl, A.; Meyer, M.; Bechmann, V.; Nerlich, M.; Angele, P. Oxidative Stress Induces Senescence in Human Mesenchymal Stem Cells. *Exp. Cell Res.* **2011**, *317*, 1541–1547, doi:10.1016/j.yexcr.2011.02.015.
8. Chen, J.-H.; Ozanne, S.E.; Hales, C.N. Methods of Cellular Senescence Induction Using Oxidative Stress. *Methods Mol. Biol. Clifton NJ* **2007**, *371*, 179–189, doi:10.1007/978-1-59745-361-5\_14.
9. Serrano, M.; Lin, A.W.; McCurrach, M.E.; Beach, D.; Lowe, S.W. Oncogenic Ras Provokes Premature Cell Senescence Associated with Accumulation of P53 and P16INK4a. *Cell* **1997**, *88*, 593–602.
10. Toussaint, O.; Dumont, P.; Remacle, J.; Dierick, J.-F.; Pascal, T.; Fripiat, C.; Magalhaes, J.P.; Zdanov, S.; Chainiaux, F. Stress-Induced Premature Senescence or Stress-Induced Senescence-like Phenotype: One in Vivo Reality, Two Possible Definitions? *ScientificWorldJournal* **2002**, *2*, 230–247, doi:10.1100/tsw.2002.100.
11. Vettorelli, S.; Passos, J.F. Telomeres and Cell Senescence - Size Matters Not. *EBioMedicine* **2017**, *21*, 14–20, doi:10.1016/j.ebiom.2017.03.027.
12. Herranz, N.; Gallage, S.; Mellone, M.; Wuestefeld, T.; Klotz, S.; Hanley, C.J.; Raguz, S.; Acosta, J.C.; Innes, A.J.; Banito, A.; et al. MTOR Regulates MAPKAPK2 Translation to Control the Senescence-Associated Secretory Phenotype. *Nat. Cell Biol.* **2015**, *17*, 1205–1217, doi:10.1038/ncb3225.
13. Astle, M.V.; Hannan, K.M.; Ng, P.Y.; Lee, R.S.; George, A.J.; Hsu, A.K.; Haupt, Y.; Hannan, R.D.; Pearson, R.B. AKT Induces Senescence in Human Cells via MTORC1 and P53 in the Absence of DNA Damage: Implications for Targeting MTOR during Malignancy. *Oncogene* **2012**, *31*, 1949–1962, doi:10.1038/onc.2011.394.
14. Castilho, R.M.; Squarize, C.H.; Chodosh, L.A.; Williams, B.O.; Gutkind, J.S. MTOR Mediates Wnt-Induced Epidermal Stem Cell Exhaustion and Aging. *Cell Stem Cell* **2009**, *5*, 279–289, doi:10.1016/j.stem.2009.06.017.
15. Walters, H.E.; Deneka-Hannemann, S.; Cox, L.S. Reversal of Phenotypes of Cellular Senescence by Pan-MTOR Inhibition. *Aging* **2016**, *8*, 231–244, doi:10.18632/aging.100872.
16. Horvath, S.; Raj, K. DNA Methylation-Based Biomarkers and the Epigenetic Clock Theory of Ageing. *Nat. Rev. Genet.* **2018**, *19*, 371, doi:10.1038/s41576-018-0004-3.
17. Horvath, S. DNA Methylation Age of Human Tissues and Cell Types. *Genome Biol.* **2013**, *14*, 3156, doi:10.1186/gb-2013-14-10-r115.
18. Dou, Z.; Ghosh, K.; Vizioli, M.G.; Zhu, J.; Sen, P.; Wangenstein, K.J.; Simithy, J.; Lan, Y.; Lin, Y.; Zhou, Z.; et al. Cytoplasmic Chromatin Triggers Inflammation in Senescence and Cancer. *Nature* **2017**, *550*, 402–406, doi:10.1038/nature24050.
19. Rodier, F.; Campisi, J. Four Faces of Cellular Senescence. *J. Cell Biol.* **2011**, *192*, 547–556, doi:10.1083/jcb.201009094.
20. Malaquin, N.; Martinez, A.; Rodier, F. Keeping the Senescence Secretome under Control: Molecular Reins on the Senescence-Associated Secretory Phenotype. *Exp. Gerontol.* **2016**, *82*, 39–49, doi:10.1016/j.exger.2016.05.010.
21. Sagiv, A.; Krizhanovsky, V. Immunosurveillance of Senescent Cells: The Bright Side of the Senescence Program. *Biogerontology* **2013**, *14*, 617–628, doi:10.1007/s10522-013-9473-0.
22. van Deursen, J.M. The Role of Senescent Cells in Ageing. *Nature* **2014**, *509*, 439–446, doi:10.1038/nature13193.

23. Antonangeli, F.; Zingoni, A.; Soriani, A.; Santoni, A. Senescent Cells: Living or Dying Is a Matter of NK Cells. *J. Leukoc. Biol.* **2019**, *105*, 1275–1283, doi:10.1002/JLB.MR0718-299R.
24. Burton, D.G.A.; Stolzing, A. Cellular Senescence: Immunosurveillance and Future Immunotherapy. *Ageing Res. Rev.* **2018**, *43*, 17–25, doi:10.1016/j.arr.2018.02.001.
25. Sagiv, A.; Biran, A.; Yon, M.; Simon, J.; Lowe, S.W.; Krizhanovsky, V. Granule Exocytosis Mediates Immune Surveillance of Senescent Cells. *Oncogene* **2013**, *32*, 1971–1977, doi:10.1038/onc.2012.206.
26. Kale, A.; Sharma, A.; Stolzing, A.; Desprez, P.-Y.; Campisi, J. Role of Immune Cells in the Removal of Deleterious Senescent Cells. *Immun. Ageing* **2020**, *17*, 16, doi:10.1186/s12979-020-00187-9.
27. Storer, M.; Mas, A.; Robert-Moreno, A.; Pecoraro, M.; Ortells, M.C.; Di Giacomo, V.; Yosef, R.; Pilpel, N.; Krizhanovsky, V.; Sharpe, J.; et al. Senescence Is a Developmental Mechanism That Contributes to Embryonic Growth and Patterning. *Cell* **2013**, *155*, 1119–1130, doi:10.1016/j.cell.2013.10.041.
28. Walters, H.E.; Yun, M.H. Rising from the Ashes: Cellular Senescence in Regeneration. *Curr. Opin. Genet. Dev.* **2020**, *64*, 94–100, doi:10.1016/j.gde.2020.06.002.
29. Davaapil, H.; Brookes, J.P.; Yun, M.H. Conserved and Novel Functions of Programmed Cellular Senescence during Vertebrate Development. *Dev. Camb. Engl.* **2017**, *144*, 106–114, doi:10.1242/dev.138222.
30. Mosteiro, L.; Pantoja, C.; Alcazar, N.; Marión, R.M.; Chondronasiou, D.; Rovira, M.; Fernandez-Marcos, P.J.; Muñoz-Martin, M.; Blanco-Aparicio, C.; Pastor, J.; et al. Tissue Damage and Senescence Provide Critical Signals for Cellular Reprogramming in Vivo. *Science* **2016**, *354*, aaf4445, doi:10.1126/science.aaf4445.
31. Yun, M.H.; Davaapil, H.; Brookes, J.P. Recurrent Turnover of Senescent Cells during Regeneration of a Complex Structure. *eLife* **2015**, *4*, e05505, doi:10.7554/eLife.05505.
32. Muñoz-Espín, D.; Cañamero, M.; Maraver, A.; Gómez-López, G.; Contreras, J.; Murillo-Cuesta, S.; Rodríguez-Baeza, A.; Varela-Nieto, I.; Ruberte, J.; Collado, M.; et al. Programmed Cell Senescence during Mammalian Embryonic Development. *Cell* **2013**, *155*, 1104–1118, doi:10.1016/j.cell.2013.10.019.
33. Campisi, J. Cellular Senescence as a Tumor-Suppressor Mechanism. *Trends Cell Biol.* **2001**, *11*, S27-31, doi:10.1016/s0962-8924(01)02151-1.
34. Ovadya, Y.; Landsberger, T.; Leins, H.; Vadai, E.; Gal, H.; Biran, A.; Yosef, R.; Sagiv, A.; Agrawal, A.; Shapira, A.; et al. Impaired Immune Surveillance Accelerates Accumulation of Senescent Cells and Aging. *Nat. Commun.* **2018**, *9*, doi:10.1038/s41467-018-07825-3.
35. Muñoz, D.P.; Yannoni, S.M.; Daemen, A.; Sun, Y.; Vakar-Lopez, F.; Kawahara, M.; Freund, A.M.; Rodier, F.; Wu, J.D.; Desprez, P.-Y.; et al. Targetable Mechanisms Driving Immuno-evasion of Persistent Senescent Cells Link Chemotherapy-Resistant Cancer to Aging. *JCI Insight* **2019**, *5*, 124716, doi:10.1172/jci.insight.124716.
36. Yousefzadeh, M.J.; Flores, R.R.; Zhu, Y.; Schmichen, Z.C.; Brooks, R.W.; Trussoni, C.E.; Cui, Y.; Angelini, L.; Lee, K.-A.; McGowan, S.J.; et al. An Aged Immune System Drives Senescence and Ageing of Solid Organs. *Nature* **2021**, *594*, 100–105, doi:10.1038/s41586-021-03547-7.
37. Coppé, J.-P.; Desprez, P.-Y.; Krtolica, A.; Campisi, J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annu. Rev. Pathol.* **2010**, *5*, 99–118, doi:10.1146/annurev-pathol-121808-102144.
38. Freund, A.; Orjalo, A.V.; Desprez, P.-Y.; Campisi, J. Inflammatory Networks during Cellular Senescence: Causes and Consequences. *Trends Mol. Med.* **2010**, *16*, 238–246, doi:10.1016/j.molmed.2010.03.003.
39. Chien, Y.; Scuoppo, C.; Wang, X.; Fang, X.; Balgley, B.; Bolden, J.E.; Premisrur, P.; Luo, W.; Chicas, A.; Lee, C.S.; et al. Control of the Senescence-Associated Secretory Phenotype by NF- $\kappa$ B Promotes Senescence and Enhances Chemosensitivity. *Genes Dev.* **2011**, *25*, 2125–2136, doi:10.1101/gad.17276711.
40. Meyer, P.; Maity, P.; Burkovski, A.; Schwab, J.; Müssel, C.; Singh, K.; Ferreira, F.F.; Krug, L.; Maier, H.J.; Wlaschek, M.; et al. A Model of the Onset of the Senescence Associated Secretory Phenotype after DNA Damage Induced Senescence. *PLOS Comput. Biol.* **2017**, *13*, e1005741, doi:10.1371/journal.pcbi.1005741.



41. Salminen, A.; Kauppinen, A.; Kaarniranta, K. Emerging Role of NF-KB Signaling in the Induction of Senescence-Associated Secretory Phenotype (SASP). *Cell. Signal.* **2012**, *24*, 835–845, doi:10.1016/j.cellsig.2011.12.006.
42. Nelson, G.; Wordworth, J.; Wang, C.; Jurk, D.; Lawless, C.; Martin-Ruiz, C.; von Zglinicki, T. A Senescent Cell Bystander Effect: Senescence-Induced Senescence. *Aging Cell* **2012**, *11*, 345–349, doi:10.1111/j.1474-9726.2012.00795.x.
43. Jeon, O.H.; David, N.; Campisi, J.; Elisseeff, J.H. Senescent Cells and Osteoarthritis: A Painful Connection. *J. Clin. Invest.* **2018**, *128*, 1229–1237, doi:10.1172/JCI95147.
44. Xu, M.; Bradley, E.W.; Weivoda, M.M.; Hwang, S.M.; Pirtskhalava, T.; Decklever, T.; Curran, G.L.; Ogrodnik, M.; Jurk, D.; Johnson, K.O.; et al. Transplanted Senescent Cells Induce an Osteoarthritis-Like Condition in Mice. *J. Gerontol. A. Biol. Sci. Med. Sci.* **2017**, *72*, 780–785, doi:10.1093/gerona/glw154.
45. Jeon, O.H.; Kim, C.; Laberge, R.-M.; Demaria, M.; Rathod, S.; Vasserot, A.P.; Chung, J.W.; Kim, D.H.; Poon, Y.; David, N.; et al. Local Clearance of Senescent Cells Attenuates the Development of Post-Traumatic Osteoarthritis and Creates a pro-Regenerative Environment. *Nat. Med.* **2017**, *23*, 775–781, doi:10.1038/nm.4324.
46. Wang, B.; Liu, Z.; Chen, V.P.; Wang, L.; Inman, C.L.; Zhou, Y.; Guo, C.; Tchkonja, T.; Rowe, D.W.; Kuchel, G.A.; et al. Transplanting Cells from Old but Not Young Donors Causes Physical Dysfunction in Older Recipients. *Aging Cell* **2020**, *19*, e13106, doi:10.1111/ace1.13106.
47. Franceschi, C.; Campisi, J. Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases. *J. Gerontol. Ser. A* **2014**, *69*, S4–S9, doi:10.1093/gerona/glu057.
48. Xu, M.; Pirtskhalava, T.; Farr, J.N.; Weigand, B.M.; Palmer, A.K.; Weivoda, M.M.; Inman, C.L.; Ogrodnik, M.B.; Hachfeld, C.M.; Fraser, D.G.; et al. Senolytics Improve Physical Function and Increase Lifespan in Old Age. *Nat. Med.* **2018**, *24*, 1246, doi:10.1038/s41591-018-0092-9.
49. Jurk, D.; Wilson, C.; Passos, J.F.; Oakley, F.; Correia-Melo, C.; Greaves, L.; Saretzki, G.; Fox, C.; Lawless, C.; Anderson, R.; et al. Chronic Inflammation Induces Telomere Dysfunction and Accelerates Ageing in Mice. *Nat. Commun.* **2014**, *5*, 4172, doi:10.1038/ncomms5172.
50. Teissier, T.; Boulanger, É. The Receptor for Advanced Glycation End-Products (RAGE) Is an Important Pattern Recognition Receptor (PRR) for Inflammaging. *Biogerontology* **2019**, *20*, 279–301, doi:10.1007/s10522-019-09808-3.
51. Teissier, T.; Quersin, V.; Gnemmi, V.; Daroux, M.; Howsam, M.; Delguste, F.; Lemoine, C.; Fradin, C.; Schmidt, A.-M.; Cauffiez, C.; et al. Knockout of Receptor for Advanced Glycation End-Products Attenuates Age-Related Renal Lesions. *Aging Cell* **2019**, *18*, e12850, doi:10.1111/ace1.12850.
52. Davalos, A.R.; Kawahara, M.; Malhotra, G.K.; Schaum, N.; Huang, J.; Ved, U.; Beausejour, C.M.; Coppe, J.-P.; Rodier, F.; Campisi, J. P53-Dependent Release of Alarmin HMGB1 Is a Central Mediator of Senescent Phenotypes. *J. Cell Biol.* **2013**, *201*, 613–629, doi:10.1083/jcb.201206006.
53. Chuprin, A.; Gal, H.; Biron-Shental, T.; Biran, A.; Amiel, A.; Rozenblatt, S.; Krizhanovsky, V. Cell Fusion Induced by ERVWE1 or Measles Virus Causes Cellular Senescence. *Genes Dev.* **2013**, *27*, 2356–2366, doi:10.1101/gad.227512.113.
54. Kohli, J.; Veenstra, I.; Demaria, M. The Struggle of a Good Friend Getting Old: Cellular Senescence in Viral Responses and Therapy. *EMBO Rep.* **2021**, *22*, e52243, doi:10.15252/embr.202052243.
55. Camell, C.D.; Yousefzadeh, M.J.; Zhu, Y.; Langhi Prata, L.G.P.; Huggins, M.A.; Pierson, M.; Zhang, L.; O’Kelly, R.D.; Pirtskhalava, T.; Xun, P.; et al. Senolytics Reduce Coronavirus-Related Mortality in Old Mice. *Science* **2021**, eabe4832, doi:10.1126/science.abe4832.
56. Cox, L.S.; Lord, J.M. Targeting Aging Cells Improves Survival. *Science* **2021**, *373*, 281–282, doi:10.1126/science.abi4474.

57. Ibler, A.E.M.; ElGhazaly, M.; Naylor, K.L.; Bulgakova, N.A.; F. El-Khamisy, S.; Humphreys, D. Typhoid Toxin Exhausts the RPA Response to DNA Replication Stress Driving Senescence and Salmonella Infection. *Nat. Commun.* **2019**, *10*, 4040, doi:10.1038/s41467-019-12064-1.
58. Corona, G.; Pizzocaro, A.; Vena, W.; Rastrelli, G.; Semeraro, F.; Isidori, A.M.; Pivonello, R.; Salonia, A.; Sforza, A.; Maggi, M. Diabetes Is Most Important Cause for Mortality in COVID-19 Hospitalized Patients: Systematic Review and Meta-Analysis. *Rev. Endocr. Metab. Disord.* **2021**, *22*, 275–296, doi:10.1007/s11154-021-09630-8.
59. Poly, T.N.; Islam, Md.M.; Yang, H.C.; Lin, M.C.; Jian, W.-S.; Hsu, M.-H.; Jack Li, Y.-C. Obesity and Mortality Among Patients Diagnosed With COVID-19: A Systematic Review and Meta-Analysis. *Front. Med.* **2021**, *8*, 620044, doi:10.3389/fmed.2021.620044.
60. Aghili, S.M.M.; Ebrahimpur, M.; Arjmand, B.; Shadman, Z.; Pejman Sani, M.; Qorbani, M.; Larijani, B.; Payab, M. Obesity in COVID-19 Era, Implications for Mechanisms, Comorbidities, and Prognosis: A Review and Meta-Analysis. *Int. J. Obes.* **2021**, *45*, 998–1016, doi:10.1038/s41366-021-00776-8.
61. Ershler, W.B.; Keller, E.T. Age-Associated Increased Interleukin-6 Gene Expression, Late-Life Diseases, and Frailty. *Annu. Rev. Med.* **2000**, *51*, 245–270, doi:10.1146/annurev.med.51.1.245.
62. Minciullo, P.L.; Catalano, A.; Mandraffino, G.; Casciaro, M.; Crucitti, A.; Maltese, G.; Morabito, N.; Lasco, A.; Gangemi, S.; Basile, G. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. *Arch. Immunol. Ther. Exp. (Warsz.)* **2016**, *64*, 111–126, doi:10.1007/s00005-015-0377-3.
63. Fagiolo, U.; Cossarizza, A.; Scala, E.; Fanales-Belasio, E.; Ortolani, C.; Cozzi, E.; Monti, D.; Franceschi, C.; Paganelli, R. Increased Cytokine Production in Mononuclear Cells of Healthy Elderly People. *Eur. J. Immunol.* **1993**, *23*, 2375–2378, doi:10.1002/eji.1830230950.
64. Seidler, S.; Zimmermann, H.W.; Bartneck, M.; Trautwein, C.; Tacke, F. Age-Dependent Alterations of Monocyte Subsets and Monocyte-Related Chemokine Pathways in Healthy Adults. *BMC Immunol.* **2010**, *11*, 30, doi:10.1186/1471-2172-11-30.
65. Gerli, R.; Monti, D.; Bistoni, O.; Mazzone, A.M.; Peri, G.; Cossarizza, A.; Di Gioacchino, M.; Cesarotti, M.E.; Doni, A.; Mantovani, A.; et al. Chemokines, STNF-Rs and SCD30 Serum Levels in Healthy Aged People and Centenarians. *Mech. Ageing Dev.* **2000**, *121*, 37–46.
66. Mariani, E.; Cattini, L.; Neri, S.; Malavolta, M.; Mocchegiani, E.; Ravaglia, G.; Facchini, A. Simultaneous Evaluation of Circulating Chemokine and Cytokine Profiles in Elderly Subjects by Multiplex Technology: Relationship with Zinc Status. *Biogerontology* **2006**, *7*, 449–459, doi:10.1007/s10522-006-9060-8.
67. Morrisette-Thomas, V.; Cohen, A.A.; Fülöp, T.; Riesco, É.; Legault, V.; Li, Q.; Milot, E.; Dusseault-Bélanger, F.; Ferrucci, L. Inflamm-Aging Does Not Simply Reflect Increases in pro-Inflammatory Markers. *Mech. Ageing Dev.* **2014**, *139*, 49–57, doi:10.1016/j.mad.2014.06.005.
68. Bandeen-Roche, K.; Walston, J.D.; Huang, Y.; Semba, R.D.; Ferrucci, L. Measuring Systemic Inflammatory Regulation in Older Adults: Evidence and Utility. *Rejuvenation Res.* **2009**, *12*, 403–410, doi:10.1089/rej.2009.0883.
69. Hsu, F.-C.; Kritchevsky, S.B.; Liu, Y.; Kanaya, A.; Newman, A.B.; Perry, S.E.; Visser, M.; Pahor, M.; Harris, T.B.; Nicklas, B.J.; et al. Association between Inflammatory Components and Physical Function in the Health, Aging, and Body Composition Study: A Principal Component Analysis Approach. *J. Gerontol. A. Biol. Sci. Med. Sci.* **2009**, *64*, 581–589, doi:10.1093/gerona/glp005.
70. Tziakas, D.N.; Chalikias, G.K.; Kaski, J.C.; Kekes, A.; Hatzinikolaou, E.I.; Stakos, D.A.; Tentes, I.K.; Kortsaris, A.X.; Hatseras, D.I. Inflammatory and Anti-Inflammatory Variable Clusters and Risk Prediction in Acute Coronary Syndrome Patients: A Factor Analysis Approach. *Atherosclerosis* **2007**, *193*, 196–203, doi:10.1016/j.atherosclerosis.2006.06.016.

71. Kip, K.E.; Marroquin, O.C.; Shaw, L.J.; Arant, C.B.; Wessel, T.R.; Olson, M.B.; Johnson, B.D.; Mulukutla, S.; Sopko, G.; Merz, C.N.B.; et al. Global Inflammation Predicts Cardiovascular Risk in Women: A Report from the Women's Ischemia Syndrome Evaluation (WISE) Study. *Am. Heart J.* **2005**, *150*, 900–906, doi:10.1016/j.ahj.2005.02.002.
72. Ferrucci, L.; Corsi, A.; Lauretani, F.; Bandinelli, S.; Bartali, B.; Taub, D.D.; Guralnik, J.M.; Longo, D.L. The Origins of Age-Related Proinflammatory State. *Blood* **2005**, *105*, 2294–2299, doi:10.1182/blood-2004-07-2599.
73. Cesari, M.; Penninx, B.W.J.H.; Pahor, M.; Lauretani, F.; Corsi, A.M.; Rhys Williams, G.; Guralnik, J.M.; Ferrucci, L. Inflammatory Markers and Physical Performance in Older Persons: The InCHIANTI Study. *J. Gerontol. A. Biol. Sci. Med. Sci.* **2004**, *59*, 242–248, doi:10.1093/gerona/59.3.m242.
74. Collerton, J.; Martin-Ruiz, C.; Davies, K.; Hilkens, C.M.; Isaacs, J.; Kolenda, C.; Parker, C.; Dunn, M.; Catt, M.; Jagger, C.; et al. Frailty and the Role of Inflammation, Immunosenescence and Cellular Ageing in the Very Old: Cross-Sectional Findings from the Newcastle 85+ Study. *Mech. Ageing Dev.* **2012**, *133*, 456–466, doi:10.1016/j.mad.2012.05.005.
75. Varadhan, R.; Yao, W.; Matteini, A.; Beamer, B.A.; Xue, Q.-L.; Yang, H.; Manwani, B.; Reiner, A.; Jenny, N.; Parekh, N.; et al. Simple Biologically Informed Inflammatory Index of Two Serum Cytokines Predicts 10 Year All-Cause Mortality in Older Adults. *J. Gerontol. A. Biol. Sci. Med. Sci.* **2014**, *69*, 165–173, doi:10.1093/gerona/glt023.
76. Weinhold, B.; R  ther, U. Interleukin-6-Dependent and -Independent Regulation of the Human C-Reactive Protein Gene. *Biochem. J.* **1997**, *327*, 425–429.
77. Li, Y.; Lu, L.; Xie, Y.; Chen, X.; Tian, L.; Liang, Y.; Li, H.; Zhang, J.; Liu, Y.; Yu, X. Interleukin-6 Knockout Inhibits Senescence of Bone Mesenchymal Stem Cells in High-Fat Diet-Induced Bone Loss. *Front. Endocrinol.* **2020**, *11*, 622950, doi:10.3389/fendo.2020.622950.
78. Cardoso, A.L.; Fernandes, A.; Aguilar-Pimentel, J.A.; de Angelis, M.H.; Guedes, J.R.; Brito, M.A.; Ortolano, S.; Pani, G.; Athanasopoulou, S.; Gonos, E.S.; et al. Towards Frailty Biomarkers: Candidates from Genes and Pathways Regulated in Aging and Age-Related Diseases. *Ageing Res. Rev.* **2018**, *47*, 214–277, doi:10.1016/j.arr.2018.07.004.
79. Xu, Z.; Castellino, F.J.; Ploplis, V.A. Plasminogen Activator Inhibitor-1 (PAI-1) Is Cardioprotective in Mice by Maintaining Microvascular Integrity and Cardiac Architecture. *Blood* **2010**, *115*, 2038–2047, doi:10.1182/blood-2009-09-244962.
80. Miles, L.A.; Parmer, R.J. PAI-1: Cardiac Friend or Foe? *Blood* **2010**, *115*, 1862–1863, doi:10.1182/blood-2010-01-261420.
81. Eren, M.; Boe, A.E.; Klyachko, E.A.; Vaughan, D.E. Role of Plasminogen Activator Inhibitor-1 in Senescence and Aging. *Semin. Thromb. Hemost.* **2014**, *40*, 645–651, doi:10.1055/s-0034-1387883.
82. Eren, M.; Boe, A.E.; Murphy, S.B.; Place, A.T.; Nagpal, V.; Morales-Nebreda, L.; Urich, D.; Quaggin, S.E.; Budinger, G.R.S.; Mutlu, G.M.; et al. PAI-1-Regulated Extracellular Proteolysis Governs Senescence and Survival in Klotho Mice. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 7090–7095, doi:10.1073/pnas.1321942111.
83. Ghosh, A.K.; Rai, R.; Park, K.E.; Eren, M.; Miyata, T.; Wilsbacher, L.D.; Vaughan, D.E. A Small Molecule Inhibitor of PAI-1 Protects against Doxorubicin-Induced Cellular Senescence. *Oncotarget* **2016**, *7*, 72443–72457, doi:10.18632/oncotarget.12494.
84. Mailliez, A.; Guilbaud, A.; Puisieux, F.; Dauchet, L.; Boulanger,   . Circulating Biomarkers Characterizing Physical Frailty: CRP, Hemoglobin, Albumin, 25OHD and Free Testosterone as Best Biomarkers. Results of a Meta-Analysis. *Exp. Gerontol.* **2020**, *139*, 111014, doi:10.1016/j.exger.2020.111014.
85. Jylh  , M.; Paavilainen, P.; Lehtim  ki, T.; Goebeler, S.; Karhunen, P.J.; Hervonen, A.; Hurme, M. Interleukin-1 Receptor Antagonist, Interleukin-6, and C-Reactive Protein as Predictors of Mortality in Nonagenarians: The Vitality 90+ Study. *J. Gerontol. Ser. A* **2007**, *62*, 1016–1021, doi:10.1093/gerona/62.9.1016.

86. Suh, Y.; Atzmon, G.; Cho, M.-O.; Hwang, D.; Liu, B.; Leahy, D.J.; Barzilai, N.; Cohen, P. Functionally Significant Insulin-like Growth Factor I Receptor Mutations in Centenarians. *Proc. Natl. Acad. Sci.* **2008**, *105*, 3438–3442.
87. Horvath, S.; Pirazzini, C.; Bacalini, M.G.; Gentilini, D.; Di Blasio, A.M.; Delledonne, M.; Mari, D.; Arosio, B.; Monti, D.; Passarino, G.; et al. Decreased Epigenetic Age of PBMCs from Italian Semi-Supercentenarians and Their Offspring. *Aging* **2015**, *7*, 1159–1170, doi:10.18632/aging.100861.
88. Atzmon, G.; Schechter, C.; Greiner, W.; Davidson, D.; Rennert, G.; Barzilai, N. Clinical Phenotype of Families with Longevity. *J. Am. Geriatr. Soc.* **2004**, *52*, 274–277, doi:10.1111/j.1532-5415.2004.52068.x.
89. Atzmon, G.; Barzilai, N.; Hollowell, J.G.; Surks, M.I.; Gabriely, I. Extreme Longevity Is Associated with Increased Serum Thyrotropin. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 1251–1254, doi:10.1210/jc.2008-2325.
90. Del Giudice, M.; Gangestad, S.W. Rethinking IL-6 and CRP: Why They Are More than Inflammatory Biomarkers, and Why It Matters. *Brain. Behav. Immun.* **2018**, *70*, 61–75, doi:10.1016/j.bbi.2018.02.013.
91. Weyh, C.; Krüger, K.; Strasser, B. Physical Activity and Diet Shape the Immune System during Aging. *Nutrients* **2020**, *12*, doi:10.3390/nu12030622.
92. Brandt, C.; Pedersen, B.K. The Role of Exercise-Induced Myokines in Muscle Homeostasis and the Defense against Chronic Diseases. *J. Biomed. Biotechnol.* **2010**, *2010*, doi:10.1155/2010/520258.
93. Steensberg, A.; Fischer, C.P.; Keller, C.; Möller, K.; Pedersen, B.K. IL-6 Enhances Plasma IL-1ra, IL-10, and Cortisol in Humans. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *285*, E433–437, doi:10.1152/ajpendo.00074.2003.
94. Starkie, R.; Ostrowski, S.R.; Jauffred, S.; Febbraio, M.; Pedersen, B.K. Exercise and IL-6 Infusion Inhibit Endotoxin-Induced TNF-Alpha Production in Humans. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2003**, *17*, 884–886, doi:10.1096/fj.02-0670fje.
95. Ubaida-Mohien, C.; Lyashkov, A.; Gonzalez-Freire, M.; Tharakan, R.; Shardell, M.; Moaddel, R.; Semba, R.D.; Chia, C.W.; Gorospe, M.; Sen, R.; et al. Discovery Proteomics in Aging Human Skeletal Muscle Finds Change in Spliceosome, Immunity, Proteostasis and Mitochondria. *eLife* **2019**, *8*, e49874, doi:10.7554/eLife.49874.
96. Dinarello, C.A. Interleukin 1 and Interleukin 18 as Mediators of Inflammation and the Aging Process. *Am. J. Clin. Nutr.* **2006**, *83*, 447S–455S, doi:10.1093/ajcn/83.2.447S.
97. Dinarello, C.A.; Novick, D.; Kim, S.; Kaplanski, G. Interleukin-18 and IL-18 Binding Protein. *Front. Immunol.* **2013**, *4*, doi:10.3389/fimmu.2013.00289.
98. Pu, Z.; Che, Y.; Zhang, W.; Sun, H.; Meng, T.; Xie, H.; Cao, L.; Hao, H. Dual Roles of IL-18 in Colitis through Regulation of the Function and Quantity of Goblet Cells. *Int. J. Mol. Med.* **2019**, *43*, 2291–2302, doi:10.3892/ijmm.2019.4156.
99. Macia, L.; Tan, J.; Vieira, A.T.; Leach, K.; Stanley, D.; Luong, S.; Maruya, M.; Ian McKenzie, C.; Hijikata, A.; Wong, C.; et al. Metabolite-Sensing Receptors GPR43 and GPR109A Facilitate Dietary Fibre-Induced Gut Homeostasis through Regulation of the Inflammasome. *Nat. Commun.* **2015**, *6*, 6734, doi:10.1038/ncomms7734.
100. Eisenmesser, E.Z.; Gottschlich, A.; Redzic, J.S.; Paukovich, N.; Nix, J.C.; Azam, T.; Zhang, L.; Zhao, R.; Kieft, J.S.; The, E.; et al. Interleukin-37 Monomer Is the Active Form for Reducing Innate Immunity. *Proc. Natl. Acad. Sci.* **2019**, *116*, 5514–5522, doi:10.1073/pnas.1819672116.
101. Yan, X.; Xie, B.; Wu, G.; Hu, J.; Wang, D.; Cai, X.; Li, J. Interleukin-37: The Effect of Anti-Inflammatory Response in Human Coronary Artery Endothelial Cells Available online: <https://www.hindawi.com/journals/mi/2019/2650590/> (accessed on 16 June 2020).
102. Henry, C.J.; Casás-Selves, M.; Kim, J.; Zaberezhnyy, V.; Aghili, L.; Daniel, A.E.; Jimenez, L.; Azam, T.; McNamee, E.N.; Clambey, E.T.; et al. Aging-Associated Inflammation Promotes Selection for Adaptive Oncogenic Events in B Cell Progenitors. *J. Clin. Invest.* **2015**, *125*, 4666–4680, doi:10.1172/JCI83024.
103. Henry, C.J.; Eisenmesser, E.; Dinarello, C.A.; DeGregori, J. Interleukin-37 Improves Aging-Associated Declines in Adaptive Immunity Leading to Enhanced Suppression of Ph+ Leukemia. *J. Immunol.* **2019**, *202*, 65.8–65.8.



104. Sapinsley, Z.J.; Ballak, D.B.; Brunt, V.E.; Zigler, M.C.; Gioscia-Ryan, R.A.; Richey, J.J.; Dinarello, C.A.; Seals, D.R. Administration of Anti-Inflammatory Interleukin-37 Ameliorates Age-Related Vascular, Metabolic and Physical Dysfunction in Mice. *FASEB J.* **2017**, *31*, 1079.3-1079.3, doi:10.1096/fasebj.31.1\_supplement.1079.3.
105. Ballak, D.B.; Brunt, V.E.; Sapinsley, Z.J.; Ziemba, B.P.; Richey, J.J.; Zigler, M.C.; Johnson, L.C.; Gioscia-Ryan, R.A.; Culp-Hill, R.; Eisenmesser, E.Z.; et al. Short-Term Interleukin-37 Treatment Improves Vascular Endothelial Function, Endurance Exercise Capacity, and Whole-Body Glucose Metabolism in Old Mice. *Aging Cell* **2020**, *19*, e13074, doi:10.1111/accel.13074.
106. Sanjabi, S.; Zenewicz, L.A.; Kamanaka, M.; Flavell, R.A. Anti- and Pro-Inflammatory Roles of TGF- $\beta$ , IL-10, and IL-22 In Immunity and Autoimmunity. *Curr. Opin. Pharmacol.* **2009**, *9*, 447–453, doi:10.1016/j.coph.2009.04.008.
107. Takahashi, T.; Ellingson, M.K.; Wong, P.; Israelow, B.; Lucas, C.; Klein, J.; Silva, J.; Mao, T.; Oh, J.E.; Tokuyama, M.; et al. Sex Differences in Immune Responses That Underlie COVID-19 Disease Outcomes. *Nature* **2020**, *588*, 315–320, doi:10.1038/s41586-020-2700-3.
108. Yu, B.; Qi, Y.; Li, R.; Shi, Q.; Satpathy, A.T.; Chang, H.Y. B Cell-Specific XIST Complex Enforces X-Inactivation and Restrains Atypical B Cells. *Cell* **2021**, *184*, 1790-1803.e17, doi:10.1016/j.cell.2021.02.015.
109. Girard, S.; Kadhim, H.; Larouche, A.; Roy, M.; Gobeil, F.; Sébire, G. Pro-Inflammatory Disequilibrium of the IL-1 Beta/IL-1ra Ratio in an Experimental Model of Perinatal Brain Damages Induced by Lipopolysaccharide and Hypoxia-Ischemia. *Cytokine* **2008**, *43*, 54–62, doi:10.1016/j.cyto.2008.04.007.
110. Arend, W.P. The Balance between IL-1 and IL-1Ra in Disease. *Cytokine Growth Factor Rev.* **2002**, *13*, 323–340, doi:10.1016/s1359-6101(02)00020-5.
111. Esmon, C.T. The Interactions between Inflammation and Coagulation. *Br. J. Haematol.* **2005**, *131*, 417–430, doi:10.1111/j.1365-2141.2005.05753.x.
112. Branchford, B.R.; Carpenter, S.L. The Role of Inflammation in Venous Thromboembolism. *Front. Pediatr.* **2018**, *6*, doi:10.3389/fped.2018.00142.
113. Engbers, M.J.; Vlieg, A.V.H.; Rosendaal, F.R. Venous Thrombosis in the Elderly: Incidence, Risk Factors and Risk Groups. *J. Thromb. Haemost.* **2010**, *8*, 2105–2112, doi:10.1111/j.1538-7836.2010.03986.x.
114. Cohen, H.J.; Harris, T.; Pieper, C.F. Coagulation and Activation of Inflammatory Pathways in the Development of Functional Decline and Mortality in the Elderly. *Am. J. Med.* **2003**, *114*, 180–187.
115. Ferrucci, L.; Harris, T.B.; Guralnik, J.M.; Tracy, R.P.; Corti, M.C.; Cohen, H.J.; Penninx, B.; Pahor, M.; Wallace, R.; Havlik, R.J. Serum IL-6 Level and the Development of Disability in Older Persons. *J. Am. Geriatr. Soc.* **1999**, *47*, 639–646.
116. Grobler, C.; Maphumulo, S.C.; Grobelaar, L.M.; Bredenkamp, J.C.; Laubscher, G.J.; Lourens, P.J.; Steenkamp, J.; Kell, D.B.; Pretorius, E. Covid-19: The Rollercoaster of Fibrin(Ogen), D-Dimer, Von Willebrand Factor, P-Selectin and Their Interactions with Endothelial Cells, Platelets and Erythrocytes. *Int. J. Mol. Sci.* **2020**, *21*, 5168, doi:10.3390/ijms21145168.
117. Coppola, R.; Mari, D.; Lattuada, A.; Franceschi, C. Von Willebrand Factor in Italian Centenarians. *Haematologica* **2003**, *88*, 39–43.
118. Mari, D.; Mannucci, P.M.; Coppola, R.; Bottasso, B.; Bauer, K.A.; Rosenberg, R.D. Hypercoagulability in Centenarians: The Paradox of Successful Aging. *Blood* **1995**, *85*, 3144–3149.
119. Mari, D.; Coppola, R.; Provenzano, R. Hemostasis Factors and Aging. *Exp. Gerontol.* **2008**, *43*, 66–73, doi:10.1016/j.exger.2007.06.014.
120. Sanada, F.; Taniyama, Y.; Muratsu, J.; Otsu, R.; Shimizu, H.; Rakugi, H.; Morishita, R. Source of Chronic Inflammation in Aging. *Front. Cardiovasc. Med.* **2018**, *5*, doi:10.3389/fcvm.2018.00012.
121. Zhang, H.; Puleston, D.J.; Simon, A.K. Autophagy and Immune Senescence. *Trends Mol. Med.* **2016**, *22*, 671–686, doi:10.1016/j.molmed.2016.06.001.



122. Wijsman, C.A.; Maier, A.B.; de Craen, A.J.M.; van den Biggelaar, A.H.J.; Westendorp, R.G.J. An Unopposed Proinflammatory Response Is Beneficial for Survival in the Oldest Old. Results of the Leiden 85-Plus Study. *J. Gerontol. Ser. A* **2011**, *66A*, 393–399, doi:10.1093/gerona/glq212.
123. Panda, A.; Arjona, A.; Sapey, E.; Bai, F.; Fikrig, E.; Montgomery, R.R.; Lord, J.M.; Shaw, A.C. Human Innate Immunosenescence: Causes and Consequences for Immunity in Old Age. *Trends Immunol.* **2009**, *30*, 325–333, doi:10.1016/j.it.2009.05.004.
124. Ogata, K.; An, E.; Shioi, Y.; Nakamura, K.; Luo, S.; Yokose, N.; Minami, S.; Dan, K. Association between Natural Killer Cell Activity and Infection in Immunologically Normal Elderly People. *Clin. Exp. Immunol.* **2001**, *124*, 392–397, doi:10.1046/j.1365-2249.2001.01571.x.
125. Hazeldine, J.; Lord, J.M. The Impact of Ageing on Natural Killer Cell Function and Potential Consequences for Health in Older Adults. *Ageing Res. Rev.* **2013**, *12*, 1069–1078, doi:10.1016/j.arr.2013.04.003.
126. Zimmermann, P.; Curtis, N. Why Is COVID-19 Less Severe in Children? A Review of the Proposed Mechanisms Underlying the Age-Related Difference in Severity of SARS-CoV-2 Infections. *Arch. Dis. Child.* **2021**, *106*, 429–439, doi:10.1136/archdischild-2020-320338.
127. Fábíán, T.K.; Hermann, P.; Beck, A.; Fejérdy, P.; Fábíán, G. Salivary Defense Proteins: Their Network and Role in Innate and Acquired Oral Immunity. *Int. J. Mol. Sci.* **2012**, *13*, 4295–4320, doi:10.3390/ijms13044295.
128. Mun, J.J.; Tam, C.; Evans, D.J.; Fleiszig, S.M.J. Modulation of Epithelial Immunity by Mucosal Fluid. *Sci. Rep.* **2011**, *1*, doi:10.1038/srep00008.
129. Voynow, J.A.; Rubin, B.K. Mucins, Mucus, and Sputum. *Chest* **2009**, *135*, 505–512, doi:10.1378/chest.08-0412.
130. Thevaranjan, N.; Puchta, A.; Schulz, C.; Naidoo, A.; Szamosi, J.C.; Verschoor, C.P.; Loukov, D.; Schenck, L.P.; Jury, J.; Foley, K.P.; et al. Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. *Cell Host Microbe* **2017**, *21*, 455–466.e4, doi:10.1016/j.chom.2017.03.002.
131. Hollander, D.; Kaunitz, J.D. The “Leaky Gut”: Tight Junctions but Loose Associations? *Dig. Dis. Sci.* **2020**, *65*, 1277–1287, doi:10.1007/s10620-019-05777-2.
132. Wu, Y.; Dong, G.; Xiao, W.; Xiao, E.; Miao, F.; Syverson, A.; Missaghian, N.; Vafa, R.; Cabrera-Ortega, A.A.; Rossa, C.; et al. Effect of Aging on Periodontal Inflammation, Microbial Colonization, and Disease Susceptibility. *J. Dent. Res.* **2016**, *95*, 460–466, doi:10.1177/0022034515625962.
133. Nadim, R.; Tang, J.; Dilmohamed, A.; Yuan, S.; Wu, C.; Bakre, A.T.; Partridge, M.; Ni, J.; Copeland, J.R.; Anstey, K.J.; et al. Influence of Periodontal Disease on Risk of Dementia: A Systematic Literature Review and a Meta-Analysis. *Eur. J. Epidemiol.* **2020**, *35*, 821–833, doi:10.1007/s10654-020-00648-x.
134. Watts, A.; Crimmins, E.M.; Gatz, M. Inflammation as a Potential Mediator for the Association between Periodontal Disease and Alzheimer’s Disease. *Neuropsychiatr. Dis. Treat.* **2008**, *4*, 865–876.
135. Liu, Y.-J.; Guo, D.-W.; Tian, L.; Shang, D.-S.; Zhao, W.-D.; Li, B.; Fang, W.-G.; Zhu, L.; Chen, Y.-H. Peripheral T Cells Derived from Alzheimer’s Disease Patients Overexpress CXCR2 Contributing to Its Transendothelial Migration, Which Is Microglial TNF- $\alpha$ -Dependent. *Neurobiol. Aging* **2010**, *31*, 175–188, doi:10.1016/j.neurobiolaging.2008.03.024.
136. Marques, F.; Sousa, J.C.; Sousa, N.; Palha, J.A. Blood–Brain-Barriers in Aging and in Alzheimer’s Disease. *Mol. Neurodegener.* **2013**, *8*, 38, doi:10.1186/1750-1326-8-38.
137. Pellicanò, M.; Larbi, A.; Goldeck, D.; Colonna-Romano, G.; Buffa, S.; Bulati, M.; Rubino, G.; Iemolo, F.; Candore, G.; Caruso, C.; et al. Immune Profiling of Alzheimer Patients. *J. Neuroimmunol.* **2012**, *242*, 52–59, doi:10.1016/j.jneuroim.2011.11.005.
138. Shang, D.S.; Yang, Y.M.; Zhang, H.; Tian, L.; Jiang, J.S.; Dong, Y.B.; Zhang, K.; Li, B.; Zhao, W.D.; Fang, W.G.; et al. Intracerebral GM-CSF Contributes to Transendothelial Monocyte Migration in APP/PS1 Alzheimer’s Disease Mice. *J. Cereb. Blood Flow Metab.* **2016**, *36*, 1978–1991, doi:10.1177/0271678X16660983.

139. Zhang, Y.-W.; Bi, L.-T.; Hou, S.-P.; Zhao, X.-L.; Song, Y.-L.; Ma, T.-H. Reduced Lung Water Transport Rate Associated with Downregulation of Aquaporin-1 and Aquaporin-5 in Aged Mice. *Clin. Exp. Pharmacol. Physiol.* **2009**, *36*, 734–738, doi:10.1111/j.1440-1681.2009.05156.x.
140. Ho, J.C.; Chan, K.N.; Hu, W.H.; Lam, W.K.; Zheng, L.; Tipoe, G.L.; Sun, J.; Leung, R.; Tsang, K.W. The Effect of Aging on Nasal Mucociliary Clearance, Beat Frequency, and Ultrastructure of Respiratory Cilia. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 983–988, doi:10.1164/ajrccm.163.4.9909121.
141. Grudzinska, F.S.; Brodlie, M.; Scholefield, B.R.; Jackson, T.; Scott, A.; Thickett, D.R.; Sapey, E. Neutrophils in Community-Acquired Pneumonia: Parallels in Dysfunction at the Extremes of Age. *Thorax* **2020**, *75*, 164–171, doi:10.1136/thoraxjnl-2018-212826.
142. Sapey, E.; Patel, J.M.; Greenwood, H.L.; Walton, G.M.; Hazeldine, J.; Sadhra, C.; Parekh, D.; Dancer, R.C.A.; Nightingale, P.; Lord, J.M.; et al. Pulmonary Infections in the Elderly Lead to Impaired Neutrophil Targeting, Which Is Improved by Simvastatin. *Am. J. Respir. Crit. Care Med.* **2017**, *196*, 1325–1336, doi:10.1164/rccm.201704-0814OC.
143. Müller, L.; Fülöp, T.; Pawelec, G. Immunosenescence in Vertebrates and Invertebrates. *Immun. Ageing A* **2013**, *10*, 12, doi:10.1186/1742-4933-10-12.
144. Kaufmann, S.H.E.; Dorhoi, A. Molecular Determinants in Phagocyte-Bacteria Interactions. *Immunity* **2016**, *44*, 476–491, doi:10.1016/j.immuni.2016.02.014.
145. Rivera, A.; Siracusa, M.C.; Yap, G.S.; Gause, W.C. Innate Cell Communication Kick-Starts Pathogen-Specific Immunity. *Nat. Immunol.* **2016**, *17*, 356–363, doi:10.1038/ni.3375.
146. Franchi, L.; Warner, N.; Viani, K.; Nuñez, G. Function of Nod-like Receptors in Microbial Recognition and Host Defense. *Immunol. Rev.* **2009**, *227*, 106–128, doi:10.1111/j.1600-065X.2008.00734.x.
147. Kato, H.; Takahashi, K.; Fujita, T. RIG-I-like Receptors: Cytoplasmic Sensors for Non-Self RNA. *Immunol. Rev.* **2011**, *243*, 91–98, doi:10.1111/j.1600-065X.2011.01052.x.
148. Kumar, S.; Ingle, H.; Prasad, D.V.R.; Kumar, H. Recognition of Bacterial Infection by Innate Immune Sensors. *Crit. Rev. Microbiol.* **2013**, *39*, 229–246, doi:10.3109/1040841X.2012.706249.
149. Plato, A.; Willment, J.A.; Brown, G.D. C-Type Lectin-Like Receptors of the Dectin-1 Cluster: Ligands and Signaling Pathways. *Int. Rev. Immunol.* **2013**, *32*, 134–156, doi:10.3109/08830185.2013.777065.
150. Roers, A.; Hiller, B.; Hornung, V. Recognition of Endogenous Nucleic Acids by the Innate Immune System. *Immunity* **2016**, *44*, 739–754, doi:10.1016/j.immuni.2016.04.002.
151. Lin, L. RAGE on the Toll Road? *Cell. Mol. Immunol.* **2006**, *3*, 351–358.
152. Cruickshanks, H.A.; McBryan, T.; Nelson, D.M.; VanderKraats, N.D.; Shah, P.P.; van Tuyn, J.; Singh Rai, T.; Brock, C.; Donahue, G.; Dunican, D.S.; et al. Senescent Cells Harbour Features of the Cancer Epigenome. *Nat. Cell Biol.* **2013**, *15*, 1495–1506, doi:10.1038/ncb2879.
153. Simon, M.; Meter, M.V.; Abulaeva, J.; Ke, Z.; Gonzalez, R.S.; Taguchi, T.; Cecco, M.D.; Leonova, K.I.; Kogan, V.; Helfand, S.L.; et al. LINE1 Derepression in Aged Wild Type and SIRT6 Deficient Mice Drives Inflammation. *Cell Metab.* **2019**, *29*, 871–885.e5, doi:10.1016/j.cmet.2019.02.014.
154. Franceschi, C.; Garagnani, P.; Vitale, G.; Capri, M.; Salvioli, S. Inflammaging and ‘Garb-Aging.’ *Trends Endocrinol. Metab.* **2017**, *28*, 199–212, doi:10.1016/j.tem.2016.09.005.
155. Dranoff, G. Cytokines in Cancer Pathogenesis and Cancer Therapy. *Nat. Rev. Cancer* **2004**, *4*, 11–22, doi:10.1038/nrc1252.
156. Markiewski, M.M.; Lambris, J.D. The Role of Complement in Inflammatory Diseases from behind the Scenes into the Spotlight. *Am. J. Pathol.* **2007**, *171*, 715–727, doi:10.2353/ajpath.2007.070166.

157. Pang, W.W.; Price, E.A.; Sahoo, D.; Beerman, I.; Maloney, W.J.; Rossi, D.J.; Schrier, S.L.; Weissman, I.L. Human Bone Marrow Hematopoietic Stem Cells Are Increased in Frequency and Myeloid-Biased with Age. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 20012–20017, doi:10.1073/pnas.1116110108.
158. Rossi, D.J.; Bryder, D.; Zahn, J.M.; Ahlenius, H.; Sonu, R.; Wagers, A.J.; Weissman, I.L. Cell Intrinsic Alterations Underlie Hematopoietic Stem Cell Aging. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 9194–9199, doi:10.1073/pnas.0503280102.
159. Geiger, H.; de Haan, G.; Florian, M.C. The Ageing Haematopoietic Stem Cell Compartment. *Nat. Rev. Immunol.* **2013**, *13*, 376–389, doi:10.1038/nri3433.
160. Jung, H.; Kim, D.O.; Byun, J.-E.; Kim, W.S.; Kim, M.J.; Song, H.Y.; Kim, Y.K.; Kang, D.-K.; Park, Y.-J.; Kim, T.-D.; et al. Thioredoxin-Interacting Protein Regulates Haematopoietic Stem Cell Ageing and Rejuvenation by Inhibiting P38 Kinase Activity. *Nat. Commun.* **2016**, *7*, 13674, doi:10.1038/ncomms13674.
161. Kim, M.; Moon, H.-B.; Spangrude, G.J. Major Age-Related Changes of Mouse Hematopoietic Stem/Progenitor Cells. *Ann. N. Y. Acad. Sci.* **2003**, *996*, 195–208, doi:10.1111/j.1749-6632.2003.tb03247.x.
162. Liang, Y.; Van Zant, G.; Szilvassy, S.J. Effects of Aging on the Homing and Engraftment of Murine Hematopoietic Stem and Progenitor Cells. *Blood* **2005**, *106*, 1479–1487, doi:10.1182/blood-2004-11-4282.
163. Xing, Z.; Ryan, M.A.; Daria, D.; Nattamai, K.J.; Van Zant, G.; Wang, L.; Zheng, Y.; Geiger, H. Increased Hematopoietic Stem Cell Mobilization in Aged Mice. *Blood* **2006**, *108*, 2190–2197, doi:10.1182/blood-2005-12-010272.
164. de Haan, G.; Lazare, S.S. Aging of Hematopoietic Stem Cells. *Blood* **2018**, *131*, 479–487, doi:10.1182/blood-2017-06-746412.
165. Lee, J.; Yoon, S.R.; Choi, I.; Jung, H. Causes and Mechanisms of Hematopoietic Stem Cell Aging. *Int. J. Mol. Sci.* **2019**, *20*, doi:10.3390/ijms20061272.
166. Mejia-Ramirez, E.; Florian, M.C. Understanding Intrinsic Hematopoietic Stem Cell Aging. *Haematologica* **2020**, *105*, 22–37, doi:10.3324/haematol.2018.211342.
167. Ergen, A.V.; Boles, N.C.; Goodell, M.A. Rantes/Ccl5 Influences Hematopoietic Stem Cell Subtypes and Causes Myeloid Skewing. *Blood* **2012**, *119*, 2500–2509, doi:10.1182/blood-2011-11-391730.
168. He, H.; Xu, P.; Zhang, X.; Liao, M.; Dong, Q.; Cong, T.; Tang, B.; Yang, X.; Ye, M.; Chang, Y.-J.; et al. Aging-Induced IL27Ra Signaling Impairs Hematopoietic Stem Cells. *Blood*, doi:10.1182/blood.2019003910.
169. Lee, B.-C.; Yu, K.-R. Impact of Mesenchymal Stem Cell Senescence on Inflammaging. *BMB Rep.* **2020**, *53*, 65–73, doi:10.5483/BMBRep.2020.53.2.291.
170. Esplin, B.L.; Shimazu, T.; Welner, R.S.; Garrett, K.P.; Nie, L.; Zhang, Q.; Humphrey, M.B.; Yang, Q.; Borghesi, L.A.; Kincade, P.W. Chronic Exposure to a TLR Ligand Injures Hematopoietic Stem Cells. *J. Immunol. Baltim. Md 1950* **2011**, *186*, 5367–5375, doi:10.4049/jimmunol.1003438.
171. Kapsenberg, M.L. Dendritic-Cell Control of Pathogen-Driven T-Cell Polarization. *Nat. Rev. Immunol.* **2003**, *3*, 984–993, doi:10.1038/nri1246.
172. Behrens, G.; Li, M.; Smith, C.M.; Belz, G.T.; Mintern, J.; Carbone, F.R.; Heath, W.R. Helper T Cells, Dendritic Cells and CTL Immunity. *Immunol. Cell Biol.* **2004**, *82*, 84–90, doi:10.1111/j.1440-1711.2004.01211.x.
173. Agrawal, A.; Agrawal, S.; Cao, J.-N.; Su, H.; Osann, K.; Gupta, S. Altered Innate Immune Functioning of Dendritic Cells in Elderly Humans: A Role of Phosphoinositide 3-Kinase-Signaling Pathway. *J. Immunol. Baltim. Md 1950* **2007**, *178*, 6912–6922.
174. Agrawal, A.; Tay, J.; Ton, S.; Agrawal, S.; Gupta, S. Increased Reactivity of Dendritic Cells from Aged Subjects to Self-Antigen, the Human DNA. *J. Immunol. Baltim. Md 1950* **2009**, *182*, 1138–1145, doi:10.4049/jimmunol.182.2.1138.

175. Agrawal, S.; Gollapudi, S.; Gupta, S.; Agrawal, A. Dendritic Cells from the Elderly Display an Intrinsic Defect in the Production of IL-10 in Response to Lithium Chloride. *Exp. Gerontol.* **2013**, *48*, 1285–1292, doi:10.1016/j.exger.2013.08.006.
176. Wong, C.; Goldstein, D.R. Impact of Aging on Antigen Presentation Cell Function of Dendritic Cells. *Curr. Opin. Immunol.* **2013**, *25*, 535–541, doi:10.1016/j.coi.2013.05.016.
177. Kornete, M.; Piccirillo, C.A. Functional Crosstalk between Dendritic Cells and Foxp3+ Regulatory T Cells in the Maintenance of Immune Tolerance. *Front. Immunol.* **2012**, *3*, doi:10.3389/fimmu.2012.00165.
178. Aydar, Y.; Balogh, P.; Tew, J.G.; Szakal, A.K. Age-Related Depression of FDC Accessory Functions and CD21 Ligand-Mediated Repair of Co-Stimulation. *Eur. J. Immunol.* **2002**, *32*, 2817–2826, doi:10.1002/1521-4141(200210)32:10<2817::AID-IMMU2817>3.0.CO;2-Z.
179. Agrawal, A.; Agrawal, S.; Gupta, S. Role of Dendritic Cells in Inflammation and Loss of Tolerance in the Elderly. *Front. Immunol.* **2017**, *8*, doi:10.3389/fimmu.2017.00896.
180. Orsini, G.; Legitimo, A.; Failli, A.; Massei, F.; Biver, P.; Consolini, R. Enumeration of Human Peripheral Blood Dendritic Cells throughout the Life. *Int. Immunol.* **2012**, *24*, 347–356, doi:10.1093/intimm/dxs006.
181. Garbe, K.; Bratke, K.; Wagner, S.; Virchow, J.C.; Lommatzsch, M. Plasmacytoid Dendritic Cells and Their Toll-like Receptor 9 Expression Selectively Decrease with Age. *Hum. Immunol.* **2012**, *73*, 493–497, doi:10.1016/j.humimm.2012.02.007.
182. Jing, Y.; Shaheen, E.; Drake, R.R.; Chen, N.; Gravenstein, S.; Deng, Y. Aging Is Associated with a Numerical and Functional Decline in Plasmacytoid Dendritic Cells, Whereas Myeloid Dendritic Cells Are Relatively Unaltered in Human Peripheral Blood. *Hum. Immunol.* **2009**, *70*, 777–784, doi:10.1016/j.humimm.2009.07.005.
183. Chatta, G.S.; Andrews, R.G.; Rodger, E.; Schrag, M.; Hammond, W.P.; Dale, D.C. Hematopoietic Progenitors and Aging: Alterations in Granulocytic Precursors and Responsiveness to Recombinant Human G-CSF, GM-CSF, and IL-3. *J. Gerontol.* **1993**, *48*, M207–212, doi:10.1093/geronj/48.5.m207.
184. Wenisch, C.; Patruta, S.; Daxböck, F.; Krause, R.; Hörl, W. Effect of Age on Human Neutrophil Function. *J. Leukoc. Biol.* **2000**, *67*, 40–45.
185. Niwa, Y.; Kasama, T.; Miyachi, Y.; Kanoh, T. Neutrophil Chemotaxis, Phagocytosis and Parameters of Reactive Oxygen Species in Human Aging: Cross-Sectional and Longitudinal Studies. *Life Sci.* **1989**, *44*, 1655–1664.
186. Simell, B.; Vuorela, A.; Ekström, N.; Palmu, A.; Reunanen, A.; Meri, S.; Käyhty, H.; Väkeväinen, M. Aging Reduces the Functionality of Anti-Pneumococcal Antibodies and the Killing of Streptococcus Pneumoniae by Neutrophil Phagocytosis. *Vaccine* **2011**, *29*, 1929–1934, doi:10.1016/j.vaccine.2010.12.121.
187. Nomellini, V.; Brubaker, A.L.; Mahbub, S.; Palmer, J.L.; Gomez, C.R.; Kovacs, E.J. Dysregulation of Neutrophil CXCR2 and Pulmonary Endothelial Icam-1 Promotes Age-Related Pulmonary Inflammation. *Aging Dis.* **2012**, *3*, 234–247.
188. Gomez, C.R.; Hirano, S.; Cutro, B.T.; Birjandi, S.; Baila, H.; Nomellini, V.; Kovacs, E.J. Advanced Age Exacerbates the Pulmonary Inflammatory Response after Lipopolysaccharide Exposure. *Crit. Care Med.* **2007**, *35*, 246–251, doi:10.1097/01.CCM.0000251639.05135.E0.
189. Naccache, P.H.; Lefebvre, J.S. A Straight Neutrophil Path to Healthy Aging? *Blood* **2014**, *123*, 154–156, doi:10.1182/blood-2013-11-538256.
190. Sapey, E.; Greenwood, H.; Walton, G.; Mann, E.; Love, A.; Aaronson, N.; Insall, R.H.; Stockley, R.A.; Lord, J.M. Phosphoinositide 3-Kinase Inhibition Restores Neutrophil Accuracy in the Elderly: Toward Targeted Treatments for Immunosenescence. *Blood* **2014**, *123*, 239–248, doi:10.1182/blood-2013-08-519520.
191. Sauce, D.; Dong, Y.; Campillo-Gimenez, L.; Casulli, S.; Bayard, C.; Autran, B.; Boddaert, J.; Appay, V.; Elbim, C. Reduced Oxidative Burst by Primed Neutrophils in the Elderly Individuals Is Associated With Increased Levels



- of the CD16bright/CD62Ldim Immunosuppressive Subset. *J. Gerontol. Ser. A* **2017**, *72*, 163–172, doi:10.1093/gerona/glw062.
192. Ogawa, K.; Suzuki, K.; Okutsu, M.; Yamazaki, K.; Shinkai, S. The Association of Elevated Reactive Oxygen Species Levels from Neutrophils with Low-Grade Inflammation in the Elderly. *Immun. Ageing A* **2008**, *5*, 13, doi:10.1186/1742-4933-5-13.
  193. Braga, P.C.; Sala, M.T.; Sasso, M.D.; Mancini, L.; Sandrini, M.C.; Annoni, G. Influence of Age on Oxidative Bursts (Chemiluminescence) of Polymorphonuclear Neutrophil Leukocytes. *Gerontology* **1998**, *44*, 192–197, doi:10.1159/000022009.
  194. Kovalenko, E.I.; Boyko, A.A.; Semenov, V.F.; Lutsenko, G.V.; Grechikhina, M.V.; Kanevskiy, L.M.; Azhikina, T.L.; Telford, W.G.; Sapozhnikov, A.M. ROS Production, Intracellular HSP70 Levels and Their Relationship in Human Neutrophils: Effects of Age. *Oncotarget* **2014**, *5*, 11800–11812.
  195. Hazeldine, J.; Harris, P.; Chapple, I.L.; Grant, M.; Greenwood, H.; Livesey, A.; Sapey, E.; Lord, J.M. Impaired Neutrophil Extracellular Trap Formation: A Novel Defect in the Innate Immune System of Aged Individuals. *Aging Cell* **2014**, *13*, 690–698, doi:10.1111/ace.12222.
  196. Brubaker, A.L.; Rendon, J.L.; Ramirez, L.; Choudhry, M.A.; Kovacs, E.J. Reduced Neutrophil Chemotaxis and Infiltration Contributes to Delayed Resolution of Cutaneous Wound Infection with Advanced Age. *J. Immunol. Baltim. Md 1950* **2013**, *190*, 1746–1757, doi:10.4049/jimmunol.1201213.
  197. Butcher, S.; Chahel, H.; Lord, J.M. Ageing and the Neutrophil: No Appetite for Killing? *Immunology* **2000**, *100*, 411–416, doi:10.1046/j.1365-2567.2000.00079.x.
  198. Wessels, I.; Jansen, J.; Rink, L.; Uciechowski, P. Immunosenescence of Polymorphonuclear Neutrophils. *Sci. World J.* **2010**, *10*, 145–160, doi:10.1100/tsw.2010.14.
  199. De Martinis, M.; Modesti, M.; Ginaldi, L. Phenotypic and Functional Changes of Circulating Monocytes and Polymorphonuclear Leucocytes from Elderly Persons. *Immunol. Cell Biol.* **2004**, *82*, 415–420, doi:10.1111/j.0818-9641.2004.01242.x.
  200. Tortorella, C.; Polignano, A.; Piazzolla, G.; Serrone, M.; Jirillo, E.; Antonaci, S. Lipopolysaccharide-, Granulocyte-Monocyte Colony Stimulating Factor and Pentoxifylline-Mediated Effects on Formyl-Methionyl-Leucine-Phenylalanine-Stimulated Neutrophil Respiratory Burst in the Elderly. *Microbios* **1996**, *85*, 189–198.
  201. Uciechowski, P.; Rink, L. Neutrophil Granulocyte Functions in the Elderly. In *Handbook on Immunosenescence: Basic Understanding and Clinical Applications*; Fulop, T., Franceschi, C., Hirokawa, K., Pawelec, G., Eds.; Springer Netherlands: Dordrecht, 2009; pp. 439–455 ISBN 978-1-4020-9063-9.
  202. Lipschitz, D.A.; Udupa, K.B.; Boxer, L.A. The Role of Calcium in the Age-Related Decline of Neutrophil Function. *Blood* **1988**, *71*, 659–665.
  203. Tanji-Matsuba, K.; van Eeden, S.F.; Saito, Y.; Okazawa, M.; Klut, M.E.; Hayashi, S.; Hogg, J.C. Functional Changes in Aging Polymorphonuclear Leukocytes. *Circulation* **1998**, *97*, 91–98, doi:10.1161/01.cir.97.1.91.
  204. Rao, K.M. Age-Related Decline in Ligand-Induced Actin Polymerization in Human Leukocytes and Platelets. *J. Gerontol.* **1986**, *41*, 561–566, doi:10.1093/geronj/41.5.561.
  205. Ito, T.K.; Yokoyama, M.; Yoshida, Y.; Nojima, A.; Kassai, H.; Oishi, K.; Okada, S.; Kinoshita, D.; Kobayashi, Y.; Fruttiger, M.; et al. A Crucial Role for CDC42 in Senescence-Associated Inflammation and Atherosclerosis. *PLoS ONE* **2014**, *9*, e102186, doi:10.1371/journal.pone.0102186.
  206. Uciechowski, P.; Rink, L. Basophil, Eosinophil, and Neutrophil Functions in the Elderly. In *Immunology of Aging*; Massoud, A., Rezaei, N., Eds.; Springer: Berlin, Heidelberg, 2014; pp. 47–63 ISBN 978-3-642-39495-9.
  207. van Beek, A.A.; Franssen, F.; Meijer, B.; de Vos, P.; Knol, E.F.; Savelkoul, H.F.J. Aged Mice Display Altered Numbers and Phenotype of Basophils, and Bone Marrow-Derived Basophil Activation, with a Limited Role for Aging-Associated Microbiota. *Immun. Ageing A* **2018**, *15*, doi:10.1186/s12979-018-0135-6.



208. He, Z.; Allers, C.; Sugimoto, C.; Ahmed, N.; Fujioka, H.; Kim, W.-K.; Didier, E.S.; Kuroda, M.J. Rapid Turnover and High Production Rate of Myeloid Cells in Adult Rhesus Macaques with Compensations during Aging. *J. Immunol. Baltim. Md 1950* **2018**, *200*, 4059–4067, doi:10.4049/jimmunol.1800207.
209. Song, C.; Vandewoude, M.; Stevens, W.; De Clerck, L.; Van der Planken, M.; Whelan, A.; Anisman, H.; Dossche, A.; Maes, M. Alterations in Immune Functions during Normal Aging and Alzheimer's Disease. *Psychiatry Res.* **1999**, *85*, 71–80, doi:10.1016/s0165-1781(98)00130-9.
210. Schwarzenbach, H.R.; Nakagawa, T.; Conroy, M.C.; de Weck, A.L. Skin Reactivity, Basophil Degranulation and IgE Levels in Ageing. *Clin. Allergy* **1982**, *12*, 465–473, doi:10.1111/j.1365-2222.1982.tb01645.x.
211. Mathur, S.K.; Schwantes, E.A.; Jarjour, N.N.; Busse, W.W. Age-Related Changes in Eosinophil Function in Human Subjects. *Chest* **2008**, *133*, 412–419, doi:10.1378/chest.07-2114.
212. Abernathy-Close, L.; Dieterle, M.G.; Vendrov, K.C.; Bergin, I.L.; Rao, K.; Young, V.B. Aging Dampens the Intestinal Innate Immune Response during Severe Clostridioides Difficile Infection and Is Associated with Altered Cytokine Levels and Granulocyte Mobilization. *Infect. Immun.* **2020**, *88*, doi:10.1128/IAI.00960-19.
213. Yagi, T.; Sato, A.; Hayakawa, H.; Ide, K. Failure of Aged Rats to Accumulate Eosinophils in Allergic Inflammation of the Airway. *J. Allergy Clin. Immunol.* **1997**, *99*, 38–47, doi:10.1016/S0091-6749(97)70298-7.
214. Karasuyama, H.; Miyake, K.; Yoshikawa, S.; Kawano, Y.; Yamanishi, Y. How Do Basophils Contribute to Th2 Cell Differentiation and Allergic Responses? *Int. Immunol.* **2018**, *30*, 391–396, doi:10.1093/intimm/dxy026.
215. Faunce, D.E.; Palmer, J.L.; Paskowicz, K.K.; Witte, P.L.; Kovacs, E.J. CD1d-Restricted NKT Cells Contribute to the Age-Associated Decline of T Cell Immunity. *J. Immunol. Baltim. Md 1950* **2005**, *175*, 3102–3109.
216. Inui, T.; Nakagawa, R.; Ohkura, S.; Habu, Y.; Koike, Y.; Motoki, K.; Kuranaga, N.; Fukasawa, M.; Shinomiya, N.; Seki, S. Age-Associated Augmentation of the Synthetic Ligand-Mediated Function of Mouse NK1.1 Ag(+) T Cells: Their Cytokine Production and Hepatotoxicity in Vivo and in Vitro. *J. Immunol. Baltim. Md 1950* **2002**, *169*, 6127–6132.
217. Borrego, F.; Alonso, M.C.; Galiani, M.D.; Carracedo, J.; Ramirez, R.; Ostos, B.; Peña, J.; Solana, R. NK Phenotypic Markers and IL2 Response in NK Cells from Elderly People. *Exp. Gerontol.* **1999**, *34*, 253–265.
218. Facchini, A.; Mariani, E.; Mariani, A.R.; Papa, S.; Vitale, M.; Manzoli, F.A. Increased Number of Circulating Leu 11+ (CD 16) Large Granular Lymphocytes and Decreased NK Activity during Human Ageing. *Clin. Exp. Immunol.* **1987**, *68*, 340–347.
219. Vitale, M.; Zamai, L.; Neri, L.M.; Galanzi, A.; Facchini, A.; Rana, R.; Cataldi, A.; Papa, S. The Impairment of Natural Killer Function in the Healthy Aged Is Due to a Postbinding Deficient Mechanism. *Cell. Immunol.* **1992**, *145*, 1–10, doi:10.1016/0008-8749(92)90307-B.
220. DelaRosa, O.; Tarazona, R.; Casado, J.G.; Alonso, C.; Ostos, B.; Peña, J.; Solana, R. Valpha24+ NKT Cells Are Decreased in Elderly Humans. *Exp. Gerontol.* **2002**, *37*, 213–217.
221. Peralbo, E.; DelaRosa, O.; Gayoso, I.; Pita, M.L.; Tarazona, R.; Solana, R. Decreased Frequency and Proliferative Response of Invariant Valpha24Vbeta11 Natural Killer T (INKT) Cells in Healthy Elderly. *Biogerontology* **2006**, *7*, 483–492, doi:10.1007/s10522-006-9063-5.
222. Campos, C.; Pera, A.; Sanchez-Correa, B.; Alonso, C.; Lopez-Fernandez, I.; Morgado, S.; Tarazona, R.; Solana, R. Effect of Age and CMV on NK Cell Subpopulations. *Exp. Gerontol.* **2014**, *54*, 130–137, doi:10.1016/j.exger.2014.01.008.
223. Chidrawar, S.M.; Khan, N.; Chan, Y.L.T.; Nayak, L.; Moss, P.A. Ageing Is Associated with a Decline in Peripheral Blood CD56bright NK Cells. *Immun. Ageing* **2006**, *3*, 10, doi:10.1186/1742-4933-3-10.
224. Solana, R.; Campos, C.; Pera, A.; Tarazona, R. Shaping of NK Cell Subsets by Aging. *Curr. Opin. Immunol.* **2014**, *29*, 56–61, doi:10.1016/j.coi.2014.04.002.

225. Almeida-Oliveira, A.; Smith-Carvalho, M.; Porto, L.C.; Cardoso-Oliveira, J.; Ribeiro, A. dos S.; Falcão, R.R.; Abdelhay, E.; Bouzas, L.F.; Thuler, L.C.S.; Ornellas, M.H.; et al. Age-Related Changes in Natural Killer Cell Receptors from Childhood through Old Age. *Hum. Immunol.* **2011**, *72*, 319–329, doi:10.1016/j.humimm.2011.01.009.
226. Le Garff-Tavernier, M.; Béziat, V.; Decocq, J.; Siguret, V.; Gandjbakhch, F.; Pautas, E.; Debré, P.; Merle-Beral, H.; Vieillard, V. Human NK Cells Display Major Phenotypic and Functional Changes over the Life Span. *Aging Cell* **2010**, *9*, 527–535, doi:10.1111/j.1474-9726.2010.00584.x.
227. Camous, X.; Pera, A.; Solana, R.; Larbi, A. NK Cells in Healthy Aging and Age-Associated Diseases Available online: <https://www.hindawi.com/journals/bmri/2012/195956/> (accessed on 28 November 2018).
228. Solana, R.; Tarazona, R.; Gayoso, I.; Lesur, O.; Dupuis, G.; Fulop, T. Innate Immunosenescence: Effect of Aging on Cells and Receptors of the Innate Immune System in Humans. *Semin. Immunol.* **2012**, *24*, 331–341, doi:10.1016/j.smim.2012.04.008.
229. Hazeldine, J.; Hampson, P.; Lord, J.M. Reduced Release and Binding of Perforin at the Immunological Synapse Underlies the Age-Related Decline in Natural Killer Cell Cytotoxicity. *Aging Cell* **2012**, *11*, 751–759, doi:10.1111/j.1474-9726.2012.00839.x.
230. Haynes, L.; Linton, P.-J.; Eaton, S.M.; Tonkonogy, S.L.; Swain, S.L. Interleukin 2, but Not Other Common  $\gamma$  Chain-Binding Cytokines, Can Reverse the Defect in Generation of Cd4 Effector T Cells from Naive T Cells of Aged Mice. *J. Exp. Med.* **1999**, *190*, 1013–1024.
231. Myśliwska, J.; Bryl, E.; Foerster, J.; Myśliwski, A. Increase of Interleukin 6 and Decrease of Interleukin 2 Production during the Ageing Process Are Influenced by the Health Status. *Mech. Ageing Dev.* **1998**, *100*, 313–328, doi:10.1016/s0047-6374(97)00154-1.
232. Whisler, R.L.; Beiqing, L.; Chen, M. Age-Related Decreases in IL-2 Production by Human T Cells Are Associated with Impaired Activation of Nuclear Transcriptional Factors AP-1 and NF-AT. *Cell. Immunol.* **1996**, *169*, 185–195, doi:10.1006/cimm.1996.0109.
233. Gounder, S.S.; Abdullah, B.J.J.; Radzuanb, N.E.I.B.M.; Zain, F.D.B.M.; Sait, N.B.M.; Chua, C.; Subramani, B. Effect of Aging on NK Cell Population and Their Proliferation at Ex Vivo Culture Condition. *Anal. Cell. Pathol. Amst.* **2018**, *2018*, 7871814, doi:10.1155/2018/7871814.
234. Albright, J.M.; Dunn, R.C.; Shults, J.A.; Boe, D.M.; Afshar, M.; Kovacs, E.J. Advanced Age Alters Monocyte and Macrophage Responses. *Antioxid. Redox Signal.* **2016**, *25*, 805–815, doi:10.1089/ars.2016.6691.
235. Ortega-Gómez, A.; Perretti, M.; Soehnlein, O. Resolution of Inflammation: An Integrated View. *EMBO Mol. Med.* **2013**, *5*, 661–674, doi:10.1002/emmm.201202382.
236. Krishnamoorthy, N.; Abdunour, R.-E.E.; Walker, K.H.; Engstrom, B.D.; Levy, B.D. Specialized Proresolving Mediators in Innate and Adaptive Immune Responses in Airway Diseases. *Physiol. Rev.* **2018**, *98*, 1335–1370, doi:10.1152/physrev.00026.2017.
237. Duffney, P.F.; Falsetta, M.L.; Rackow, A.R.; Thatcher, T.H.; Phipps, R.P.; Sime, P.J. Key Roles for Lipid Mediators in the Adaptive Immune Response. *J. Clin. Invest.* **2018**, *128*, 2724–2731, doi:10.1172/JCI97951.
238. Krishnamoorthy, N.; Burkett, P.R.; Dalli, J.; Abdunour, R.-E.E.; Colas, R.; Ramon, S.; Phipps, R.P.; Petasis, N.A.; Kuchroo, V.K.; Serhan, C.N.; et al. Cutting Edge: Maresin-1 Engages Regulatory T Cells to Limit Type 2 Innate Lymphoid Cell Activation and Promote Resolution of Lung Inflammation. *J. Immunol. Baltim. Md 1950* **2015**, *194*, 863–867, doi:10.4049/jimmunol.1402534.
239. Chiurchiù, V.; Leuti, A.; Dalli, J.; Jacobsson, A.; Battistini, L.; Maccarrone, M.; Serhan, C.N. Proresolving Lipid Mediators Resolvin D1, Resolvin D2, and Maresin 1 Are Critical in Modulating T Cell Responses. *Sci. Transl. Med.* **2016**, *8*, 353ra111, doi:10.1126/scitranslmed.aaf7483.
240. Rea, I.M.; Gibson, D.S.; McGilligan, V.; McNerlan, S.E.; Alexander, H.D.; Ross, O.A. Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines. *Front. Immunol.* **2018**, *9*, doi:10.3389/fimmu.2018.00586.

241. Duvall, M.G.; Levy, B.D. DHA- and EPA-Derived Resolvins, Protectins, and Maresins in Airway Inflammation. *Eur. J. Pharmacol.* **2016**, *785*, 144–155, doi:10.1016/j.ejphar.2015.11.001.
242. Moro, K.; Nagahashi, M.; Ramanathan, R.; Takabe, K.; Wakai, T. Resolvins and Omega Three Polyunsaturated Fatty Acids: Clinical Implications in Inflammatory Diseases and Cancer. *World J. Clin. Cases* **2016**, *4*, 155–164, doi:10.12998/wjcc.v4.i7.155.
243. Serhan, C.N.; Petasis, N.A. Resolvins and Protectins in Inflammation-Resolution. *Chem. Rev.* **2011**, *111*, 5922–5943, doi:10.1021/cr100396c.
244. Börgeson, E.; McGillicuddy, F.C.; Harford, K.A.; Corrigan, N.; Higgins, D.F.; Maderna, P.; Roche, H.M.; Godson, C. Lipoxin A4 Attenuates Adipose Inflammation. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2012**, *26*, 4287–4294, doi:10.1096/fj.12-208249.
245. Gangemi, S.; Pescara, L.; D'Urbano, E.; Basile, G.; Nicita-Mauro, V.; Davì, G.; Romano, M. Aging Is Characterized by a Profound Reduction in Anti-Inflammatory Lipoxin A4 Levels. *Exp. Gerontol.* **2005**, *40*, 612–614, doi:10.1016/j.exger.2005.04.004.
246. Doyle, R.; Sadlier, D.M.; Godson, C. Pro-Resolving Lipid Mediators: Agents of Anti-Ageing? *Semin. Immunol.* **2018**, *40*, 36–48, doi:10.1016/j.smim.2018.09.002.
247. Arnardottir, H.H.; Dalli, J.; Colas, R.A.; Shinohara, M.; Serhan, C.N. Aging Delays Resolution of Acute Inflammation in Mice: Reprogramming the Host Response with Novel Nano-Proresolving Medicines. *J. Immunol. Baltim. Md 1950* **2014**, *193*, 4235–4244, doi:10.4049/jimmunol.1401313.
248. Dennis, E.A.; Norris, P.C. Eicosanoid Storm in Infection and Inflammation. *Nat. Rev. Immunol.* **2015**, *15*, 511–523, doi:10.1038/nri3859.
249. Serhan, C.N. Pro-Resolving Lipid Mediators Are Leads for Resolution Physiology. *Nature* **2014**, *510*, 92–101, doi:10.1038/nature13479.
250. Dalli, J.; Serhan, C.N. Pro-Resolving Mediators in Regulating and Conferring Macrophage Function. *Front. Immunol.* **2017**, *8*, doi:10.3389/fimmu.2017.01400.
251. Chen, D.; Chao, D.L.; Rocha, L.; Kolar, M.; Huu, V.A.N.; Krawczyk, M.; Dasyani, M.; Wang, T.; Jafari, M.; Jabari, M.; et al. The Lipid Elongation Enzyme ELOVL2 Is a Molecular Regulator of Aging in the Retina. *Aging Cell* **2020**, *19*, e13100, doi:10.1111/accel.13100.
252. Bacalini, M.G.; Deelen, J.; Pirazzini, C.; De Cecco, M.; Giuliani, C.; Lanzarini, C.; Ravaioli, F.; Marasco, E.; van Heemst, D.; Suchiman, H.E.D.; et al. Systemic Age-Associated DNA Hypermethylation of ELOVL2 Gene: In Vivo and In Vitro Evidences of a Cell Replication Process. *J. Gerontol. Ser. A* **2017**, *72*, 1015–1023, doi:10.1093/gerona/glw185.
253. Garagnani, P.; Bacalini, M.G.; Pirazzini, C.; Gori, D.; Giuliani, C.; Mari, D.; Di Blasio, A.M.; Gentilini, D.; Vitale, G.; Collino, S.; et al. Methylation of ELOVL2 Gene as a New Epigenetic Marker of Age. *Aging Cell* **2012**, *11*, 1132–1134, doi:10.1111/accel.12005.
254. Slieker, R.C.; Relton, C.L.; Gaunt, T.R.; Slagboom, P.E.; Heijmans, B.T. Age-Related DNA Methylation Changes Are Tissue-Specific with ELOVL2 Promoter Methylation as Exception. *Epigenetics Chromatin* **2018**, *11*, doi:10.1186/s13072-018-0191-3.
255. Ginhoux, F.; Williams, M. Tissue-Resident Macrophage Ontogeny and Homeostasis. *Immunity* **2016**, *44*, 439–449, doi:10.1016/j.immuni.2016.02.024.
256. Nayar, S.; Dasgupta, P.; Galustian, C. Extending the Lifespan and Efficacies of Immune Cells Used in Adoptive Transfer for Cancer Immunotherapies-A Review. *Oncoimmunology* **2015**, *4*, e1002720, doi:10.1080/2162402X.2014.1002720.
257. Sathe, P.; Shortman, K. The Steady-State Development of Splenic Dendritic Cells. *Mucosal Immunol.* **2008**, *1*, 425–431, doi:10.1038/mi.2008.56.

258. Dimri, G.P.; Lee, X.; Basile, G.; Acosta, M.; Scott, G.; Roskelley, C.; Medrano, E.E.; Linskens, M.; Rubelj, I.; Pereira-Smith, O. A Biomarker That Identifies Senescent Human Cells in Culture and in Aging Skin in Vivo. *Proc. Natl. Acad. Sci.* **1995**, *92*, 9363–9367, doi:10.1073/pnas.92.20.9363.
259. Majumdar, A.; Cruz, D.; Asamoah, N.; Buxbaum, A.; Sohar, I.; Lobel, P.; Maxfield, F.R. Activation of Microglia Acidifies Lysosomes and Leads to Degradation of Alzheimer Amyloid Fibrils. *Mol. Biol. Cell* **2007**, *18*, 1490–1496, doi:10.1091/mbc.e06-10-0975.
260. Majumdar, A.; Capetillo-Zarate, E.; Cruz, D.; Gouras, G.K.; Maxfield, F.R. Degradation of Alzheimer's Amyloid Fibrils by Microglia Requires Delivery of CIC-7 to Lysosomes. *Mol. Biol. Cell* **2011**, *22*, 1664–1676, doi:10.1091/mbc.E10-09-0745.
261. Franceschi, C.; Bonafè, M.; Valensin, S.; Olivieri, F.; De Luca, M.; Ottaviani, E.; De Benedictis, G. Inflamm-Aging. An Evolutionary Perspective on Immunosenescence. *Ann. N. Y. Acad. Sci.* **2000**, *908*, 244–254.
262. Takahashi, I.; Ohmoto, E.; Aoyama, S.; Takizawa, M.; Oda, Y.; Nonaka, K.; Nakada, H.; Yorimitsu, S.; Kimura, I. Monocyte Chemiluminescence and Macrophage Precursors in the Aged. *Acta Med. Okayama* **1985**, *39*, 447–451, doi:10.18926/AMO/31506.
263. Hearps, A.C.; Martin, G.E.; Angelovich, T.A.; Cheng, W.-J.; Maisa, A.; Landay, A.L.; Jaworowski, A.; Crowe, S.M. Aging Is Associated with Chronic Innate Immune Activation and Dysregulation of Monocyte Phenotype and Function. *Aging Cell* **2012**, *11*, 867–875, doi:10.1111/j.1474-9726.2012.00851.x.
264. Nyugen, J.; Agrawal, S.; Gollapudi, S.; Gupta, S. Impaired Functions of Peripheral Blood Monocyte Subpopulations in Aged Humans. *J. Clin. Immunol.* **2010**, *30*, 806–813, doi:10.1007/s10875-010-9448-8.
265. Linehan, E.; Dombrowski, Y.; Snoddy, R.; Fallon, P.G.; Kissenpfennig, A.; Fitzgerald, D.C. Aging Impairs Peritoneal but Not Bone Marrow-Derived Macrophage Phagocytosis. *Aging Cell* **2014**, *13*, 699–708, doi:10.1111/accel.12223.
266. Takahashi, R.; Totsuka, S.; Ishigami, A.; Kobayashi, Y.; Nagata, K. Attenuated Phagocytosis of Secondary Necrotic Neutrophils by Macrophages in Aged and SMP30 Knockout Mice. *Geriatr. Gerontol. Int.* **2016**, *16*, 135–142, doi:10.1111/ggi.12436.
267. Kohut, M.L.; Senchina, D.S.; Madden, K.S.; Martin, A.E.; Felten, D.L.; Moynihan, J.A. Age Effects on Macrophage Function Vary by Tissue Site, Nature of Stimulant, and Exercise Behavior. *Exp. Gerontol.* **2004**, *39*, 1347–1360, doi:10.1016/j.exger.2004.07.001.
268. Gomez, C.R.; Karavitis, J.; Palmer, J.L.; Faunce, D.E.; Ramirez, L.; Nomellini, V.; Kovacs, E.J. Interleukin-6 Contributes to Age-Related Alteration of Cytokine Production by Macrophages. *Mediators Inflamm.* **2010**, *2010*, 475139, doi:10.1155/2010/475139.
269. Liang, S.; Domon, H.; Hosur, K.B.; Wang, M.; Hajishengallis, G. Age-Related Alterations in Innate Immune Receptor Expression and Ability of Macrophages to Respond to Pathogen Challenge in Vitro. *Mech. Ageing Dev.* **2009**, *130*, 538–546, doi:10.1016/j.mad.2009.06.006.
270. Chelvarajan, R.L.; Liu, Y.; Popa, D.; Getchell, M.L.; Getchell, T.V.; Stromberg, A.J.; Bondada, S. Molecular Basis of Age-Associated Cytokine Dysregulation in LPS-Stimulated Macrophages. *J. Leukoc. Biol.* **2006**, *79*, 1314–1327, doi:10.1189/jlb.0106024.
271. Boehmer, E.D.; Goral, J.; Faunce, D.E.; Kovacs, E.J. Age-Dependent Decrease in Toll-like Receptor 4-Mediated Proinflammatory Cytokine Production and Mitogen-Activated Protein Kinase Expression. *J. Leukoc. Biol.* **2004**, *75*, 342–349, doi:10.1189/jlb.0803389.
272. Boehmer, E.D.; Meehan, M.J.; Cutro, B.T.; Kovacs, E.J. Aging Negatively Skews Macrophage TLR2- and TLR4-Mediated pro-Inflammatory Responses without Affecting the IL-2-Stimulated Pathway. *Mech. Ageing Dev.* **2005**, *126*, 1305–1313, doi:10.1016/j.mad.2005.07.009.



273. Boyd, A.R.; Shivshankar, P.; Jiang, S.; Berton, M.T.; Orihuela, C.J. Age-Related Defects in TLR2 Signaling Diminish the Cytokine Response by Alveolar Macrophages during Murine Pneumococcal Pneumonia. *Exp. Gerontol.* **2012**, *47*, 507–518, doi:10.1016/j.exger.2012.04.004.
274. Vande Walle, L.; Lamkanfi, M. Pyroptosis. *Curr. Biol.* **2016**, *26*, R568–R572, doi:10.1016/j.cub.2016.02.019.
275. Shi, J.; Zhao, Y.; Wang, K.; Shi, X.; Wang, Y.; Huang, H.; Zhuang, Y.; Cai, T.; Wang, F.; Shao, F. Cleavage of GSDMD by Inflammatory Caspases Determines Pyroptotic Cell Death. *Nature* **2015**, *526*, 660–665, doi:10.1038/nature15514.
276. Franchi, L.; Muñoz-Planillo, R.; Núñez, G. Sensing and Reacting to Microbes through the Inflammasomes. *Nat. Immunol.* **2012**, *13*, 325–332, doi:10.1038/ni.2231.
277. Franchi, L.; Eigenbrod, T.; Muñoz-Planillo, R.; Nuñez, G. The Inflammasome: A Caspase-1-Activation Platform That Regulates Immune Responses and Disease Pathogenesis. *Nat. Immunol.* **2009**, *10*, 241–247, doi:10.1038/ni.1703.
278. Kayagaki, N.; Stowe, I.B.; Lee, B.L.; O'Rourke, K.; Anderson, K.; Warming, S.; Cuellar, T.; Haley, B.; Roose-Girma, M.; Phung, Q.T.; et al. Caspase-11 Cleaves Gasdermin D for Non-Canonical Inflammasome Signalling. *Nature* **2015**, *526*, 666–671, doi:10.1038/nature15541.
279. He, W.; Wan, H.; Hu, L.; Chen, P.; Wang, X.; Huang, Z.; Yang, Z.-H.; Zhong, C.-Q.; Han, J. Gasdermin D Is an Executor of Pyroptosis and Required for Interleukin-1 $\beta$  Secretion. *Cell Res.* **2015**, *25*, 1285–1298, doi:10.1038/cr.2015.139.
280. Guo, H.; Callaway, J.B.; Ting, J.P.-Y. Inflammasomes: Mechanism of Action, Role in Disease, and Therapeutics. *Nat. Med.* **2015**, *21*, 677–687, doi:10.1038/nm.3893.
281. Wen, H.; Gris, D.; Lei, Y.; Jha, S.; Zhang, L.; Huang, M.T.-H.; Brickey, W.J.; Ting, J.P.-Y. Fatty Acid-Induced NLRP3-ASC Inflammasome Activation Interferes with Insulin Signaling. *Nat. Immunol.* **2011**, *12*, 408–415, doi:10.1038/ni.2022.
282. Vandanmagsar, B.; Youm, Y.-H.; Ravussin, A.; Galgani, J.E.; Stadler, K.; Mynatt, R.L.; Ravussin, E.; Stephens, J.M.; Dixit, V.D. The NLRP3 Inflammasome Instigates Obesity-Induced Inflammation and Insulin Resistance. *Nat. Med.* **2011**, *17*, 179–188, doi:10.1038/nm.2279.
283. Bauernfeind, F.; Niepmann, S.; Knolle, P.A.; Hornung, V. Aging-Associated TNF Production Primes Inflammasome Activation and NLRP3-Related Metabolic Disturbances. *J. Immunol.* **2016**, *197*, 2900–2908, doi:10.4049/jimmunol.1501336.
284. Zhou, R.; Yazdi, A.S.; Menu, P.; Tschopp, J. A Role for Mitochondria in NLRP3 Inflammasome Activation. *Nature* **2011**, *469*, 221–225, doi:10.1038/nature09663.
285. Shimada, K.; Crother, T.R.; Karlin, J.; Dagvadorj, J.; Chiba, N.; Chen, S.; Ramanujan, V.K.; Wolf, A.J.; Vergnes, L.; Ojcius, D.M.; et al. Oxidized Mitochondrial DNA Activates the NLRP3 Inflammasome during Apoptosis. *Immunity* **2012**, *36*, 401–414, doi:10.1016/j.immuni.2012.01.009.
286. Youm, Y.-H.; Grant, R.W.; McCabe, L.R.; Albarado, D.C.; Nguyen, K.Y.; Ravussin, A.; Pistell, P.; Newman, S.; Carter, R.; Laque, A.; et al. Canonical Nlrp3 Inflammasome Links Systemic Low-Grade Inflammation to Functional Decline in Aging. *Cell Metab.* **2013**, *18*, 519–532, doi:10.1016/j.cmet.2013.09.010.
287. Youm, Y.-H.; Kanneganti, T.-D.; Vandanmagsar, B.; Zhu, X.; Ravussin, A.; Adijiang, A.; Owen, J.S.; Thomas, M.J.; Francis, J.; Parks, J.S.; et al. The Nlrp3 Inflammasome Promotes Age-Related Thymic Demise and Immunosenescence. *Cell Rep.* **2012**, *1*, 56–68, doi:10.1016/j.celrep.2011.11.005.
288. Fuster, J.J.; MacLauchlan, S.; Zuriaga, M.A.; Polackal, M.N.; Ostriker, A.C.; Chakraborty, R.; Wu, C.-L.; Sano, S.; Muralidharan, S.; Rius, C.; et al. Clonal Hematopoiesis Associated with TET2 Deficiency Accelerates Atherosclerosis Development in Mice. *Science* **2017**, *355*, 842–847, doi:10.1126/science.aag1381.



289. Mejias, N.H.; Martinez, C.C.; Stephens, M.E.; de Rivero Vaccari, J.P. Contribution of the Inflammasome to Inflammaging. *J. Inflamm. Lond. Engl.* **2018**, *15*, 23, doi:10.1186/s12950-018-0198-3.
290. Franklin, B.S.; Bossaller, L.; De Nardo, D.; Ratter, J.M.; Stutz, A.; Engels, G.; Brenker, C.; Nordhoff, M.; Mirandola, S.R.; Al-Amoudi, A.; et al. The Adaptor ASC Has Extracellular and “prionoid” Activities That Propagate Inflammation. *Nat. Immunol.* **2014**, *15*, 727–737, doi:10.1038/ni.2913.
291. Kleinnijenhuis, J.; Quintin, J.; Preijers, F.; Joosten, L.A.B.; Ifrim, D.C.; Saeed, S.; Jacobs, C.; van Loenhout, J.; de Jong, D.; Stunnenberg, H.G.; et al. Bacille Calmette-Guerin Induces NOD2-Dependent Nonspecific Protection from Reinfection via Epigenetic Reprogramming of Monocytes. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 17537–17542, doi:10.1073/pnas.1202870109.
292. Netea, M.G.; van der Meer, J.W.M. Trained Immunity: An Ancient Way of Remembering. *Cell Host Microbe* **2017**, *21*, 297–300, doi:10.1016/j.chom.2017.02.003.
293. Netea, M.G.; Joosten, L.A.B.; Latz, E.; Mills, K.H.G.; Natoli, G.; Stunnenberg, H.G.; O'Neill, L.A.J.; Xavier, R.J. Trained Immunity: A Program of Innate Immune Memory in Health and Disease. *Science* **2016**, *352*, aaf1098, doi:10.1126/science.aaf1098.
294. Zhong, C.; Yang, X.; Feng, Y.; Yu, J. Trained Immunity: An Underlying Driver of Inflammatory Atherosclerosis. *Front. Immunol.* **2020**, *11*, doi:10.3389/fimmu.2020.00284.
295. Zhang, Q.; Cao, X. Epigenetic Regulation of the Innate Immune Response to Infection. *Nat. Rev. Immunol.* **2019**, *19*, 417–432, doi:10.1038/s41577-019-0151-6.
296. Wen, H.; Dou, Y.; Hogaboam, C.M.; Kunkel, S.L. Epigenetic Regulation of Dendritic Cell-Derived Interleukin-12 Facilitates Immunosuppression after a Severe Innate Immune Response. *Blood* **2008**, *111*, 1797–1804, doi:10.1182/blood-2007-08-106443.
297. He, X.; Xu, J.; Li, G.; Li, M.; Li, L.; Pei, Z.; Zhang, L.; Hu, X. NLRP3-Dependent Microglial Training Impaired the Clearance of Amyloid-Beta and Aggravated the Cognitive Decline in Alzheimer's Disease. *Cell Death Dis.* **2020**, *11*, 1–11, doi:10.1038/s41419-020-03072-x.
298. Vineis, P.; Robinson, O.; Chadeau-Hyam, M.; Dehghan, A.; Mudway, I.; Dagnino, S. What Is New in the Exposome? *Environ. Int.* **2020**, *143*, 105887, doi:10.1016/j.envint.2020.105887.
299. Shiels, P.G.; Buchanan, S.; Selman, C.; Stenvinkel, P. Allostatic Load and Ageing: Linking the Microbiome and Nutrition with Age-Related Health. *Biochem. Soc. Trans.* **2019**, *47*, 1165–1172, doi:10.1042/BST20190110.
300. Shiels, P.G.; McGuinness, D.; Eriksson, M.; Kooman, J.P.; Stenvinkel, P. The Role of Epigenetics in Renal Ageing. *Nat. Rev. Nephrol.* **2017**, *13*, 471–482, doi:10.1038/nrneph.2017.78.
301. Heijmans, B.T.; Tobi, E.W.; Stein, A.D.; Putter, H.; Blauw, G.J.; Susser, E.S.; Slagboom, P.E.; Lumey, L.H. Persistent Epigenetic Differences Associated with Prenatal Exposure to Famine in Humans. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 17046–17049, doi:10.1073/pnas.0806560105.
302. Tsurumi, A.; Li, W. Global Heterochromatin Loss. *Epigenetics* **2012**, *7*, 680–688, doi:10.4161/epi.20540.
303. Villeponteau, B. The Heterochromatin Loss Model of Aging. *Exp. Gerontol.* **1997**, *32*, 383–394, doi:10.1016/S0531-5565(96)00155-6.
304. Djeghloul, D.; Kuranda, K.; Kuzniak, I.; Barbieri, D.; Naguibneva, I.; Choisy, C.; Bories, J.-C.; Dosquet, C.; Pla, M.; Vanneaux, V.; et al. Age-Associated Decrease of the Histone Methyltransferase SUV39H1 in HSC Perturbs Heterochromatin and B Lymphoid Differentiation. *Stem Cell Rep.* **2016**, *6*, 970–984, doi:10.1016/j.stemcr.2016.05.007.
305. Sidler, C.; Woycicki, R.; Li, D.; Wang, B.; Kovalchuk, I.; Kovalchuk, O. A Role for SUV39H1-Mediated H3K9 Trimethylation in the Control of Genome Stability and Senescence in WI38 Human Diploid Lung Fibroblasts. *Aging* **2014**, *6*, 545–563, doi:10.18632/aging.100678.

306. Jintaridth, P.; Mutirangura, A. Distinctive Patterns of Age-Dependent Hypomethylation in Interspersed Repetitive Sequences. *Physiol. Genomics* **2010**, *41*, 194–200, doi:10.1152/physiolgenomics.00146.2009.
307. Zhang, Y.; Wilson, R.; Heiss, J.; Breitling, L.P.; Saum, K.-U.; Schöttker, B.; Holleczeck, B.; Waldenberger, M.; Peters, A.; Brenner, H. DNA Methylation Signatures in Peripheral Blood Strongly Predict All-Cause Mortality. *Nat. Commun.* **2017**, *8*, 14617, doi:10.1038/ncomms14617.
308. Gowers, I.R.; Walters, K.; Kiss-Toth, E.; Read, R.C.; Duff, G.W.; Wilson, A.G. Age-Related Loss of CpG Methylation in the Tumour Necrosis Factor Promoter. *Cytokine* **2011**, *56*, 792–797, doi:10.1016/j.cyto.2011.09.009.
309. Steegenga, W.T.; Boekschoten, M.V.; Lute, C.; Hooiveld, G.J.; de Groot, P.J.; Morris, T.J.; Teschendorff, A.E.; Butcher, L.M.; Beck, S.; Müller, M. Genome-Wide Age-Related Changes in DNA Methylation and Gene Expression in Human PBMCs. *AGE* **2014**, *36*, 9648, doi:10.1007/s11357-014-9648-x.
310. Bradburn, S.; McPhee, J.; Bagley, L.; Carroll, M.; Slevin, M.; Al-Shanti, N.; Barnouin, Y.; Hogrel, J.-Y.; Pääsuke, M.; Gapeyeva, H.; et al. Dysregulation of C-X-C Motif Ligand 10 during Aging and Association with Cognitive Performance. *Neurobiol. Aging* **2018**, *63*, 54–64, doi:10.1016/j.neurobiolaging.2017.11.009.
311. Khalil, H.; Tazi, M.; Caution, K.; Ahmed, A.; Kanneganti, A.; Assani, K.; Kopp, B.; Marsh, C.; Dakhallallah, D.; Amer, A.O. Aging Is Associated with Hypermethylation of Autophagy Genes in Macrophages. *Epigenetics* **2016**, *11*, 381–388, doi:10.1080/15592294.2016.1144007.
312. Stranks, A.J.; Hansen, A.L.; Panse, I.; Mortensen, M.; Ferguson, D.J.P.; Puleston, D.J.; Shenderov, K.; Watson, A.S.; Veldhoen, M.; Phadwal, K.; et al. Autophagy Controls Acquisition of Aging Features in Macrophages. *J. Innate Immun.* **2015**, *7*, 375–391, doi:10.1159/000370112.
313. Iwasaki, A.; Medzhitov, R. Control of Adaptive Immunity by the Innate Immune System. *Nat. Immunol.* **2015**, *16*, 343–353, doi:10.1038/ni.3123.
314. Lai, A.Y.; Kondo, M. T and B Lymphocyte Differentiation from Hematopoietic Stem Cell. *Semin. Immunol.* **2008**, *20*, 207–212, doi:10.1016/j.smim.2008.05.002.
315. Andersen, M.H.; Schrama, D.; Thor Straten, P.; Becker, J.C. Cytotoxic T Cells. *J. Invest. Dermatol.* **2006**, *126*, 32–41, doi:10.1038/sj.jid.5700001.
316. Hoyer, S.; Prommersberger, S.; Pfeiffer, I.A.; Schuler-Thurner, B.; Schuler, G.; Dörrie, J.; Schaft, N. Concurrent Interaction of DCs with CD4(+) and CD8(+) T Cells Improves Secondary CTL Expansion: It Takes Three to Tango. *Eur. J. Immunol.* **2014**, *44*, 3543–3559, doi:10.1002/eji.201444477.
317. Zhang, N.; Bevan, M.J. CD8+ T Cells: Foot Soldiers of the Immune System. *Immunity* **2011**, *35*, 161, doi:10.1016/j.immuni.2011.07.010.
318. LeBien, T.W.; Tedder, T.F. B Lymphocytes: How They Develop and Function. *Blood* **2008**, *112*, 1570–1580, doi:10.1182/blood-2008-02-078071.
319. Takahama, Y. Journey through the Thymus: Stromal Guides for T-Cell Development and Selection. *Nat. Rev. Immunol.* **2006**, *6*, 127–135, doi:10.1038/nri1781.
320. Bayly-Jones, C.; Bubeck, D.; Dunstone, M.A. The Mystery behind Membrane Insertion: A Review of the Complement Membrane Attack Complex. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2017**, *372*, doi:10.1098/rstb.2016.0221.
321. Frank, M.M.; Joiner, K.; Hammer, C. The Function of Antibody and Complement in the Lysis of Bacteria. *Rev. Infect. Dis.* **1987**, *9 Suppl 5*, S537–545.
322. Radbruch, A.; Muehlinghaus, G.; Luger, E.O.; Inamine, A.; Smith, K.G.C.; Dörner, T.; Hiepe, F. Competence and Competition: The Challenge of Becoming a Long-Lived Plasma Cell. *Nat. Rev. Immunol.* **2006**, *6*, 741–750, doi:10.1038/nri1886.
323. Cancro, M.P.; Hao, Y.; Scholz, J.L.; Riley, R.L.; Frasca, D.; Dunn-Walters, D.K.; Blomberg, B.B. B Cells and Aging: Molecules and Mechanisms. *Trends Immunol.* **2009**, *30*, 313–318, doi:10.1016/j.it.2009.04.005.

324. Koohy, H.; Bolland, D.J.; Matheson, L.S.; Schoenfelder, S.; Stellato, C.; Dimond, A.; Várnai, C.; Chovanec, P.; Chessa, T.; Denizot, J.; et al. Genome Organization and Chromatin Analysis Identify Transcriptional Downregulation of Insulin-like Growth Factor Signaling as a Hallmark of Aging in Developing B Cells. *Genome Biol.* **2018**, *19*, doi:10.1186/s13059-018-1489-y.
325. Stephan, R.P.; Lill-Elghanian, D.A.; Witte, P.L. Development of B Cells in Aged Mice: Decline in the Ability of pro-B Cells to Respond to IL-7 but Not to Other Growth Factors. *J. Immunol. Baltim. Md 1950* **1997**, *158*, 1598–1609.
326. Labrie, J.E.; Sah, A.P.; Allman, D.M.; Cancro, M.P.; Gerstein, R.M. Bone Marrow Microenvironmental Changes Underlie Reduced RAG-Mediated Recombination and B Cell Generation in Aged Mice. *J. Exp. Med.* **2004**, *200*, 411–423, doi:10.1084/jem.20040845.
327. Frasca, D.; Diaz, A.; Romero, M.; Blomberg, B.B. The Generation of Memory B Cells Is Maintained, but the Antibody Response Is Not, in the Elderly after Repeated Influenza Immunizations. *Vaccine* **2016**, *34*, 2834–2840, doi:10.1016/j.vaccine.2016.04.023.
328. Hao, Y.; O'Neill, P.; Naradikian, M.S.; Scholz, J.L.; Cancro, M.P. A B-Cell Subset Uniquely Responsive to Innate Stimuli Accumulates in Aged Mice. *Blood* **2011**, *118*, 1294–1304, doi:10.1182/blood-2011-01-330530.
329. Rubtsov, A.V.; Rubtsova, K.; Fischer, A.; Meehan, R.T.; Gillis, J.Z.; Kappler, J.W.; Marrack, P. Toll-like Receptor 7 (TLR7)–Driven Accumulation of a Novel CD11c<sup>+</sup> B-Cell Population Is Important for the Development of Autoimmunity. *Blood* **2011**, *118*, 1305–1315, doi:10.1182/blood-2011-01-331462.
330. Ma, S.; Wang, C.; Mao, X.; Hao, Y. B Cell Dysfunction Associated With Aging and Autoimmune Diseases. *Front. Immunol.* **2019**, *10*, doi:10.3389/fimmu.2019.00318.
331. Anthony, R.M.; Rutitzky, L.I.; Urban, J.F.; Stadecker, M.J.; Gause, W.C. Protective Immune Mechanisms in Helminth Infection. *Nat. Rev. Immunol.* **2007**, *7*, 975–987, doi:10.1038/nri2199.
332. Sallusto, F.; Geginat, J.; Lanzavecchia, A. Central Memory and Effector Memory T Cell Subsets: Function, Generation, and Maintenance. *Annu. Rev. Immunol.* **2004**, *22*, 745–763, doi:10.1146/annurev.immunol.22.012703.104702.
333. Chi, H. Regulation and Function of MTOR Signalling in T Cell Fate Decisions. *Nat. Rev. Immunol.* **2012**, *12*, 325–338, doi:10.1038/nri3198.
334. Mannick, J.B.; Del Giudice, G.; Lattanzi, M.; Valiante, N.M.; Praestgaard, J.; Huang, B.; Lonetto, M.A.; Maecker, H.T.; Kovarik, J.; Carson, S.; et al. MTOR Inhibition Improves Immune Function in the Elderly. *Sci. Transl. Med.* **2014**, *6*, 268ra179, doi:10.1126/scitranslmed.3009892.
335. Mannick, J.B.; Morris, M.; Hockey, H.-U.P.; Roma, G.; Beibel, M.; Kulmatycki, K.; Watkins, M.; Shavlakadze, T.; Zhou, W.; Quinn, D.; et al. TORC1 Inhibition Enhances Immune Function and Reduces Infections in the Elderly. *Sci. Transl. Med.* **2018**, *10*, doi:10.1126/scitranslmed.aaq1564.
336. Mannick, J.B.; Teo, G.; Bernardo, P.; Quinn, D.; Russell, K.; Klickstein, L.; Marshall, W.; Shergill, S. Targeting the Biology of Ageing with MTOR Inhibitors to Improve Immune Function in Older Adults: Phase 2b and Phase 3 Randomised Trials. *Lancet Healthy Longev.* **2021**, *2*, e250–e262, doi:10.1016/S2666-7568(21)00062-3.
337. Walters, H.E.; Cox, L.S. MTORC Inhibitors as Broad-Spectrum Therapeutics for Age-Related Diseases. *Int. J. Mol. Sci.* **2018**, *19*, doi:10.3390/ijms19082325.
338. Min, H.; Montecino-Rodriguez, E.; Dorshkind, K. Reduction in the Developmental Potential of Intrathymic T Cell Progenitors with Age. *J. Immunol. Baltim. Md 1950* **2004**, *173*, 245–250, doi:10.4049/jimmunol.173.1.245.
339. Sudo, K.; Ema, H.; Morita, Y.; Nakauchi, H. Age-Associated Characteristics of Murine Hematopoietic Stem Cells. *J. Exp. Med.* **2000**, *192*, 1273–1280, doi:10.1084/jem.192.9.1273.
340. Sun, L.; Brown, R.; Chen, S.; Zhuge, Q.; Su, D.-M. Aging Induced Decline in T-Lymphopoiesis Is Primarily Dependent on Status of Progenitor Niches in the Bone Marrow and Thymus. *Aging* **2012**, *4*, 606–619, doi:10.18632/aging.100487.

341. Fletcher, A.L.; Seach, N.; Reiseger, J.J.; Lowen, T.E.; Hammett, M.V.; Scott, H.S.; Boyd, R.L. Reduced Thymic Aire Expression and Abnormal NF-KB2 Signaling in a Model of Systemic Autoimmunity. *J. Immunol.* **2009**, *182*, 2690–2699, doi:10.4049/jimmunol.0801752.
342. Latchney, S.E.; Calvi, L.M. The Aging Hematopoietic Stem Cell Niche: Phenotypic and Functional Changes and Mechanisms That Contribute to Hematopoietic Aging. *Semin. Hematol.* **2017**, *54*, 25–32, doi:10.1053/j.seminhematol.2016.10.001.
343. Thomas, R.; Wang, W.; Su, D.-M. Contributions of Age-Related Thymic Involution to Immunosenescence and Inflammaging. *Immun. Ageing A* **2020**, *17*, doi:10.1186/s12979-020-0173-8.
344. Hale, J.S.; Boursalian, T.E.; Turk, G.L.; Fink, P.J. Thymic Output in Aged Mice. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 8447–8452, doi:10.1073/pnas.0601040103.
345. Goronzy, J.J.; Lee, W.-W.; Weyand, C.M. Aging and T-Cell Diversity. *Exp. Gerontol.* **2007**, *42*, 400–406, doi:10.1016/j.exger.2006.11.016.
346. Swain, S.; Clise-Dwyer, K.; Haynes, L. Homeostasis and the Age-Associated Defect of CD4 T Cells. *Semin. Immunol.* **2005**, *17*, 370–377, doi:10.1016/j.smim.2005.05.007.
347. Merani, S.; Pawelec, G.; Kuchel, G.A.; McElhaney, J.E. Impact of Aging and Cytomegalovirus on Immunological Response to Influenza Vaccination and Infection. *Front. Immunol.* **2017**, *8*, 784, doi:10.3389/fimmu.2017.00784.
348. Nguyen, T.H.O.; Sant, S.; Bird, N.L.; Grant, E.J.; Clemens, E.B.; Koutsakos, M.; Valkenburg, S.A.; Gras, S.; Lappas, M.; Jaworowski, A.; et al. Perturbed CD8+ T Cell Immunity across Universal Influenza Epitopes in the Elderly. *J. Leukoc. Biol.* **2018**, *103*, 321–339, doi:10.1189/jlb.5MA0517-207R.
349. Pawelec, G. Immunosenescence and Cancer. *Biogerontology* **2017**, *18*, 717–721, doi:10.1007/s10522-017-9682-z.
350. den Braber, I.; Mugwagwa, T.; Vriskoop, N.; Westera, L.; Mögling, R.; de Boer, A.B.; Willems, N.; Schrijver, E.H.R.; Spierenburg, G.; Gaiser, K.; et al. Maintenance of Peripheral Naive T Cells Is Sustained by Thymus Output in Mice but Not Humans. *Immunity* **2012**, *36*, 288–297, doi:10.1016/j.immuni.2012.02.006.
351. van der Geest, K.S.M.; Abdulahad, W.H.; Tete, S.M.; Lorencetti, P.G.; Horst, G.; Bos, N.A.; Kroesen, B.-J.; Brouwer, E.; Boots, A.M.H. Aging Disturbs the Balance between Effector and Regulatory CD4+ T Cells. *Exp. Gerontol.* **2014**, *60*, 190–196, doi:10.1016/j.exger.2014.11.005.
352. Liu, S.; Liu, F.; Zhang, B.; Yan, P.; Rowan, B.G.; Abdel-Mageed, A.B.; Steele, C.; Jazwinski, S.M.; Moroz, K.; Norton, E.B.; et al. CD4+ T Helper 17 Cell Response of Aged Mice Promotes Prostate Cancer Cell Migration and Invasion. *The Prostate* **2020**, *80*, 764–776, doi:10.1002/pros.23990.
353. Bharath, L.P.; Agrawal, M.; McCambridge, G.; Nicholas, D.A.; Hasturk, H.; Liu, J.; Jiang, K.; Liu, R.; Guo, Z.; Deeney, J.; et al. Metformin Enhances Autophagy and Normalizes Mitochondrial Function to Alleviate Aging-Associated Inflammation. *Cell Metab.* **2020**, doi:10.1016/j.cmet.2020.04.015.
354. Kroemer, G.; Zitvogel, L. CD4+ T Cells at the Center of Inflammaging. *Cell Metab.* **2020**, doi:10.1016/j.cmet.2020.04.016.
355. Coder, B.D.; Wang, H.; Ruan, L.; Su, D.-M. Thymic Involution Perturbs Negative Selection Leading to Autoreactive T Cells That Induce Chronic Inflammation. *J. Immunol. Baltim. Md 1950* **2015**, *194*, 5825–5837, doi:10.4049/jimmunol.1500082.
356. Xia, J.; Wang, H.; Guo, J.; Zhang, Z.; Coder, B.; Su, D.-M. Age-Related Disruption of Steady-State Thymic Medulla Provokes Autoimmune Phenotype via Perturbing Negative Selection. *Aging Dis.* **2012**, *3*, 248–259.
357. Coder, B.; Su, D.-M. Thymic Involution beyond T-Cell Insufficiency. *Oncotarget* **2015**, *6*, 21777–21778.
358. Gallegos, A.M.; Bevan, M.J. Central Tolerance: Good but Imperfect. *Immunol. Rev.* **2006**, *209*, 290–296, doi:10.1111/j.0105-2896.2006.00348.x.
359. Oh, J.; Wang, W.; Thomas, R.; Su, D.-M. Capacity of Treg Generation Is Not Impaired in the Atrophied Thymus. *PLoS Biol.* **2017**, *15*, e2003352, doi:10.1371/journal.pbio.2003352.



360. Kalia, V.; Sarkar, S. Regulation of Effector and Memory CD8 T Cell Differentiation by IL-2—A Balancing Act. *Front. Immunol.* **2018**, *0*, doi:10.3389/fimmu.2018.02987.
361. Goronzy, J.J.; Fang, F.; Cavanagh, M.M.; Qi, Q.; Weyand, C.M. Naive T Cell Maintenance and Function in Human Aging. *J. Immunol. Baltim. Md 1950* **2015**, *194*, 4073–4080, doi:10.4049/jimmunol.1500046.
362. Martínez-Zamudio, R.I.; Dewald, H.K.; Vasilopoulos, T.; Gittens-Williams, L.; Fitzgerald-Bocarsly, P.; Herbig, U. Senescence-Associated  $\beta$ -Galactosidase Reveals the Abundance of Senescent CD8<sup>+</sup> T Cells in Aging Humans. *Aging Cell* **2021**, *20*, e13344, doi:10.1111/accel.13344.
363. Perez-Lanzon, M.; Zitvogel, L.; Kroemer, G. Failure of Immunosurveillance Accelerates Aging. *Oncoimmunology* **2019**, *8*, doi:10.1080/2162402X.2019.1575117.
364. Ouyang, Q.; Wagner, W.M.; Voehringer, D.; Wikby, A.; Klatt, T.; Walter, S.; Müller, C.A.; Pircher, H.; Pawelec, G. Age-Associated Accumulation of CMV-Specific CD8<sup>+</sup> T Cells Expressing the Inhibitory Killer Cell Lectin-like Receptor G1 (KLRG1). *Exp. Gerontol.* **2003**, *38*, 911–920, doi:10.1016/S0531-5565(03)00134-7.
365. Voehringer, D.; Koschella, M.; Pircher, H. Lack of Proliferative Capacity of Human Effector and Memory T Cells Expressing Killer Cell Lectinlike Receptor G1 (KLRG1). *Blood* **2002**, *100*, 3698–3702, doi:10.1182/blood-2002-02-0657.
366. Desdín-Micó, G.; Soto-Herederó, G.; Aranda, J.F.; Oller, J.; Carrasco, E.; Gabandé-Rodríguez, E.; Blanco, E.M.; Alfranca, A.; Cussó, L.; Desco, M.; et al. T Cells with Dysfunctional Mitochondria Induce Multimorbidity and Premature Senescence. *Science* **2020**, *368*, 1371–1376, doi:10.1126/science.aax0860.
367. Moreau, J.-F.; Pradeu, T.; Grignolio, A.; Nardini, C.; Castiglione, F.; Tieri, P.; Capri, M.; Salvioli, S.; Taupin, J.-L.; Garagnani, P.; et al. The Emerging Role of ECM Crosslinking in T Cell Mobility as a Hallmark of Immunosenescence in Humans. *Ageing Res. Rev.* **2017**, *35*, 322–335, doi:10.1016/j.arr.2016.11.005.
368. Pereira, B.I.; De Maeyer, R.P.H.; Covre, L.P.; Nehar-Belaid, D.; Lanna, A.; Ward, S.; Marches, R.; Chambers, E.S.; Gomes, D.C.O.; Riddell, N.E.; et al. Sestrins Induce Natural Killer Function in Senescent-like CD8<sup>+</sup> T Cells. *Nat. Immunol.* **2020**, *21*, 684–694, doi:10.1038/s41590-020-0643-3.
369. Quinn, K.M.; Linterman, M.A. Senescence Blurs the Line between Innate and Adaptive Immune Cells. *Immunol. Cell Biol.* **2020**, *98*, 431–433, doi:10.1111/imcb.12341.
370. Zhou, D.; Borsa, M.; Simon, A.K. Hallmarks and Detection Techniques of Cellular Senescence and Cellular Aging in Immune Cells. *Aging Cell* **2021**, *20*, e13316, doi:10.1111/accel.13316.
371. Nilsson, M.I.; Bourgeois, J.M.; Nederveen, J.P.; Leite, M.R.; Hettinga, B.P.; Bujak, A.L.; May, L.; Lin, E.; Crozier, M.; Rusiecki, D.R.; et al. Lifelong Aerobic Exercise Protects against Inflammaging and Cancer. *PLoS One* **2019**, *14*, e0210863, doi:10.1371/journal.pone.0210863.
372. Lavin, K.M.; Perkins, R.K.; Jemiolo, B.; Raue, U.; Trappe, S.W.; Trappe, T.A. Effects of Aging and Lifelong Aerobic Exercise on Basal and Exercise-Induced Inflammation. *J. Appl. Physiol.* **2019**, *128*, 87–99, doi:10.1152/jappphysiol.00495.2019.
373. Duggal, N.A.; Pollock, R.D.; Lazarus, N.R.; Harridge, S.; Lord, J.M. Major Features of Immunesenescence, Including Reduced Thymic Output, Are Ameliorated by High Levels of Physical Activity in Adulthood. *Aging Cell* **2018**, *17*, doi:10.1111/accel.12750.
374. Beyer, I.; Mets, T.; Bautmans, I. Chronic Low-Grade Inflammation and Age-Related Sarcopenia. *Curr. Opin. Clin. Nutr. Metab. Care* **2012**, *15*, 12, doi:10.1097/MCO.0b013e32834dd297.
375. Tartibian, B.; Hajizadeh Maleki, B.; Kanaley, J.; Sadeghi, K. Long-Term Aerobic Exercise and Omega-3 Supplementation Modulate Osteoporosis through Inflammatory Mechanisms in Post-Menopausal Women: A Randomized, Repeated Measures Study. *Nutr. Metab.* **2011**, *8*, 71, doi:10.1186/1743-7075-8-71.



376. Nicklas, B.J.; Hsu, F.-C.; Brinkley, T.J.; Church, T.; Goodpaster, B.H.; Kritchevsky, S.B.; Pahor, M. Exercise Training and Plasma C-Reactive Protein and Interleukin-6 in Elderly People. *J. Am. Geriatr. Soc.* **2008**, *56*, 2045–2052, doi:10.1111/j.1532-5415.2008.01994.x.
377. Bautmans, I.; Njemini, R.; Vasseur, S.; Chabert, H.; Moens, L.; Demanet, C.; Mets, T. Biochemical Changes in Response to Intensive Resistance Exercise Training in the Elderly. *Gerontology* **2005**, *51*, 253–265, doi:10.1159/000085122.
378. Girven, M.; Dugdale, H.F.; Owens, D.J.; Hughes, D.C.; Stewart, C.E.; Sharples, A.P. L-Glutamine Improves Skeletal Muscle Cell Differentiation and Prevents Myotube Atrophy After Cytokine (TNF- $\alpha$ ) Stress Via Reduced P38 MAPK Signal Transduction. *J. Cell. Physiol.* **2016**, *231*, 2720–2732, doi:10.1002/jcp.25380.
379. Dalle, S.; Rossmeislova, L.; Koppo, K. The Role of Inflammation in Age-Related Sarcopenia. *Front. Physiol.* **2017**, *8*, doi:10.3389/fphys.2017.01045.
380. Minuzzi, L.G.; Rama, L.; Chupel, M.U.; Rosado, F.; Dos Santos, J.V.; Simpson, R.; Martinho, A.; Paiva, A.; Teixeira, A.M. Effects of Lifelong Training on Senescence and Mobilization of T Lymphocytes in Response to Acute Exercise. *Exerc. Immunol. Rev.* **2018**, *24*, 72–84.
381. Spielmann, G.; McFarlin, B.K.; O'Connor, D.P.; Smith, P.J.W.; Pircher, H.; Simpson, R.J. Aerobic Fitness Is Associated with Lower Proportions of Senescent Blood T-Cells in Man. *Brain. Behav. Immun.* **2011**, *25*, 1521–1529, doi:10.1016/j.bbi.2011.07.226.
382. Chan, R.S.M.; Yu, B.W.M.; Leung, J.; Lee, J.S.W.; Auyeung, T.W.; Kwok, T.; Woo, J. How Dietary Patterns Are Related to Inflammaging and Mortality in Community-Dwelling Older Chinese Adults in Hong Kong — A Prospective Analysis. *J. Nutr. Health Aging* **2019**, *23*, 181–194, doi:10.1007/s12603-018-1143-0.
383. Alonso-Pedrero, L.; Ojeda-Rodríguez, A.; Martínez-González, M.A.; Zalba, G.; Bes-Rastrollo, M.; Marti, A. Ultra-Processed Food Consumption and the Risk of Short Telomeres in an Elderly Population of the Seguimiento Universidad de Navarra (SUN) Project. *Am. J. Clin. Nutr.* **2020**, *111*, 1259–1266, doi:10.1093/ajcn/nqaa075.
384. Ojeda-Rodríguez, A.; Zazpe, I.; Alonso-Pedrero, L.; Zalba, G.; Guillen-Grima, F.; Martinez-Gonzalez, M.A.; Marti, A. Association between Diet Quality Indexes and the Risk of Short Telomeres in an Elderly Population of the SUN Project. *Clin. Nutr.* **2020**, *39*, 2487–2494, doi:10.1016/j.clnu.2019.11.003.
385. Fontana, L.; Partridge, L.; Longo, V.D. Dietary Restriction, Growth Factors and Aging: From Yeast to Humans. *Science* **2010**, *328*, 321–326, doi:10.1126/science.1172539.
386. Kim, D.H.; Bang, E.; Jung, H.J.; Noh, S.G.; Yu, B.P.; Choi, Y.J.; Chung, H.Y. Anti-Aging Effects of Calorie Restriction (CR) and CR Mimetics Based on the Senoinflammation Concept. *Nutrients* **2020**, *12*, doi:10.3390/nu12020422.
387. Chung, H.Y.; Kim, H.J.; Kim, J.W.; Yu, B.P. The Inflammation Hypothesis of Aging. *Ann. N. Y. Acad. Sci.* **2001**, *928*, 327–335, doi:10.1111/j.1749-6632.2001.tb05662.x.
388. Mattison, J.A.; Roth, G.S.; Beasley, T.M.; Tilmont, E.M.; Handy, A.M.; Herbert, R.L.; Longo, D.L.; Allison, D.B.; Young, J.E.; Bryant, M.; et al. Impact of Caloric Restriction on Health and Survival in Rhesus Monkeys from the NIA Study. *Nature* **2012**, *489*, 318–321, doi:10.1038/nature11432.
389. Mattison, J.A.; Colman, R.J.; Beasley, T.M.; Allison, D.B.; Kemnitz, J.W.; Roth, G.S.; Ingram, D.K.; Weindruch, R.; de Cabo, R.; Anderson, R.M. Caloric Restriction Improves Health and Survival of Rhesus Monkeys. *Nat. Commun.* **2017**, *8*, 14063, doi:10.1038/ncomms14063.
390. Colman, R.J.; Anderson, R.M.; Johnson, S.C.; Kastman, E.K.; Kosmatka, K.J.; Beasley, T.M.; Allison, D.B.; Cruzen, C.; Simmons, H.A.; Kemnitz, J.W.; et al. Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys. *Science* **2009**, *325*, 201–204, doi:10.1126/science.1173635.
391. Meydani, S.N.; Das, S.K.; Pieper, C.F.; Lewis, M.R.; Klein, S.; Dixit, V.D.; Gupta, A.K.; Villareal, D.T.; Bhapkar, M.; Huang, M.; et al. Long-Term Moderate Calorie Restriction Inhibits Inflammation without Impairing Cell-

- Mediated Immunity: A Randomized Controlled Trial in Non-Obese Humans. *Aging* **2016**, *8*, 1416–1426, doi:10.18632/aging.100994.
392. Cox, L.S.; Mattison, J.A. Increasing Longevity through Caloric Restriction or Rapamycin Feeding in Mammals: Common Mechanisms for Common Outcomes? *Aging Cell* **2009**, *8*, 607–613, doi:10.1111/j.1474-9726.2009.00509.x.
  393. Lührs, H.; Gerke, T.; Müller, J.G.; Melcher, R.; Schaubert, J.; Boxberge, F.; Scheppach, W.; Menzel, T. Butyrate Inhibits NF-KappaB Activation in Lamina Propria Macrophages of Patients with Ulcerative Colitis. *Scand. J. Gastroenterol.* **2002**, *37*, 458–466, doi:10.1080/003655202317316105.
  394. Parada Venegas, D.; De la Fuente, M.K.; Landskron, G.; González, M.J.; Quera, R.; Dijkstra, G.; Harmsen, H.J.M.; Faber, K.N.; Hermoso, M.A. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front. Immunol.* **2019**, *10*, doi:10.3389/fimmu.2019.00277.
  395. Pirozzi, C.; Francisco, V.; Guida, F.D.; Gómez, R.; Lago, F.; Pino, J.; Meli, R.; Gualillo, O. Butyrate Modulates Inflammation in Chondrocytes via GPR43 Receptor. *Cell. Physiol. Biochem.* **2018**, *51*, 228–243, doi:10.1159/000495203.
  396. Inan, M.S.; Rasoulpour, R.J.; Yin, L.; Hubbard, A.K.; Rosenberg, D.W.; Giardina, C. The Luminal Short-Chain Fatty Acid Butyrate Modulates NF-KappaB Activity in a Human Colonic Epithelial Cell Line. *Gastroenterology* **2000**, *118*, 724–734, doi:10.1016/s0016-5085(00)70142-9.
  397. Lee, C.; Kim, B.G.; Kim, J.H.; Chun, J.; Im, J.P.; Kim, J.S. Sodium Butyrate Inhibits the NF-Kappa B Signaling Pathway and Histone Deacetylation, and Attenuates Experimental Colitis in an IL-10 Independent Manner. *Int. Immunopharmacol.* **2017**, *51*, 47–56, doi:10.1016/j.intimp.2017.07.023.
  398. Asnicar, F.; Berry, S.E.; Valdes, A.M.; Nguyen, L.H.; Piccinno, G.; Drew, D.A.; Leeming, E.; Gibson, R.; Le Roy, C.; Khatib, H.A.; et al. Microbiome Connections with Host Metabolism and Habitual Diet from 1,098 Deeply Phenotyped Individuals. *Nat. Med.* **2021**, *27*, 321–332, doi:10.1038/s41591-020-01183-8.
  399. Fahy, G.M.; Brooke, R.T.; Watson, J.P.; Good, Z.; Vasanawala, S.S.; Maecker, H.; Leipold, M.D.; Lin, D.T.S.; Kobor, M.S.; Horvath, S. Reversal of Epigenetic Aging and Immunosenescent Trends in Humans. *Aging Cell* **2019**, *18*, e13028, doi:10.1111/accel.13028.
  400. Keren, Z.; Naor, S.; Nussbaum, S.; Golan, K.; Itkin, T.; Sasaki, Y.; Schmidt-Supprian, M.; Lapidot, T.; Melamed, D. B-Cell Depletion Reactivates B Lymphopoiesis in the BM and Rejuvenates the B Lineage in Aging. *Blood* **2011**, *117*, 3104–3112, doi:10.1182/blood-2010-09-307983.
  401. Avivi, I.; Zisman-Rozen, S.; Naor, S.; Dai, I.; Benhamou, D.; Shahaf, G.; Tabibian-Keissar, H.; Rosenthal, N.; Rakovsky, A.; Hanna, A.; et al. Depletion of B Cells Rejuvenates the Peripheral B-Cell Compartment but Is Insufficient to Restore Immune Competence in Aging. *Aging Cell* **2019**, *18*, e12959, doi:10.1111/accel.12959.
  402. Wahlestedt, M.; Erlandsson, E.; Kristiansen, T.; Lu, R.; Brakebusch, C.; Weissman, I.L.; Yuan, J.; Martin-Gonzalez, J.; Bryder, D. Clonal Reversal of Ageing-Associated Stem Cell Lineage Bias via a Pluripotent Intermediate. *Nat. Commun.* **2017**, *8*, doi:10.1038/ncomms14533.
  403. Aiello, A.; Farzaneh, F.; Candore, G.; Caruso, C.; Davinelli, S.; Gambino, C.M.; Ligotti, M.E.; Zareian, N.; Accardi, G. Immunosenescence and Its Hallmarks: How to Oppose Aging Strategically? A Review of Potential Options for Therapeutic Intervention. *Front. Immunol.* **2019**, *10*, doi:10.3389/fimmu.2019.02247.
  404. Cox, L.S.; Bellantuono, I.; Lord, J.M.; Sapey, E.; Mannick, J.B.; Partridge, L.; Gordon, A.L.; Steves, C.J.; Witham, M.D. Tackling Immunosenescence to Improve COVID-19 Outcomes and Vaccine Response in Older Adults. *Lancet Healthy Longev.* **2020**, *1*, e55–e57, doi:10.1016/S2666-7568(20)30011-8.
  405. Amor, C.; Feucht, J.; Leibold, J.; Ho, Y.-J.; Zhu, C.; Alonso-Curbelo, D.; Mansilla-Soto, J.; Boyer, J.A.; Li, X.; Giavridis, T.; et al. Senolytic CAR T Cells Reverse Senescence-Associated Pathologies. *Nature* **2020**, 1–6, doi:10.1038/s41586-020-2403-9.

406. Song, P.; An, J.; Zou, M.-H. Immune Clearance of Senescent Cells to Combat Ageing and Chronic Diseases. *Cells* **2020**, *9*, 671, doi:10.3390/cells9030671.
407. Baar, M.P.; Brandt, R.M.C.; Putavet, D.A.; Klein, J.D.D.; Derks, K.W.J.; Bourgeois, B.R.M.; Stryeck, S.; Rijksen, Y.; van Willigenburg, H.; Feijtel, D.A.; et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell* **2017**, *169*, 132-147.e16, doi:10.1016/j.cell.2017.02.031.
408. Muñoz-Espín, D.; Rovira, M.; Galiana, I.; Giménez, C.; Lozano-Torres, B.; Paez-Ribes, M.; Llanos, S.; Chaib, S.; Muñoz-Martín, M.; Uceró, A.C.; et al. A Versatile Drug Delivery System Targeting Senescent Cells. *EMBO Mol. Med.* **2018**, *10*, doi:10.15252/emmm.201809355.
409. Chang, J.; Wang, Y.; Shao, L.; Laberge, R.-M.; Demaria, M.; Campisi, J.; Janakiraman, K.; Sharpless, N.E.; Ding, S.; Feng, W.; et al. Clearance of Senescent Cells by ABT263 Rejuvenates Aged Hematopoietic Stem Cells in Mice. *Nat. Med.* **2016**, *22*, 78-83, doi:10.1038/nm.4010.
410. Harrison, D.E.; Strong, R.; Sharp, Z.D.; Nelson, J.F.; Astle, C.M.; Flurkey, K.; Nadon, N.L.; Wilkinson, J.E.; Frenkel, K.; Carter, C.S.; et al. Rapamycin Fed Late in Life Extends Lifespan in Genetically Heterogeneous Mice. *Nature* **2009**, *460*, 392-395, doi:10.1038/nature08221.
411. Laberge, R.-M.; Sun, Y.; Orjalo, A.V.; Patil, C.K.; Freund, A.; Zhou, L.; Curran, S.C.; Davalos, A.R.; Wilson-Edell, K.A.; Liu, S.; et al. MTOR Regulates the Pro-Tumorigenic Senescence-Associated Secretory Phenotype by Promoting IL1A Translation. *Nat. Cell Biol.* **2015**, *17*, 1049-1061, doi:10.1038/ncb3195.
412. Rolt, A.; Nair, A.; Cox, L.S. Optimisation of a Screening Platform for Determining IL-6 Inflammatory Signalling in the Senescence-Associated Secretory Phenotype (SASP). *Biogerontology* **2019**, *20*, 359-371, doi:10.1007/s10522-019-09796-4.
413. Davis, T.; Haughton, M.F.; Jones, C.J.; Kipling, D. Prevention of Accelerated Cell Aging in the Werner Syndrome. *Ann. N. Y. Acad. Sci.* **2006**, *1067*, 243-247, doi:10.1196/annals.1354.031.
414. Alimbetov, D.; Davis, T.; Brook, A.J.C.; Cox, L.S.; Faragher, R.G.A.; Nurgozhin, T.; Zhumadilov, Z.; Kipling, D. Suppression of the Senescence-Associated Secretory Phenotype (SASP) in Human Fibroblasts Using Small Molecule Inhibitors of P38 MAP Kinase and MK2. *Biogerontology* **2016**, *17*, 305-315, doi:10.1007/s10522-015-9610-z.
415. Correia-Melo, C.; Birch, J.; Fielder, E.; Rahmatika, D.; Taylor, J.; Chapman, J.; Lagnado, A.; Carroll, B.M.; Miwa, S.; Richardson, G.; et al. Rapamycin Improves Healthspan but Not Inflammaging in Nfkb1-/- Mice. *Aging Cell* **2019**, *18*, e12882, doi:10.1111/acer.12882.
416. Zhang, X.-J.; Qin, J.-J.; Cheng, X.; Shen, L.; Zhao, Y.-C.; Yuan, Y.; Lei, F.; Chen, M.-M.; Yang, H.; Bai, L.; et al. In-Hospital Use of Statins Is Associated with a Reduced Risk of Mortality among Individuals with COVID-19. *Cell Metab.* **2020**, *32*, 176-187.e4, doi:10.1016/j.cmet.2020.06.015.
417. Puleston, D.J.; Zhang, H.; Powell, T.J.; Lipina, E.; Sims, S.; Panse, I.; Watson, A.S.; Cerundolo, V.; Townsend, A.R.; Klenerman, P.; et al. Autophagy Is a Critical Regulator of Memory CD8+ T Cell Formation. *eLife* **2014**, *3*, doi:10.7554/eLife.03706.
418. Alsaleh, G.; Panse, I.; Swadling, L.; Zhang, H.; Richter, F.C.; Meyer, A.; Lord, J.; Barnes, E.; Klenerman, P.; Green, C.; et al. Autophagy in T Cells from Aged Donors Is Maintained by Spermidine and Correlates with Function and Vaccine Responses. *eLife* **2020**, *9*, e57950, doi:10.7554/eLife.57950.
419. Zhang, H.; Alsaleh, G.; Feltham, J.; Sun, Y.; Napolitano, G.; Riffelmacher, T.; Charles, P.; Frau, L.; Hublitz, P.; Yu, Z.; et al. Polyamines Control EIF5A Hypusination, TFEB Translation, and Autophagy to Reverse B Cell Senescence. *Mol. Cell* **2019**, *76*, 110-125.e9, doi:10.1016/j.molcel.2019.08.005.
420. Moiseeva, O.; Deschênes-Simard, X.; St-Germain, E.; Igelmann, S.; Huot, G.; Cadar, A.E.; Bourdeau, V.; Pollak, M.N.; Ferbeyre, G. Metformin Inhibits the Senescence-Associated Secretory Phenotype by Interfering with IKK/NF-KB Activation. *Aging Cell* **2013**, *12*, 489-498, doi:10.1111/acer.12075.

421. Bannister, C.A.; Holden, S.E.; Jenkins-Jones, S.; Morgan, C.L.; Halcox, J.P.; Schernthaner, G.; Mukherjee, J.; Currie, C.J. Can People with Type 2 Diabetes Live Longer than Those without? A Comparison of Mortality in People Initiated with Metformin or Sulphonylurea Monotherapy and Matched, Non-Diabetic Controls. *Diabetes Obes. Metab.* **2014**, *16*, 1165–1173, doi:10.1111/dom.12354.
422. Glossmann, H.H.; Lutz, O.M.D. Metformin and Aging: A Review. *Gerontology* **2019**, *65*, 581–590, doi:10.1159/000502257.
423. Barzilai, N.; Crandall, J.P.; Kritchevsky, S.B.; Espeland, M.A. Metformin as a Tool to Target Aging. *Cell Metab.* **2016**, *23*, 1060–1065, doi:10.1016/j.cmet.2016.05.011.
424. Menegazzo, L.; Scattolini, V.; Cappellari, R.; Bonora, B.M.; Albiero, M.; Bortolozzi, M.; Romanato, F.; Ceolotto, G.; Vigili de Kreutzberg, S.; Avogaro, A.; et al. The Antidiabetic Drug Metformin Blunts NETosis in Vitro and Reduces Circulating NETosis Biomarkers in Vivo. *Acta Diabetol.* **2018**, *55*, 593–601, doi:10.1007/s00592-018-1129-8.
425. Martin-Montalvo, A.; Mercken, E.M.; Mitchell, S.J.; Palacios, H.H.; Mote, P.L.; Scheibye-Knudsen, M.; Gomes, A.P.; Ward, T.M.; Minor, R.K.; Blouin, M.-J.; et al. Metformin Improves Healthspan and Lifespan in Mice. *Nat. Commun.* **2013**, *4*, 2192, doi:10.1038/ncomms3192.
426. He, M.; Chiang, H.-H.; Luo, H.; Zheng, Z.; Qiao, Q.; Wang, L.; Tan, M.; Ohkubo, R.; Mu, W.-C.; Zhao, S.; et al. An Acetylation Switch of the NLRP3 Inflammasome Regulates Aging-Associated Chronic Inflammation and Insulin Resistance. *Cell Metab.* **2020**, *31*, 580-591.e5, doi:10.1016/j.cmet.2020.01.009.
427. Luo, H.; Mu, W.-C.; Karki, R.; Chiang, H.-H.; Mohrin, M.; Shin, J.J.; Ohkubo, R.; Ito, K.; Kanneganti, T.-D.; Chen, D. Mitochondrial Stress-Initiated Aberrant Activation of the NLRP3 Inflammasome Regulates the Functional Deterioration of Hematopoietic Stem Cell Aging. *Cell Rep.* **2019**, *26*, 945-954.e4, doi:10.1016/j.celrep.2018.12.101.
428. Marín-Aguilar, F.; Castejón-Vega, B.; Alcocer-Gómez, E.; Lendines-Cordero, D.; Cooper, M.A.; de la Cruz, P.; Andújar-Pulido, E.; Pérez-Alegre, M.; Muntané, J.; Pérez-Pulido, A.J.; et al. NLRP3 Inflammasome Inhibition by MCC950 in Aged Mice Improves Health via Enhanced Autophagy and PPAR $\alpha$  Activity. *J. Gerontol. A. Biol. Sci. Med. Sci.* **2020**, *75*, 1457–1464, doi:10.1093/gerona/glz239.
429. Zahid, A.; Li, B.; Kombe, A.J.K.; Jin, T.; Tao, J. Pharmacological Inhibitors of the NLRP3 Inflammasome. *Front. Immunol.* **2019**, *10*, 2538, doi:10.3389/fimmu.2019.02538.
430. Mitchell, S.J.; Bernier, M.; Aon, M.A.; Cortassa, S.; Kim, E.Y.; Fang, E.F.; Palacios, H.H.; Ali, A.; Navas-Enamorado, I.; Di Francesco, A.; et al. Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice. *Cell Metab.* **2018**, *27*, 667-676.e4, doi:10.1016/j.cmet.2018.02.001.
431. Miller, R.A.; Harrison, D.E.; Astle, C.M.; Baur, J.A.; Boyd, A.R.; de Cabo, R.; Fernandez, E.; Flurkey, K.; Javors, M.A.; Nelson, J.F.; et al. Rapamycin, But Not Resveratrol or Simvastatin, Extends Life Span of Genetically Heterogeneous Mice. *J. Gerontol. Ser. A* **2011**, *66A*, 191–201, doi:10.1093/gerona/glq178.
432. Schmidt, C. GSK/Sirtis Compounds Dogged by Assay Artifacts. *Nat. Biotechnol.* **2010**, *28*, 185–186, doi:10.1038/nbt0310-185.
433. Kitada, M.; Ogura, Y.; Monno, I.; Koya, D. Sirtuins and Type 2 Diabetes: Role in Inflammation, Oxidative Stress, and Mitochondrial Function. *Front. Endocrinol.* **2019**, *0*, doi:10.3389/fendo.2019.00187.
434. Aman, Y.; Qiu, Y.; Tao, J.; Fang, E.F. Therapeutic Potential of Boosting NAD<sup>+</sup> in Aging and Age-Related Diseases. *Transl. Med. Aging* **2018**, *2*, 30–37, doi:10.1016/j.tma.2018.08.003.
435. Martens, C.R.; Denman, B.A.; Mazzo, M.R.; Armstrong, M.L.; Reisdorph, N.; McQueen, M.B.; Chonchol, M.; Seals, D.R. Chronic Nicotinamide Riboside Supplementation Is Well-Tolerated and Elevates NAD<sup>+</sup> in Healthy Middle-Aged and Older Adults. *Nat. Commun.* **2018**, *9*, 1286, doi:10.1038/s41467-018-03421-7.
436. Foster, A.D.; Sivarapatna, A.; Gress, R.E. The Aging Immune System and Its Relationship with Cancer. *Aging Health* **2011**, *7*, 707–718, doi:10.2217/ahe.11.56.

437. Sayed, N., Huang, Y., Nguyen, K. *et al.* An inflammatory aging clock (iAge) based on deep learning tracks multimorbidity, immunosenescence, frailty and cardiovascular aging. *Nat Aging* **1**, 598–615 (2021). <https://doi.org/10.1038/s43587-021-00082-y>