



Immunosenescence and inflammaging in the aging process: age-related diseases or longevity? / Santoro A.; Bientinesi E.; Monti D. *in*: AGEING RESEARCH REVIEWS. ISSN 1568-1637. STAMPA. - 71:(2021), pp. 101422.1-101422.19. [10.1016/j.arr.2021.101422]

ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE  
DELLA RICERCA

## Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

Immunosenescence and inflammaging in the aging process: age-related diseases or longevity?

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

*Availability:*

This version is available at: <https://hdl.handle.net/11585/871469> since: 2022-02-27

*Published:*

DOI: <http://doi.org/10.1016/j.arr.2021.101422>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

This is the accepted manuscript of:

Santoro A, Bientinesi E, Monti D.

*Immunosenescence and inflammaging in the aging process: age-related diseases or longevity?*

Ageing Res Rev. 2021 Nov;71:101422

The final published version is available online at: <https://doi.org/10.1016/j.arr.2021.101422>

#### Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

1 **Immunosenescence and inflammaging in the aging process: age-related diseases or longevity?**

2

3 **Authors:** Aurelia Santoro<sup>1</sup>, Elisa Bientinesi<sup>2</sup> and Daniela Monti<sup>2</sup>

4

5 **Affiliations:**

6 <sup>1</sup>Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna  
7 40126, Italy

8 <sup>2</sup>Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of  
9 Florence, Florence 50134, Italy

10

11 **Corresponding Author:** Daniela Monti, Department of Experimental and Clinical Biomedical  
12 Sciences, University of Florence, Viale Morgagni 50, 50134 Florence, Italy.

13 [daniela.monti@unifi.it](mailto:daniela.monti@unifi.it)

14

15

16 **Keywords:** Aging, Longevity, Centenarians, Innate immunity, Immunosenescence, Inflammaging,  
17 COVID-19

18

19 **Highlights:**

- 20 • Immunosenescence represents a dynamic process describing the changes occurring in the  
21 innate and adaptive immune system with age.
- 22 • Immunosenescence has been considered detrimental for a long time due to its contribution to  
23 inflammaging. However, in the last years, a new positive connotation has been emphasized.  
24 Immunosenescence can indeed be considered an adaptive process that can remodel the  
25 immune system in response to many stimuli humans are exposed to during life.
- 26 • Inflammaging, i.e. the age-related increase of pro-inflammatory molecules, is the major  
27 contributor to age-related diseases and represents an example of remodeling because it could  
28 be considered the result of the imbalance between inflammatory and anti-inflammatory  
29 networks.
- 30 • Centenarians are the best example of successful aging, because they avoid or escape age-  
31 related disease and are characterized by an optimal balance between pro- and anti-  
32 inflammatory factors
- 33 • Immunosenescence and inflammaging are highly heterogeneous processes. Genetic  
34 background (including sex) and environmental factors (infections, nutrition, lifestyle, stress,  
35 pollution, etc.) modulate the extent of each individual's adaptive responses.
- 36 • Immunosenescence and inflammaging are intimately interconnected, and the adaptive  
37 mechanisms that they generate as complex network (adaptation *vs* maladaptation) can  
38 determine the susceptibility to several diseases, including COVID-19 and the large  
39 heterogeneity of the pathological phenotype.

40

41 **Abstract:**

42 During aging the immune system (IS) undergoes remarkable changes that collectively are known as  
43 immunosenescence. It is a multifactorial and dynamic phenomenon that affects both natural and  
44 acquired immunity and plays a critical role in most chronic diseases in older people. For a long time,  
45 immunosenescence has been considered detrimental because it may lead to a low-grade, sterile  
46 chronic inflammation we proposed to call "inflammaging" and a progressive reduction in the ability  
47 to trigger effective antibody and cellular responses against infections and vaccinations. Recently,  
48 many scientists revised this negative meaning because it can be considered an essential  
49 adaptation/remodeling resulting from the lifelong immunological biography of single individuals  
50 from an evolutionary perspective. Inflammaging can be considered an adaptive process because it  
51 can trigger an anti-inflammatory response to counteract the age-related pro-inflammatory  
52 environment. Centenarians represent a valuable model to study the beneficial changes occurring in  
53 the IS with age. These extraordinary individuals reached the extreme limits of human life by slowing  
54 down the aging process and, in most cases, delaying, avoiding or surviving the major age-associated  
55 diseases. They indeed show a complex and heterogeneous phenotype determined by an improved  
56 ability to adapt and remodel in response to harmful stimuli. This review aims to point out the intimate  
57 relationship between immunosenescence and inflammaging and how these processes impact  
58 unsuccessful aging rather than longevity. We also describe the gut microbiota age-related changes as  
59 one of the significant triggers of inflammaging and the sex/gender differences in the immune system  
60 of the elderly, contributing to the sex/gender disparity in terms of epidemiology, pathophysiology,  
61 symptoms and severity of age-related diseases. Finally, we discuss how these phenomena could  
62 influence the susceptibility to COVID-19 infection.

63

## 64 **Introduction**

65

66 Demographic estimations predict that the coronavirus disease 2019 (COVID-19) pandemic will lower  
67 healthy life expectancy worldwide, particularly in socio-economic disadvantaged people (Harper,  
68 2021). One hundred twenty-five million people were aged 80 years, and the oldest old and  
69 centenarians were the segment of the elderly population that was increasing the fastest. Until 2019,  
70 estimation reported that by 2050, the world's population aged 60 years and older is expected to total  
71 2 billion, up from 900 million in 2015 ([https://www.who.int/news-room/fact-sheets/detail/aging-and-](https://www.who.int/news-room/fact-sheets/detail/aging-and-health)  
72 [health](https://www.who.int/news-room/fact-sheets/detail/aging-and-health)). The COVID-19 virus pandemic has caused many deaths worldwide, and the oldest-old are  
73 the most vulnerable (Marcon et al., 2020), also considering that the total deaths have been  
74 underestimated by more than a factor of 1.5 (Modi et al., 2021). Supposing that the prevalence of the  
75 infection continues to grow, this could strongly impact life expectancy, breaking the secular trend  
76 and resulting in a decline in lifespan with different rates among countries (Marois et al., 2020;  
77 Andrasfay and Goldman, 2021). However, the aging of the population and the post-COVID syndrome  
78 are currently two of the main socio-economic burdens that society and the healthcare system will  
79 have to manage over the following years.

80 Aging is one of the most intricate and complex biological phenomena that can impact many organ  
81 and systems' functions and represent the main risk factor for geriatric diseases (Kennedy et al., 2014).  
82 The recent conceptualization of Geroscience envisages that few selected and interconnected  
83 biological processes represent the critical pillars of aging and age-related diseases. Among these,  
84 inflammation, alteration of metabolic pathways and stress adaptation play a role (Kennedy et al.,  
85 2014). The immune system (IS) exhibits remarkable changes during aging called  
86 "immunosenescence", a multifactorial phenomenon that affects both natural and acquired immunity  
87 and play a critical role in most chronic diseases in the elderly (Franceschi et al., 1995a; De Martinis  
88 et al., 2005; Barbè-Tuana et al., 2020).

89 Immunosenescence is a dynamic process where several IS functions are reduced, whereas others  
90 remain unchanged or increased (Paolisso et al., 2000). For several years, immunosenescence has been  
91 considered detrimental because it may lead to a low-grade, sterile chronic inflammation we proposed  
92 to call "inflammaging" and a progressive reduction in the ability to trigger effective antibody and  
93 cellular responses against infections and vaccinations (Franceschi et al., 2000a; Franceschi and  
94 Campisi, 2014; Franceschi et al., 2017a; Fulop et al., 2018).

95 Moreover, inflammaging is crucially involved in the aetiology and progression of age-related  
96 diseases, often presented with multimorbidity and may finally lead to organ failure and death (Furman  
97 et al., 2019).

99 As immunosenescence proceeds, older people also become more susceptible to infectious diseases  
100 and cancer. Indeed, aged people and the oldest-old have an augmented risk for developing and dying  
101 from viral infections such as influenza and COVID-19 (Chen et al., 2020). Adults with chronic  
102 inflammatory conditions have a heightened risk for developing severe COVID-19 and dying (Huang  
103 et al., 2020). The interconnection between immunity and senescence is now receiving unprecedented  
104 emphasis during the COVID-19 pandemic, bringing to the fore the critical need to combat  
105 immunosenescence and improve older people's immune function and resilience.

106 Many gerontologists have now revised the negative meaning of immunosenescence (Pawelec,  
107 2020a). From an evolutionary perspective, the age-related changes of the IS can indeed be considered  
108 an adaptation/remodeling rather than solely detrimental (Franceschi and Grignolio, 2010; Fulop et  
109 al., 2020). In this framework, aging represents a continuum without precise borders. The extremes  
110 are represented on one side by patients with age-associated diseases, where inflammation plays a  
111 pathogenic role. On the other side, long-lived individuals delayed or avoided such conditions due to  
112 an effective anti-inflammatory response (Franceschi et al., 2018a). Several factors, such as genetics,  
113 nutrition, exercise, exposure to microorganisms, sex (biological-related), gender (cultural-related)  
114 and human cytomegalovirus (HCMV) status, can influence immunosenescence. (Sansoni et al., 2014,  
115 Vescovini et al., 2007; Pawelec, 2020b). Therefore, it could be conceptualized that the age-related  
116 immune changes may be a mix of adaptation/resilience and maladaptation, closely related to the  
117 immunobiography (Franceschi et al. 2017a; Fulop et al., 2018). However, not the all-elderly  
118 population will suffer from these age-related diseases: more and more individuals are reaching very  
119 old age, such as centenarians ( $\geq 100$  years old) having a relatively well-functioning IS (Monti et al.,  
120 2000; Sizzano et al., 2018). Centenarians show a complex and heterogeneous phenotype determined  
121 by an improved ability to adapt and remodel in response to physical and chemical agents,  
122 psychological stress and biological stimuli such as viral, bacterial and tumour antigens (Franceschi et  
123 al., 2017b; Franceschi et al., 2017c). These extraordinary individuals reached the extreme limits of  
124 human life by slowing down the aging process and, in most cases, delaying, avoiding or surviving  
125 the major age-associated diseases. Centenarians show a lower prevalence of cancer (Salvioli et al.,  
126 2009), cardiovascular diseases (Olivieri et al., 2008), insulin resistance and diabetes (Paolisso et al.,  
127 2001), and they manage to delay the onset of dementia, Alzheimer's disease and osteoporotic fractures  
128 of about one or two decades on average (Evert et al., 2003; Passeri et al., 2003). On the other extreme,  
129 aging is accompanied by augmented morbidity due to a decreased ability of the IS to cope with new  
130 antigenic challenges and control chronic infections. Indeed, mortality due to infectious diseases  
131 continues to accelerate in very late life, different from all the other mortality causes (Pawelec et al.,

132 2006). The age-associated immune deregulation is due to changes in innate and adaptive immunity  
133 (Franceschi et al., 1995b; Alberti et al., 2006; Nasi et al., 2006; Ostan et al., 2008; Sansoni et al.,  
134 2008) and is associated with chronically elevated markers of systemic inflammation (Cevenini et al.,  
135 2013).

136 The present review aims to summarise recent advancements in immunosenescence. Particular  
137 attention is devoted to the intimate relationship between immunosenescence and inflammaging and  
138 how these processes impact unsuccessful aging rather than longevity. We also describe the gut  
139 microbiota age-related changes as one of the significant triggers of inflammaging and the sex/gender  
140 differences in the immune system of the elderly, contributing to the sex/gender disparity in terms of  
141 epidemiology, pathophysiology, symptoms and severity of age-related diseases. Finally, we discuss  
142 how these phenomena could influence the susceptibility to COVID-19 infection.

143

## 144 **2. Age-associated changes in innate immunity**

145 The IS may schematically be divided into an ancestral/ innate part, mainly represented by neutrophils,  
146 monocytes, natural killer (NK) and dendritic cells (DC), and into a phylogenetically recent part  
147 represented by adaptive immunity (B and T lymphocytes). For a long time, innate immunity was  
148 considered unaffected by aging. Still, several studies have demonstrated that crucial components of  
149 the innate IS undergo profound changes related to an increased risk of infections and higher infection-  
150 related mortality. In fact, the aging process seems to hit both branches of the IS (Franceschi et al.,  
151 2000b), and innate cells play a crucial role in inducing inflammaging (**Figure 1**). One hypothesis says  
152 it is because of the constant immune challenges over the lifetime leading to a higher basal activation  
153 state of the innate IS (Fulop et al., 2017). In addition to exogenous antigens, damaged  
154 macromolecules, organelles, and cell debris can serve as damage-associated molecular patterns  
155 (DAMPs) to induce innate immunity through the NF- $\kappa$ B pathway and the induction of the canonical  
156 NLRP3 inflammasome (Youm et al., 2013).

### 157 **2.1 Neutrophils: age-related changes**

158 Neutrophils represent the first line of defence of the innate immune response and kill invading  
159 microbes. Neutrophils are recruited to the sites of infection to rapidly carry out their microbicidal  
160 activity, which relies on several mechanisms such as phagocytosis, degranulation of antimicrobial  
161 proteins, and the release of neutrophil extracellular traps (NETs) (Amulic et al., 2012).

162 Age-related profound alterations in functions of these cells have been described and account for the  
163 increased frequency of infection in the elderly (Brubaker et al., 2013). The microbicidal activity of  
164 neutrophils from elderly individuals is significantly reduced (Simell et al., 2011, Wenisch et al., 2000)  
165 due to impaired phagocytosis (Butcher et al. 2001; Wenisch et al., 2000), degranulation (McLaughlin



166 et al., 1986), and ROS production (Fulop et al., 2004). Elderly individuals also display a reduced  
167 capability to NET formation owing to the increased release of neutrophil elastase via degranulation,  
168 an enzyme critical for NET formation. Moreover, a diminished respiratory burst of neutrophils from  
169 elderly subjects due to diminished NADPH oxidase and myeloperoxidase (MPO) activity can provide  
170 an additional explanation (Ortmann et al., 2018). A decreased NETosis is frequently associated with  
171 sepsis, explaining why elderly individuals are more susceptible to invasive bacterial disease following  
172 skin and soft tissue infection. Excessive NETosis has been suggested to play an essential role in the  
173 pathogenesis of many infectious, inflammatory, and autoimmune diseases, but there is insufficient  
174 evidence to support this hypothesis (Vorobjeva et al., 2020). Recently, Rodriguez-Rosales have  
175 demonstrated that in the blood of patients with psoriasis, different neutrophils subsets resembling  
176 maturation stages are present. The CD10<sup>neg</sup>CD16<sup>low</sup>CD11b<sup>low</sup> fraction increased in psoriatic patients,  
177 and this subset showed the morphology of aged neutrophils, though the lack of CD10 expression is  
178 associated with immaturity. The aged neutrophils (CD10<sup>neg</sup>) are accumulated in the skin and have a  
179 pro-inflammatory effect on T cells mediated by NET formation. NET soluble mediators induce IL-  
180 17 release by T cells and contribute to psoriasis development and inflammation (Rodriguez-Rosales  
181 et al., 2021).

182

183

184 Similarly, an increased number of immature neutrophils are associated with severe acute respiratory  
185 distress syndrome and could be a non-negligible source of IL-6 during COVID-19-induced cytokine  
186 storm (Carissimo et al., 2021). In addition, during SARS-CoV-19 infection, a substantial decrease in  
187 T-cells was observed, especially in subsets with cytolytic activity such as CD8 and  $\gamma\delta$  T-cells. In  
188 particular, VD2, a  $\gamma\delta$  T-cells subset, showed a general decrease in the periphery with disease severity.  
189 These cells can actively recruit and activate neutrophils to the site of infection or inflammation  
190 (Carissimo et al., 2021). In aging, a reduction of VD2 T-cell counts in blood have been shown, and  
191 the presence of inflammaging could explain why elderly individuals are more susceptible to severe  
192 COVID-19 (Carissimo et al., 2021).

193 Interestingly, centenarians show well-preserved neutrophil functions, such as bacterial phagocytosis,  
194 chemotaxis and superoxide production, comparable to those of young subjects (Alonso-Fernandez et  
195 al., 2008). Moreover, monocyte chemotaxis towards formyl-methionyl-leucyl-phenylalanine (f-  
196 MLP), adrenocorticotrophic hormone (ACTH), and corticotrophin-releasing hormone (CRH) were  
197 well preserved in centenarians (Genedani et al., 2008).

198 **2.2 Monocytes/macrophages: age-associated changes**

199 The fundamental role of innate cells, in particular macrophages, is further supported by recent  
200 findings indicating that they also display a form of memory (trained immunity) so that these cells  
201 could be able to mount augmented inflammatory responses upon activation by recognition of Danger-  
202 Associated Molecular Patterns (DAMPs) or alarmins (Franceschi et al. 2017a; Netea and van der  
203 Meer, 2017). At present, it is unknown whether cells of the innate IS undergo an age-related increase  
204 in such memory responses; however, it is possible to hypothesize a role for trained immunity in  
205 inflammaging (Franceschi et al. 2017a). Therefore, the progressive age-related up-regulation of  
206 macrophages and macrophages derived cells throughout the body could explain, at least in part, the  
207 pro-inflammatory status, which characteristically accompanies the aging process (Franceschi et al.,  
208 2000a). Thus, a reduced life span and health span can be envisaged with hyperactivation of the innate  
209 immunity response.

210 Aging has not been shown to significantly alter the absolute number and the frequency of overall  
211 monocytes in humans. However, it does determine significant changes in the relative distribution of  
212 their subsets and their functionality as a significant age-related reduction of reactive oxygen species  
213 (ROS) production and phagocytosis capability (Hearps et al., 2012). In addition, the macrophages  
214 show many age-related functional changes, among which a reduced expression of the principal Toll-  
215 Like Receptors (TLRs). TLRs can recognize pathogen patterns from viruses, bacteria, or fungi, induce  
216 NF- $\kappa$ B pro-inflammatory signalling, release different cytokines, and activate innate immunity to  
217 eliminate antigens (Panda et al., 2009; Shaw et al., 2011). Thus, age-associated reduction in TLR-  
218 induced IL-6 and TNF- $\alpha$  production, particularly in response to engagement of TLR1/2 and an  
219 increased release of TNF- $\alpha$  upon TLR4 stimulation, has been demonstrated (Panda et al., 2009).

220 Moreover, in human monocytes from aged people, the stimulation of TLR1/2, TLR2/6, TLR4, or  
221 TLR5 induces higher levels of IL-8 (Qian et al., 2012). Such dysregulation appears to be caused by  
222 alterations in surface TLR expression and downstream signalling: TLR1 expression declines with  
223 age, and activation of MAPK and ERK1/1 pathways by TLR1/2 triggering is severely reduced in cells  
224 from elderly subjects (van Duin et al., 2007). In contrast, downstream signalling of TLR5 has been  
225 shown to increase, leading to inflammatory responses in the elderly (Qian et al., 2012). However,  
226 findings of different research groups demonstrated a heightened pro-inflammatory milieu in old and  
227 long-lived individuals, with higher serum levels of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6  
228 and IL-1 $\beta$ , and other markers (C-reactive protein, clotting factors) (Fagiolo et al., 1993; Franceschi  
229 et al., 2007; Morrisette-Thomas et al., 2014). A possible explanation for this apparent paradox, i.e.,  
230 augment of pro-inflammatory cytokines with concomitant defects of TLRs function, could be due to  
231 different tissue cell types, such as adipocytes, producing cytokines, also if the  
232 monocytes/macrophages are the primary sources of IL-6 (Maggio et al., 2006). In humans, adipose

233 tissue indeed undergoes substantial immune-metabolic changes with aging increasing the pro-  
234 inflammatory pathways related to both acquired and innate immunity (Trim et al., 2021). In visceral  
235 white adipose tissue and liver have been demonstrated an age-related accumulation of pro-  
236 inflammatory M1-like macrophages characterized by increased expression of CD38, a NAD-  
237 consuming enzyme able to reduce tissue NAD levels (Covarrubias et al., 2020). This polarization of  
238 macrophages can be due to increased inflammaging determined by the accumulation of senescent  
239 cells. These active metabolic cells produce immune-modulator factors that promote CD38 expression  
240 in M1 resident macrophages, thus regulating immune cell homing, innate immune responses  
241 (Covarrubias et al., 2020). In addition, an increased level of endotoxins and others PAMPS with aging  
242 can activate innate immune cells that promote inflammatory state and consequently the CD38  
243 expression by tissue-resident M1-like macrophages, and hence enhanced NADase activity and  
244 contribute to the NAD decline associated with aging (Covarrubias et al., 2020). Therefore, the source  
245 of pro-inflammatory cytokines in aging might depend on the complex interplay of immunologic,  
246 hormonal, and neuroendocrine factors *in vivo* (Stout et al., 2005; Straub and Mocchegiani, 2004). The  
247 release of cytokines by monocytes/macrophages might be modulated by adipokines (Lago et al.,  
248 2008), adrenal hormones (Jurberg et al., 2018), whose circulating levels are impaired with age  
249 (Sergio, 2008).

250 Three different monocyte subsets can be individuated based on their phenotype: classical  
251 ( $CD14^+CD16^-$ , which are 90% of circulating monocytes), intermediate ( $CD14^+CD16^+$ ), and non-  
252 classical ( $CD14^{dim}CD16^+$ ) monocytes (Hearps et al., 2012). Aging affects the relative distribution of  
253 monocyte subsets, with a marked reduction of the classical subset and an increase in the number of  
254 intermediate and non-classical monocytes with profound dysregulation in cytokines secretion after  
255 TLRs activation of monocytes (Hearps et al., 2012).

256 On the contrary, Costantini et al. suggest that healthy aging is associated with a significantly increased  
257 proportion of total monocytes, without significant changes in the frequency of the three subsets  
258 (Costantini et al., 2018). These authors also investigated the inflammatory (M1) and anti-  
259 inflammatory (M2) profiles in the three monocyte subsets through the expression of CD80 and  
260 CD163. CD80 is expressed on M1 macrophages, whereas CD163 is expressed on M2. The results  
261 indicate a reduction in  $CD163^+$  and  $CD80^-CD163^-$  cells in classical monocytes and an increase in  
262  $CD163^+$  cells in non-classical monocytes, suggesting different age-related trends for classical and  
263 non-classical M2 monocytes (Costantini et al., 2018). However, since classical monocytes account  
264 for 80-90% of circulating monocytes, healthy aging seems to be characterized by a reduced proportion  
265 of M2 monocytes. On the contrary, old patients with acute myocardial infarction showed in the  
266 classical monocyte subset a significant increase of  $CD163^+$  cells having an inflammatory role in

267 atherosclerosis and cardiac remodeling. Moreover, CD80<sup>+</sup> monocytes (M1) increased significantly in  
268 intermediate and non-classical subsets, underlining as a pro-inflammatory polarization of monocytes  
269 and consequent M1/M2 imbalance could play a role in cardiovascular diseases' pathogenesis  
270 (Costantini et al., 2018).

271 Recently, an in-depth global analysis revealed alterations after stimulation of monocytes sorted from  
272 healthy adult and old individuals with TLR4, TLR7/8, and RIG-I agonists. A reduced release of IFN-  
273  $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , CCL20, and CCL8 and higher expression of CX3CR1 was observed, while no age  
274 effects on unstimulated monocyte subsets were evidenced (Metcalf et al., 2017). Besides, high TNF-  
275  $\alpha$  plasma levels promoted the egress of immature monocytes from bone marrow that can produce,  
276 when stimulated with bacterial products *in vivo*, high levels of TNF- $\alpha$ , thus reinforcing inflammaging  
277 (Putcha et al., 2016).

278 A key role in inflammaging might also be played by single nucleotide polymorphisms (SNPs) in the  
279 promoter regions of genes encoding for IL-6 and IFN- $\gamma$ . Bonafè et al. found that the IL-6 promoter  
280 genetic variability at -174 C/G locus and its effect on IL-6 serum levels in older people, including  
281 centenarians, contribute to inflammaging. They demonstrated that genetically predisposed  
282 individuals to produce high levels of IL-6 during aging, *i.e.* C- men at IL-6 -174 C/G locus, have a  
283 reduced ability to reach the extreme limits of the human lifespan. On the other hand, the capability of  
284 producing low levels of IL-6 throughout the lifespan (C+ individuals) appears to be beneficial for  
285 longevity, at least in men. Women experience higher IL-6 serum levels later in life than men, and  
286 the age-related increase of IL-6 serum levels in women is entirely independent of -174 C/G locus  
287 activity (Bonafè et al., 2001). Moreover, the +874 A allele for the IFN- $\gamma$  gene is associated with low  
288 IFN- $\gamma$  production and is positively associated with longevity in male and female centenarians (Lio et  
289 al. 2002). Specifically, genetic variants that tend to increase anti-inflammatory cytokines and those  
290 that decrease pro-inflammatory cytokines have been associated with successful aging and are more  
291 common among persons attaining the oldest ages (Lio et al., 2002).

292 However, while the SNPs mentioned above may yield some insights into a person's predisposition  
293 for inflammaging, many other variables can play a role, and it is essential to consider them. The  
294 lifelong immunological experiences and stimuli that each individual was exposed to (Franceschi et  
295 al. 2017a), age (Sansoni et al., 2008), gender (Ostan et al., 2016), different geographical and historical  
296 settings, diet and stress levels (Calder et al., 2011; Franceschi et al., 2018b; Santoro et al., 2020a), as  
297 well as the composition of gut-associated commensal bacteria (*i.e.*, the microbiome) (Kau et al., 2011;  
298 Santoro et al., 2020b) are key factors contributing to inflammaging. This condition is known with the  
299 term immunobiography, which should help understand the enormous heterogeneity of the immune  
300 phenotype in older adults (Franceschi et al., 2017a).

### 301 **2.3 NK cells: age-related changes**

302 NK cells are innate lymphoid cells (ILC) representing 10-15% of peripheral blood lymphocytes.  
303 They participate in the early defense against intracellular pathogens and tumour cells and are  
304 cytotoxic non-T lymphocytes characterized by the expression of CD56 and/or CD16 (Solana et al.,  
305 2012b) and share many features with ILC1 such as their capacity to produce IFN- $\gamma$  (Spits et al., 2016).  
306 According to differential expression of surface markers CD56 and CD16, three NK subsets can be  
307 identified. In the subset of CD56<sup>bright</sup>CD16<sup>neg/dim</sup> cells, the cells are more immature and secrete  
308 cytokines and chemokines, whereas the main NK cell subset CD56<sup>dim</sup>CD16<sup>+</sup> is made up of mature  
309 NK cells with high cytotoxic capacity after direct contact with tumour or virus-infected target cells  
310 (Cooper et al., 2004). Furthermore, a scarce subset of NK cells, devoid of CD56 expression and  
311 displaying a reduced functional capacity, has been identified in healthy controls and chronic viral  
312 infections such as HIV and hepatitis C virus (HCV) (Solana et al., 2012b). Many data of changes in  
313 NK-cell phenotype and function with old age have been reported but frequently inconsistent. An  
314 increase in the number of mature NK cells with a significant reduction in the immature NK cell subset  
315 probably due to the impaired production of new NK cells was observed with advanced age (Le Garff-  
316 Tavernier et al., 2010; Gayoso et al., 2011). Thus, the decline in CD56<sup>bright</sup> NK cells and the increase  
317 in the CD56<sup>dim</sup>CD57<sup>+</sup> subset support that the population of NK cells suffers a process of remodeling  
318 with a reduction in the output of more immature CD56<sup>bright</sup> cells and an accumulation of highly  
319 differentiated CD56<sup>dim</sup>CD57<sup>+</sup> NK cells (Solana et al., 2012b). Both age and persistent CMV  
320 infection contribute to the NK cell phenotypical and functional changes observed in the elderly.  
321 Aging does not change total NK cell cytotoxicity, probably due to the increased frequencies of mature  
322 NK cells, but impairment of NK cell cytotoxicity on a per-cell basis due to the decreased expression  
323 of activating receptors has been reported (Hazeldine et al., 2013). In centenarians, the increase of the  
324 high-activity NK subset is mirrored by exceptionally well-preserved cytotoxicity, and it can be  
325 speculated that the preserved NK activity can help reach far advanced age in good conditions (Sansoni  
326 et al., 1992). The age-related increase of cells bearing NK markers and non-MHC-restricted T  
327 lymphocytes could be interpreted as an adaptative mechanism to cope with the decrease of T cells  
328 related to the thymic involution. Human NK cells from healthy subjects over 90 years of age,  
329 however, are still able to secrete the chemotactic cytokines MIP-1 $\alpha$ , Rantes, and IL-8 and can also  
330 effectively release these chemokines in response to IL-12 and IL-2, but their production remains  
331 lower than that observed in young subjects (Mariani et al., 2002). Many studies have shown that the  
332 functions of NK cells extend, beyond their role in anti-viral and tumour immunity, into such areas as  
333 immune regulation, the initiation of adaptive immune responses, and the clearance of senescent cells  
334 (Hazeldine et al. 2013). Thus, several features of the aging process, such as the reduced efficacy of

335 vaccination, the appearance of senescent cells and the higher rates of fungal infection, may be  
336 attributable in part to the decline in NK cell function that accompanies human aging.

#### 337 **2.4 Dendritic cells: age-related changes**

338 Dendritic cells (DCs) are professional APCs classified as myeloid DCs (mDCs) or plasmacytoid DCs  
339 (pDCs) having different functional activities: mDCs, producing IL-12, induce helper T cell type 1  
340 (Th1) and cytotoxic T lymphocyte (CTL) responses, whereas pDCs produce IFN- $\alpha/\beta$  in response to  
341 bacteria and viruses (Banchereau et al., 2000). Age-related changes in the number and frequency of  
342 mDCs and pDCs were discordantly reported (Jing et al., 2009; Perez-Cabezas et al., 2007). Both  
343 mDCs and pDCs from elderly individuals showed a significant impairment in secreting TNF- $\alpha$ , IL-  
344 6, and IL-12 (p40) in response to TLRs. Moreover, the lower release of IFNs and pro-inflammatory  
345 cytokines from pDCs have been associated with a reduced response to the influenza vaccine (Panda  
346 et al., 2010). However, basal production of pro-inflammatory cytokines in the absence of TLR  
347 engagement is higher in cells from older than young individuals, suggesting a dysregulation of  
348 cytokine production that may limit further activation through TLR engagement (Metcalf et al., 2017).  
349 In conclusion, the picture that emerges is a profound dysregulation of innate immune functions, with  
350 some functions down-regulated and others up-regulated or even enhanced. In particular, an increase  
351 in the basal production of pro-inflammatory cytokines, observed in different cell types, could be a  
352 significant contributor to the age-related increase of the levels of such molecules observed in several  
353 cohorts of elderly subjects (Salvioli et al., 2013)

354

#### 355 **3. Age-associated changes in adaptive immunity**

356 The adaptive IS is constituted by two types of responses: the cell-mediated immune response, which  
357 is carried out by T cells, and the humoral immune response controlled by activated B cells and  
358 antibodies. T cells play a crucial role in orchestrating the immune responses and are subdivided into  
359 CD4<sup>+</sup> and CD8<sup>+</sup>T cell populations with different functions (Das et al., 2017). CD4<sup>+</sup>T cells are crucial  
360 in achieving a regulated effective immune response to pathogens and possess effector functions (Das  
361 et al., 2017). Naive CD4<sup>+</sup> T cells may differentiate into one of several lineages of T helper (Th) cells,  
362 including Th1, Th2, Th17, and Treg, as defined by their pattern of cytokine production and function  
363 (Zhu et al., 2010). CD8<sup>+</sup> T cells constitute an essential branch of adaptive immunity contributing to  
364 the clearance of intracellular pathogens and providing long-term protection (Mitrücker et al.,  
365 2014). Alterations of adaptive responses have been described in aging, and the T cells compartment  
366 is the most affected and contributes to inflammaging (Franceschi, 2017d; Tu and Rao, 2016). The T-  
367 lymphocyte compartment has been studied extensively concerning immunosenescence and will be  
368 treated in this paragraph.

369 Two main changes in the adaptive IS characterize aging: i) a decrease in naïve T cells that leads to  
370 the shrinking of the TCR repertoire, ii) an increase in memory T cells primed by different antigens  
371 and upregulation of pro-inflammatory molecules.

372 A decrease in regenerative capacity is one of aging hallmarks and contributes to reducing  
373 hematopoietic cells (Lopez-Otin et al., 2013). A good example is an age-related decline in  
374 hematopoiesis, causing a diminished production of adaptive immune cells (Lopez-Otin et al., 2013).  
375 An increase with age in the frequency of myeloid-biased differentiation at the expense of lymphoid  
376 specificity and function is demonstrated in humans (Pang et al., 2011). These changes influence the  
377 T and B repertoire and are responsible, at least partly, to reduce T and B cell number.

378 T cell repertoire is compounded by thymic involution and the decline in its function after puberty  
379 (Palmer, 2013). The release of new naïve cells by thymus is vanishingly rare in the elderly. The  
380 reduced thymus output seems to be the primary explanation for the increased incidence of infections,  
381 cancers, vaccination failure, and reduced capacity to respond to neoantigens (Appay and Sauce, 2014;  
382 Pawelec, 2017). Steinmann et al. observed that thymus atrophy begins at the age of one year, and  
383 shrinks in volume by about 3% per year until middle age, then shrinking by <1% per years through  
384 the rest of life, however, the presence of thymic tissue has been described in a 107 years old subject  
385 (Steinmann et al., 1985). An evaluation of thymic output is based on quantifying recent thymic  
386 emigrants characterized by the expression of TCR rearrangement excision circles (TRECs). TREC<sup>+</sup>  
387 lymphocytes present in the periphery indicate the organ's functionality since mature T cells that leave  
388 the thymus and enter the circulation can display TRECs in more than 70% of the cases. They have  
389 been detected in older people, up to 80 years, indicating that there may be a continuous thymic output  
390 of naïve T cells, even in advanced age (Douek et al., 2000). Nasi et al. analyzed the content of TREC  
391 in peripheral blood mononuclear cells (PBMCs) from centenarians, compared with young and  
392 middle-aged donors, and found a dramatic reduction in the number of TREC<sup>+</sup> cells. However, a well  
393 detectable number of TREC<sup>+</sup> lymphocytes was present in 4 centenarians out of 25, suggesting that  
394 such cells could derive from residues of thymic lymphopoietic islets (Nasi et al. 2006).

395 Recent data show that mechanisms can partially maintain naïve T compartment as the homeostatic  
396 proliferation (Appay and Sauce, 2014). Homeostatic proliferation effectively maintains the naïve  
397 CD4<sup>+</sup> T cell pool in humans in healthy aging, but less so in respect of naïve CD8<sup>+</sup> T cells (Goronzy  
398 et al., 2015). It is clear that homeostatic proliferation does not allow the production of new  
399 specificities but can only maintain the repertoire's richness. Moreover, during homeostatic  
400 proliferation, the selected clones could have a higher affinity for self-antigens and lead to  
401 autoreactivity in older individuals (Goronzy and Weyand, 2012). Qi and colleagues have  
402 demonstrated that naive repertoire richness until 70 years old decline slowly, but age plays a role in

403 the unequal size of the observed clones, which is more prevalent in naïve CD8<sup>+</sup> T cells than their  
404 CD4<sup>+</sup> T cells cell counterparts (Qi et al., 2014). In the past years, several studies, including ours  
405 (Cossarizza et al., 1997), used the expression of CD45 isoforms, CD45RA and CD45R0, to define  
406 naïve/unprimed and memory/experienced T cells, respectively. Consequently, it was reported that a  
407 well-preserved number of naïve T cells can be still present in people of advanced age, including  
408 centenarians (Cossarizza et al., 1996; Cossarizza et al., 1997).

409 With increasing age, our body tends to allocate resources differently, reducing the energy  
410 consumption of many metabolically active organs and tissues such as the thymus, muscles, bone  
411 marrow and redirect energy to other functions and activities to support the organism's survival.

412 Exposure to new pathogens is maximal during the first years of life but less likely in later life when  
413 immune memory for previously encountered pathogens is more prevalent and more important for  
414 survival (Pawelec, 2018, Shanley et al., 2009). Therefore, resources must be preferentially allocated  
415 to combat these "usual" related pathogens on the memory side of the IS rather than spending energy  
416 on a useless struggle, which can be interrupted in any case by the destruction of the invading  
417 organism.

418 The second hallmark of immunosenescence is the expansion of memory T cells in response to latent  
419 viruses affecting T cell repertoire diversity. The life-long chronic antigen load causes the filling of  
420 the immunological space by a T lymphocytes population with a late-differentiated phenotype and the  
421 T cell repertoire's shrinkage. The body hosts many latent infections, which can re-activate from time  
422 to time under specific conditions such as human cytomegalovirus (CMV) (Larbi et al., 2014). CMV  
423 infection has a more significant impact than age in expanding CD4<sup>+</sup> and CD8<sup>+</sup> effector memory T  
424 cells, particularly the latter, increasing oligoclonality during normal human aging (Pawelec, 2001,  
425 Hadrup et al., 2006; Vescovini et al., 2004; Sadighi and Akha, 2018). We have seen that CMV-driven  
426 CD8<sup>+</sup> T cell reactivity is correlated with increasing numbers of late differentiated CD28<sup>-</sup>CD8<sup>+</sup> T  
427 cells in the elderly, including centenarians (Fagnoni et al., 1996). This parameter makes up a  
428 substantial part of the highly discussed Immune Risk Phenotype (IRP) considered for the potential  
429 prediction of increased morbidity and death (Wikby et al., 2006). Pawelec, in collaboration with  
430 OCTO/NONA study group in Jönköping, Sweden, identified some simple immunological markers  
431 associated with the survival of the very elderly over 2, 4 and 6 years from baseline at 85 years of age  
432 (Pawelec et al., 2003). They found that a cluster of markers named IRP characterized by an excess of  
433 late-stage differentiated CD8<sup>+</sup>CD27<sup>-</sup>CD28<sup>-</sup> T cells reactive to CMV antigens, a reduced T cell  
434 proliferative response to mitogens, an inverted CD4:8 ratio and CMV-seropositivity together with a  
435 deficit of B cells, was weakly associated with 2, 4 and 6-year all-cause mortality at follow-up  
436 (Pawelec et al., 2001b).



437 Moreover, higher serum levels of IL-6 and cognitive impairment assessment were more closely  
438 associated with mortality than the IRP. However, the survival of those individuals from the  
439 OCTO/NONA studies who were both in the IRP and had higher IL 6 levels and cognitive impairment  
440 was the worst of any subjects studied (Wikby et al., 2006). Consistent with this, none of the  
441 OCTO/NONA subjects in the IRP group who survived become centenarians (Strindhall et al., 2007).  
442 However, the IRP is not widely accepted because it was not confirmed in the Leiden 85-Plus study,  
443 a prospective population-based cohort study of individuals aged 85 years living in Leiden  
444 (Derhovanessian et al., 2013). Thus, immune parameters associated with survival may vary in diverse  
445 populations at different ages (Pawelec, 2012a). These observations emphasize the concept that the  
446 immunosenescence and the consequent inflammaging are hugely heterogeneous and represent a  
447 continuum remodeling in response to unpredicted long-time exposures to external and/or internal  
448 stressors determining the so-called immunobiography. Consequently, the immunosenescence and  
449 inflammaging can be more or less severe, leading to a wide range of outcomes from overt diseases  
450 where inflammation plays a pathogenic role in successful aging (e.g., centenarians) (Franceschi et al.,  
451 2018a).

452 CMV chronic infection in nonagenarians and centenarians was characterized by highly variable  
453 frequency and an absolute number of CD8<sup>+</sup> T cells that, occasionally, were strikingly expanded.  
454 Moreover, most anti-CMV CD8<sup>+</sup> T cells did not bear the CD28 molecule, thus supporting the  
455 hypothesis that the age-related expansion of CD28<sup>-</sup> T cells may depend, at least in part, on repeated  
456 rounds of cellular replication for the ongoing immune response against CMV (Vescovini et al., 2004).  
457 This determines the phenomenon of memory cell inflation, leading to the emergence of vast  
458 populations of resting effector CD8<sup>+</sup> and, to a lesser extent, CD4<sup>+</sup> cells. These inflated CMV-specific  
459 memory T cells maintain their efficient effector functions for the individual's lifetime, and they are  
460 not exhausted (Nikolich-Zugich et al., 2017).

461 Overall, CMV immune changes may play a role in immunological fitness and, particularly, during  
462 co-infection and vaccination. Additionally, a systematic review of the relation between CMV-  
463 infection and immunosenescence in western people aged fifty and older showed that CMV seems to  
464 enhance immunosenescence. This evidence is based on the high levels of the highly differentiated  
465 effector memory T cells and T effector memory re-expressing CD45RA cells (TEMRA) in the CD8<sup>+</sup>  
466 and CD4<sup>+</sup> T cell pools. At the same time, there is a decrease in central memory cells (Weltevrede et  
467 al., 2016). Although CMV was once considered the leading cause of age-related immune changes in  
468 the elderly, accumulating data are still quite contradictory. The current opinion is that CMV infection  
469 does not seem to be only detrimental (Derhovanessian et al., 2013; Solana et al., 2012a; Pawelec et  
470 al., 2012b), but it may be considered a recurrent stimulation that maintains sustained immunological

471 alertness and favours a better immune response (Pawelec et al., 2012b). The global response to the  
472 many various CMV antigens has been linked to better survival (Bajwa et al., 2017), suggesting that  
473 the increased number of committed memory T cells may not be considered unequivocally detrimental  
474 or related only to aging.

475 One of the essential features of aging is the notion of senescent cells (Campisi et al., 2014). During  
476 aging, senescent cells, *i.e.* differentiated CD 28<sup>-</sup> T cells, induced by a repeated pathogen encounter  
477 during chronological aging, and end-stage differentiated senescent T cells, are characterized by a  
478 progressive reduction of telomere length and a proliferative arrest, tend to accumulate (Akbar et al.,  
479 2016). These cells have been previously considered to be inactive. However, recent data have shown  
480 that they are metabolically active, arising with age in the body and produce large amounts of pro-  
481 inflammatory cytokines (a phenomenon called senescence-associated secretory phenotype, SASP) as  
482 stated by the inflammaging (Akbar et al., 2016). Thus, chronic antigenic stimulation leads both to the  
483 phenomenon of inflammaging and the increase of the number of senescent T cells (Callender et al.,  
484 2018). One additional consequence of chronic stimulation is the phenomenon of exhaustion,  
485 characterized by inadequate responses to proliferative stimuli and the expression of inhibitory  
486 receptors, such as PD-1, CTLA-4, KLRG1 and many others on T cell subsets (Vasudev et al., 2014).  
487 Another component that may favour inflammaging is the compromised ability of CD4<sup>+</sup> T cells to  
488 differentiate into functional subsets, resulting in many dysregulated responses. Two of these are the  
489 reduced cognate help to B cells with consequent reduced humoral immunity and the increased ratio  
490 of the pro-inflammatory Th17 cells and the immunosuppressive T regulatory cells, favouring a basal  
491 pro-inflammatory status (Schmitt et al., 2013; Bektas et al., 2017).

492 Thus, changes in the TH17/Treg ratios and altered cytokine expression during aging may contribute  
493 to an imbalance between the pro-inflammatory and anti-inflammatory immune response (Schmitt et  
494 al., 2013), indicating higher susceptibility to developing inflammatory diseases with increasing age.  
495 Like the T cell pools, the B cell compartment also undergoes age-related changes. Peripheral B cell  
496 number and percentages decline significantly, and specific humoral immune responses against  
497 extracellular pathogens and vaccines are impaired. In particular, B cell repertoire diversity,  
498 immunoglobulin isotypes and receptor repertoire are affected by age (Bulati et al. 2011; Frasca et al.,  
499 2020). In aging, the transcriptional factor E47 that controls B cell functions is down-regulated,  
500 reducing the activation-induced cytidine deaminase (AID), which induces class switch recombination  
501 and Ig somatic hypermutation. Moreover, it might also be responsible for diminished antibodies  
502 avidity and antibody-mediated protection (Frasca et al., 2016). However, this defect might be mainly  
503 linked to reduced B cells interaction with CD40L<sup>+</sup> T helper cells because, in older adults, the  
504 memory/effector T cells show a reduced expression of CD40L, necessary for B cells cooperation

505 (Colonna-Romano et al., 2003). As well-known and described above, T cell function impairment has  
506 *per se* paramount importance in immunosenescence and contribute to an age-related decrease in  
507 antibody responses of elderly individuals.

508 Furthermore, it has been reported that elevated levels of TNF- $\alpha$ , typical of inflammaging, can cause  
509 human unstimulated B cells from elderly individuals to release significantly higher levels of TNF- $\alpha$   
510 than those from young subjects and render them unable to respond to exogenous antigens, mitogens  
511 or vaccines. (Frasca et al., 2014). Regarding the major circulating B-cell subsets have been identified  
512 four populations: naive [IgD<sup>+</sup>CD27<sup>-</sup>], IgM memory [IgD<sup>+</sup>CD27<sup>+</sup>], switched memory [IgD<sup>-</sup>CD27<sup>+</sup>],  
513 and late/exhausted memory [IgD<sup>-</sup>CD27<sup>-</sup>] (Ademokun et al., 2010).

514 It has been shown that the percentage of switched memory B cells, the predictors of optimal antibody  
515 responses, decreases with age (Frasca 2020), while the percentage of late/exhausted memory B cells,  
516 the antigen-experienced and pro-inflammatory B-cell subset, increases (Fecteau et al., 2006.  
517 Colonna-Romano et al., 2009). These senescent cells have stable cell cycle arrest, shorter telomeres  
518 and secrete pro-inflammatory cytokines before stimulation and are "refractory" to undergo in vitro  
519 class switch when stimulated with antigens and mitogens (Fecteau et al., 2006, Colonna-Romano et  
520 al., 2009). For a detailed description of B-cell age-related changes, please refer to Frasca et al., 2020.  
521 Finally, concerning sex, steroid hormones, few studies have analyzed post-menopausal IS. However,  
522 age-related changes in the IS are different between men and women, and some data show that  
523 immunosenescence develops earlier in men than in women, possibly because women have a higher  
524 life expectancy than men (Ostan et al., 2016). The IS changes related to sex/gender are described in  
525 detail in BOX 1 and **Figure 2**.

526

#### 527 **4. Inflammaging as the dark side of immunosenescence**

528 The changes of the IS occurring with age and characterizing immunosenescence should be considered  
529 a dynamic process involved in the adaptation to exogenous and endogenous detrimental stimuli to  
530 which our body is exposed lifelong and the major contributor to inflammaging (Franceschi et al.  
531 2000a, Shaw et al., 2010, Franceschi et al., 2014; 2018, Monti et al., 2017). Inflammaging is one of  
532 the seven pillars of the aging process described by Kennedy and collaborators and characterize the  
533 major age-related diseases (Kennedy et al., 2014, Franceschi and Campisi, 2014) and representing an  
534 example of remodeling because it could be considered as the result of the imbalance between  
535 inflammatory and anti-inflammatory networks (Franceschi et al., 2007). Over more than 20 years  
536 from its discovery, in addition to immunosenescence, several biological basic mechanisms that  
537 contribute to inflammaging have been described and can be summarised as follow: i) accumulation  
538 with age, in many tissues, of senescent cells secreting pro-inflammatory mediators that can spread

539 the senescent phenotype to the neighbouring cells (Coppe et al., 2008; Song et al., 2020) and promote  
540 age-related diseases (Campisi and d'Adda di Fagagna, 2007); ii) age-related increased production of  
541 cell debris and components resulting from cell death or damage, such as nucleic acids, mitochondrial  
542 DNA (mtDNA), cardiolipin, mitochondria, Heat Shock Proteins and other proteins, that collectively  
543 are known as DAMPs, and are recognized by innate immune receptors like TLRs, NOD-Like  
544 Receptors (NLR) and cGMP-AMP synthase (cGAS). The accumulation with age of DAMPs has been  
545 called "Garb-aging" and can trigger innate immunity and the production of pro-inflammatory  
546 cytokines (Pinti et al., 2014; Franceschi et al., 2017); iii) the concurrently age-related decreased  
547 disposal capability (Franceschi et al., 2017). With age, autophagy and other pathways regulating  
548 proteostasis, such as proteasome activity (Mishto et al., 2006a; Mishto et al., 2006b), are reduced,  
549 contributing to the accumulation of misfolded protein aggregates activating inflammatory pathways.  
550 iv) telomere shortening and nuclear DNA damage, mediated by ROS and other agents, trigger DNA  
551 repair response and the production of pro-inflammatory compounds (Vitale et al., 2013); v) pro-  
552 inflammatory circulating microRNA (inflammaMIR) (Olivieri et al., 2013); vi) age-related  
553 accumulation in the blood of pro-inflammatory agalactosylated N-glycans, which represent one of  
554 the most powerful markers of biological age in humans (Dall'Olio et al., 2013); vii) enhanced  
555 activation of the coagulation pathway contributes to the rise of inflammatory tone increasing the risk  
556 for arterial and venous thrombosis in older people; viii) impaired regulation of complement pathway  
557 may induce a local inflammatory reaction in many degenerative diseases (i.e. the age-related macular  
558 degeneration) (Gallenga et al., 2014); ix) excess of energy/nutrients that drives to an inflammatory  
559 process coordinated by metabolic cells called "metaflammation" (Franceschi et al., 2018b, Cevenini  
560 et al. 2013). x) age-related gut microbiota dysbiosis represents a driving force for the homeostasis of  
561 the IS and an important source of inflammatory stimuli during aging (Biagi et al., 2010; Biagi et al.,  
562 2016). An in-depth description of gut microbiota remodeling in aging and centenarians will be  
563 illustrated in the next section of this review.

564 The key player in this inflammatory response is represented by the macrophage, which expresses  
565 many receptors for DAMPs and is present in virtually all the organs and tissues of the body and thus  
566 likely is responsible for local inflammaging (Sochocka et al. 2017). Macrophages can acquire  
567 memory-like characteristics upon activation by recognizing DAMPs (trained immunity), responding  
568 to different antigen exposure, and modulating fibrotic and inflammatory processes (Jeljeli et al.,  
569 2019). Compared to young people elderly are characterized by increased production of pro-  
570 inflammatory cytokines such as Interleukin (IL)-1 $\beta$ , IL-6 and Tumor Necrosis Factor (TNF)- $\alpha$   
571 (Fagiolo et al., 1993). Although these cytokines, together with IL-8 and CRP, can be considered the  
572 most relevant circulating biomarkers of inflammaging (Ferrucci and Fabbri, 2018), recently it has

573 been identified an immune signature for age-related chronic inflammation (Sayed et al., 2021) able  
574 to track multiple diseases and immunosenescence and predict multimorbidity. A major contributor to  
575 this inflammatory clock is CXCL9, a T-cell chemoattractant produced by neutrophils, macrophages,  
576 and endothelial cells (Sayed et al., 2021).

577 Starting from the life in utero and lasting during the entire life, the IS starts to record all the  
578 immunological experiences and stimuli it was exposed to (immunobiography) and plays out  
579 inflammatory responses to cope with and neutralize the large variety of stressors (Franceschi et al.,  
580 2017a; Santoro et al., 2020a).

581 This could help understand and interpret the individual heterogeneity of immune responses (to  
582 infections and vaccinations) that becomes particularly evident at old age and could affect both  
583 immunosenescence and inflammaging (Franceschi et al., 2017a). The phenotype of older adults is  
584 very complex and dynamic, continuously balancing between adaptive robustness and accumulating  
585 frailty (Franceschi et al., 2000; Ginaldi et al., 2005).

586 When kept under a certain threshold, this chronic inflammatory stimulation should not be considered  
587 detrimental (Furman et al., 2019) because it pushes a secondary adaptive activation of anti-  
588 inflammatory networks (Franceschi et al., 2007; Franceschi et al., 2018a). The strength of the adaptive  
589 response is likely critical to determine different aging trajectories and the net outcome: unsuccessful  
590 aging and age-associated diseases rather than successful aging and longevity.

591 The anti-inflammatory response represents a dynamic and active process able to trigger specific  
592 molecular pathways aimed to inhibit and resolve dangerous inflammation (Perretti and D'Acquisto,  
593 2006). Consistent with this, the development of age-related diseases and frailty is a result of excessive  
594 stimulation of pro-inflammatory responses but also an ineffective anti-inflammatory reaction  
595 (Morrisette-Thomas et al., 2014), while the attaining of longevity and successful aging is determined  
596 by a reduced predisposition to stimulate inflammatory pathways in addition to an effective anti-  
597 inflammatory response. In other words, individuals who have a very well preserved and organized  
598 anti-inflammatory activity are able to counteract the age-related increase of inflammatory markers  
599 (inflammaging), and the probability to develop age-related diseases is highly reduced or delayed or  
600 show less severe consequences (Franceschi et al., 2007) (**Figure 3**).

601 Centenarians that represent the best example of successful aging have a large quantity of circulating  
602 anti-inflammatory molecules such as Transforming Growth Factor (TGF)-b1, IL-10, IL-1 receptor  
603 antagonist (IL-1RA), adiponectin, cortisol, anti-inflammatory arachidonic acid compounds, including  
604 HETE and EET, mitokines (FGF21, GDF15 and HN) (Salvioli et al., 2009; Gerli et al., 2000;  
605 Genedani et al., 2008; Meazza et al., 2011; Collino et al., 2013; Morrisette-Thomas et al., 2014; Conte  
606 et al., 2019). However, this anti-inflammatory state is effectively triggered to counterbalance the

607 concomitant increased levels of inflammatory molecules in plasma, such as IL6, IL-15, IL18, IL18  
608 binding protein, IL22, CRP, serum-amyloid A, fibrinogen, Von Willebrand factor, resistin and  
609 leukotrienes (Bonafè et al., 2001; Franceschi et al., 2007; Gangemi et al., 2005; Collino et al., 2013;  
610 Basile et al., 2012). For a detailed review on inflammaging and longevity, please refer to Monti et al.  
611 (2017). It is still unknown whether this optimal balance is a characteristic of these individuals during  
612 their entire life due to both lifestyle and genetic background or if they acquire this ability in the later  
613 phase of life due to an adaptive strategy. A recent whole-genome sequencing analysis from our group  
614 showed that individuals aged more than 105 years have a peculiar genetic background associated with  
615 DNA repair system and clonal haematopoiesis that could likely represent important factors for healthy  
616 aging (Garagnani et al., 2021).

617 Inflammaging is a systemic physiological process involving most of the cells and the organs of the  
618 body (Cevenini et al., 2010; Cevenini et al., 2013). A variety of tissues (adipose tissue, muscle),  
619 organs (brain, liver), systems (immune system) and ecosystems (skin, oral, lung, gut and genito-  
620 urinary tract microbiota) contributes differently to the onset and progression of inflammaging with  
621 specific site organs-restricted and/or systemic effects (Cevenini et al., 2013; Santoro et al., 2020b).  
622 For instance, it is well known that adipose tissue (Franceschi, 2017d) not only increases quantitatively  
623 with age throughout the body with marked differences between males and females (Ponti et al., 2020;  
624 Santoro et al., 2018a) but also has been recognized as an endocrine source of mediators (hormones,  
625 acute-phase proteins, cytokines, adipokines and growth factors) (Calder et al., 2011). In particular,  
626 the accumulation of abdominal fat (visceral rather than subcutaneous) can establish and sustain a  
627 chronic low-grade inflammation (Santoro et al., 2018a) and contributing to metabolic diseases (Ostan  
628 et al., 2013). Obesity-associated inflammation has also been an additional factor for COVID-19  
629 patients (Frasca et al., 2021). Interestingly, it has been found that single nucleotide polymorphism  
630 R293Q in the cGAS/STING pathway is associated with a decreased risk for obesity-associated  
631 cardiovascular disease in age-advanced subjects (Hamann et al., 2020), suggesting that this STING  
632 variant decreases the sensitivity of the innate IS towards DAMPS reducing the risk of age-related  
633 diseases.

634 Aging is not uniform, neither across tissues nor among individuals. People at the same chronological  
635 age could possess different aging rates due to a unique complex interaction among intrinsic and  
636 extrinsic factors (genetic vs environment) determining the so-called "biological age" (Hamczyk et al.,  
637 2020). Several studies are currently investigating the biomarkers defining biological age (Cohen et  
638 al., 2020). However, what is becoming more evident is that the level of inflammaging (tightly  
639 associated with biological age rather than chronological age) represents a critical factor in the large

640 inter-individual variability of the elderly and predicting the development of age-related diseases  
641 (Franceschi, 2018b, Lehallier et al., 2019, Deelin, 2019).

642 The most common triggers of inflammaging include chronic infections (e.g. persistent viral infection  
643 by CMV; Sansoni et al., 2014), physical inactivity, (visceral) obesity, intestinal dysbiosis, diet, social  
644 isolation, psychological stress, early life adversity (Merz and Turner 2021), disturbed sleep and  
645 disrupted circadian rhythm, and exposure to xenobiotics such as air pollutants, hazardous waste  
646 products, industrial chemicals and tobacco smoking (Furman et al., 2019). Therefore, the biological  
647 markers of inflammaging can vary according to environmental, cultural, and geographical settings  
648 that reflect worldwide (Batista et al., 2020; Franceschi et al., 2018c), and there is an urgent need to  
649 find tools to investigate inflammaging at personal level. Interestingly, several data report that sex and  
650 gender impact the immune response at old age and, consequently, on inflammaging (see BOX 1). In  
651 this framework, it is easy to understand that personalized strategies are needed to counteract  
652 inflammaging. Among the non-pharmacological approaches, dietary and physical activity  
653 interventions are the most encouraged. Accordingly, calorie restriction, intermitting fasting,  
654 adherence to healthy dietary patterns such as Mediterranean diet, meal timing, and frequency  
655 combined with an adequate amount of physical activity have likely advantageous effects on health  
656 (Marseglia et al., 2018; Jennings et al., 2018; Jennings et al., 2019) and longevity (Santoro et al.,  
657 2020a) also because they directly increase the abundance of specific taxa of the gut microbiota and  
658 of specific microbial metabolites associated with reduced frailty and pro-inflammatory markers and  
659 improved cognitive function (Ghosh et al., 2020).

660

#### 661 **4. 1 Gut microbiota: at the crossroad among inflammaging, immunosenescence and longevity**

662 The commensal microbiota associated with the intestinal tract (GM) is currently the most studied in  
663 humans. Microbes in the gut are fundamental for the digestion function, the biosynthesis of vitamins  
664 and amino acids (Mardinoglu et al., 2015; Soto-Martin et al., 2020) and the modulation of fat storage  
665 and improve the ability of our body to extract nutrients from food (Martinez-Guryn et al., 2018);  
666 moreover, they can strongly control innate and specific immunity.

667 GM alterations in composition and function occurring during aging and as a consequence of age-  
668 related diseases (Lakshminarayanan et al., 2014), called dysbiosis, could impact on inflammaging  
669 due to the continuous stimulation of the IS, which causes immunosenescence (Santoro et al., 2020).

670 Overall, this inflammatory environment contributes to the progression of various pathological  
671 conditions in older adults and makes the host more susceptible to dangerous bacteria (Bischoff, 2016).

672 The synergism between GM and immune cells has a remarkable impact on the host's health and  
673 immune defense. The microbiota is continuously adapting to its environment throughout the lifetime

674 and is largely heterogeneous among individuals due to genetics and lifestyle factors. Diet, place and  
675 country of residence (Claesson et al., 2012; Ghosh et al., 2020), physical activity (Huang et al., 2019),  
676 smoking (Lee et al., 2018), sleep quality (Smith et al., 2019), mental health (Barandouzi et al., 2020)  
677 and medication (Sun et al., 2019) are key factors able to modulate GM from birth to advanced age.  
678 The GM composition of healthy adults is constituted at 90% by Bacteroidetes (Bacteroides,  
679 Prevotella) and Firmicutes (Clostridium, Faecalibacterium, Lactobacilli, Ruminococcus), and the  
680 residual 10% by Actinobacteria (Bifidobacterium), Proteobacteria (Escherichia, Helicobacter,  
681 Shigella) and Verrucomicrobia (Akkermansia) phyla (Qin et al., 2010). This composition drastically  
682 changes with aging. Indeed, pathophysiological changes in the gastrointestinal tract, lifestyle  
683 modification, nutrition (Claesson et al., 2012), behaviour, immunosenescence, and inflammaging  
684 strongly impact GM, eventually pushing maladaptive variants (Claesson et al., 2011). Specifically,  
685 the main age-associated changes of GM (summarized in **Table 1**) regard a decrease in biodiversity  
686 with a progressive loss of Short Chain Fatty Acids (SCFAs) producing bacteria with anti-  
687 inflammatory abilities and an increase of pathobionts (potential harmful bacteria). On the whole, these  
688 modifications set a vicious circle, further boosting inflammation and reduce the capability of older  
689 people to positively adapt to the different environmental events because of the decline of metabolic  
690 alternatives, for example, for SCFAs production.

691 Centenarians, the best example of successful adaptation, represent a valuable model to explore how  
692 the microbiota component can successfully maintain or re-establish a mutualistic relationship with  
693 the human host, along with the occurrence of age-related variations. Indeed, the gut microbiota of  
694 centenarians emerges as a peculiar ecosystem, different from that of elderly and adults, specifically  
695 adapted to a highly aged host (Biagi et al., 2017; Biagi and Santoro, 2021). Interestingly, the  
696 comparison of GM composition of centenarians from different countries has shown that while  
697 lifestyle, ethnicity and geography undoubtedly impact such extreme microbiota adaptive variations,  
698 common signatures of longevity emerge among the studied populations (Santoro et al., 2018b).

699 The microbiota of centenarians was studied for the first time by our group in 2010 and revealed that  
700 centenarians (almost all women) showed all the modifications associated with aging itself (Biagi et  
701 al., 2010, 2012, 2013). Moreover, an increase of pro-inflammatory IL-6 and IL-8 was found (Biagi  
702 et al. 2010). The typical age modification above described, *i.e.* the reduction in the abundance of  
703 known health-promoting bacteria belonging to the genera *Faecalibacterium*, *Roseburia*,  
704 *Coprococcus*, and an increase in the proportion of subdominant species, including putative pro-  
705 inflammatory bacteria (*Enterobacteriaceae* and *Desulfovibrionaceae* families) were also present in  
706 centenarians. At the same time, the gut microbiota of centenarians and, especially, semi-  
707 supercentenarians ( $\geq 105$  years old) showed some peculiarities that might be able to contribute



708 somehow to the maintenance of health during the extreme phases of life. Indeed, the GM of the  
709 exceptional survivors presented a higher prevalence of *Bifidobacterium*, a long time a known  
710 probiotic group of bacteria, as well as higher abundances of subdominant members of the human gut  
711 ecosystem that have been explored only recently, such as *Akkermansia* and *Christensenellaceae*  
712 (Biagi et al., 2016).

713 *Akkermansia muciniphila* is a mucin-degrading bacterium whose abundance in the human gut has  
714 been inversely correlated to several metabolic disease states (Geerlings et al., 2018). Also,  
715 *Christensenellaceae* has been associated with a state of metabolic health for the host, notably  
716 correlated to a lean phenotype and a reduced visceral adipose tissue (Oki et al., 2016; Tavella et al.,  
717 2021). Other Authors came to similar results when analyzing the microbiota of centenarians living in  
718 rural or isolated areas of China and Japan (Kong et al., 2016; Odamaki et al., 2016). Recently, studies  
719 involving centenarians from a rural area of India (Tuikhar et al., 2019) and Russia (Kashtanova et al.,  
720 2020) have been published, and the results were similar to those Italian, Chinese and Japanese  
721 populations in terms of increased biodiversity but with some peculiarities owing to the different  
722 lifestyle and nutritional habits.

723 The neutralization of inflammaging is one of the most hypothesized methods by which the microbiota  
724 is supposed to contribute to healthy aging (Biagi et al., 2013; Franceschi et al., 2018b). In a recent  
725 study using germ-free mice, the microbiota of old mice was transferred into young mice inducing  
726 inflammation in the young ones. However, the levels of inflammation were negatively correlated with  
727 the abundance of *Akkermansia* in the original microbiota (Fransen et al., 2017). Moreover,  
728 metagenomics studies exploring the function of the GM of centenarians conducted on two different  
729 Italian cohorts of centenarians from Sardinia and Bologna showed an augmented capability for  
730 glycolysis and SCFA production (Wu et al., 2019; Rampelli et al. 2020) also associated with functions  
731 related to the degradation of xenobiotics (Rampelli et al. 2020). Metabonomics approaches revealed  
732 that centenarians display a marked decrease in tryptophan concentration with a unique alteration of  
733 specific glycerophospholipids and sphingolipids and increased excretion of urine  
734 phenylacetylglutamine (PAG) and p-cresol sulfate (PCS) (Collino et al., 2013; Montoliu et al., 2014).  
735 Furthermore, centenarians and their offsprings are characterized by a specific profile of Volatile  
736 organic compounds (VOCs) in urine and faeces (Conte et al., 2020). In the authors' opinion, such  
737 features of microbial metabolisms might be involved in maintaining and prolonging metabolic and  
738 immunological health, adapting to the environment, and ultimately favouring longevity. The  
739 possibilities to exploit such data for human health maintenance during aging are still being explored,  
740 and exciting scenarios can be envisaged. *Akkermansia* and *Christensenella* represent promising  
741 health-promoting strategies and have been listed among the so-called "next-generation probiotics",

742 *i.e.* bacteria other than the traditional *Lactobacillus* and *Bifidobacterium*, which could become part  
743 of more innovative and targeted probiotic strategies (O'Toole et al., 2017; Chang et al., 2019). In  
744 addition, the possibility of using microbiota transplantation to promote those features in the gut  
745 ecosystem that are known to be linked to longevity could be another possibility to sustain the IS to  
746 counteract inflammation and promote or restore healthiness.

747

## 748 **5. Can Immunosenescence and Inflammaging increase COVID-19 susceptibility?**

749 One of the most important observations in the COVID-19 pandemic is the differential susceptibility  
750 to illness. We know that individuals at the greatest risk are older persons (mainly men) affected by  
751 multimorbidities, including hypertension, diabetes, and/or obesity (Zhou et al., 2020; Gemmati et al.,  
752 2020). However, not all infected aged people will progress to the severe stage and will not die, but as  
753 yet, the why is not clear. Understanding the remodeling and adaptation or maladaptation of IS with  
754 age during the COVID-19 pandemic is fundamental because it could explain the different  
755 susceptibility among aged people and the different responses to vaccines (Ciabattini et al., 2020). In  
756 other words, it could help us to distinguish better which changes of IS may be detrimental or  
757 beneficial. As we highlighted several times in this review, older people are characterized by extreme  
758 heterogeneity due to the numerous and different exposure factors encountered lifelong that can  
759 determine each individual's different immune responses (immunobiography) (Franceschi et al.,  
760 2017a). With aging, these factors capable of eliciting inflammatory responses increase unabated,  
761 leading to high levels of pro-inflammatory mediators, which are believed to contribute to the  
762 pathogenesis of many, if not all, age-associated diseases and the progression of the aging process.  
763 (Franceschi and Campisi, 2014). Age-related gut microbiota dysbiosis represents a source of pro-  
764 inflammatory factors and may play an essential role in determining the course of COVID-19 (Ferreira  
765 et al., 2020). Inflammaging, associated with immunosenescence, likely results from the imbalance  
766 between the production of pro-and anti-inflammatory mediators. This is a sort of adaptive mechanism  
767 to a person's lifelong exposure to stressors, whereby inflammation continuously triggers anti-  
768 inflammatory responses (Spazzafumo et al., 2013). Inflammation could, in turn, be considered a sort  
769 of hormetic response, having positive outcomes at low doses (physiological inflammation) at young  
770 and adult ages and becoming detrimental during the postreproductive period, especially in people  
771 who, as a result of genetic background and/or unhealthy lifestyle, are not able to maintain an optimal  
772 balance between inflammaging and anti-inflammaging (Santoro et al., 2020a; Martucci et al., 2017).  
773 Thus, inflammaging and immunosenescence, characterized by a loss in adaptive immune functions,  
774 could be predisposing conditions that sustain the mechanism by which the SARS-CoV-2 escape the

775 immune surveillance and leads to serious COVID-19. This reinforces the need to find treatments that  
776 stimulate the innate immune response to protect the organism from infections.

777 In other words, the aged persons who have developed a phenotype characterized by a higher level of  
778 plasma inflammatory mediators leading to comorbidities ((e.g., hypertension, cardiovascular  
779 diseases, obesity, diabetes) could present higher COVID-19 susceptibility with severe complications  
780 and explain the high mortality rates in this cohort (**Figure 4**). In addition, to systemic risk factors for  
781 higher COVID-19 severity in the elderly, it is also essential to consider changes that occur locally in  
782 the lung with age. Recently, data regarding the aging human lung's transcriptomic features and  
783 cellular landscape concerning SARS- CoV-2 have been obtained (Chow et al., 2021). Lung aging is  
784 transcriptionally characterized by increased cell adhesion and heightened stress responses, along with  
785 reduced mitochondria and diminished cellular replication. Moreover, many age-related alterations in  
786 cellular composition, including cells implicated in response to SARS-COV-2, have been  
787 demonstrated. These changes highlighted a reduced regenerative capacity with a progressive loss of  
788 lung parenchyma during aging and an augmented risk for chronic obstructive pulmonary disease and  
789 pulmonary fibrosis. Furthermore, among immune cells, proliferating natural killer (NK)/T cells  
790 decreased with age, whereas IGSF21+ dendritic cells increased with age. NK and T cells are  
791 fundamental in response to SARS-CoV-2, and their decrease may contribute to the increased risk of  
792 COVID-19 morbidity and mortality in older patients. Chow et al. have also demonstrated that some  
793 age-associated genes are enriched among genes directly regulated by SARS-CoV-2 infection in vitro  
794 and in vivo, suggesting transcriptional parallels between the aging lung and SARS-CoV-2 infection  
795 (Chow et al., 2021).

796 Moreover, a central lesson from aging medicine suggests that the biological age, rather than  
797 chronological age, of affected patients, might be critical in systematically assessing COVID-19  
798 infections to avoid excess mortality. At present, it is possible to quantify biological age using various  
799 proteomic, epigenetic and inflammatory biomarkers, which should help us predict the risk of  
800 developing major age-related diseases and susceptibility to Covid-19. (Sayed et al., 2021; Lehallier  
801 et al., 2019; Horvath et al., 2015).

802 Centenarians have a peculiar state/degree of inflammaging, which is much lower than predicted by  
803 their chronological age and is biased toward anti-inflammaging. The production of anti-inflammatory  
804 molecules and cells formed lifelong represent an adaptive, compensatory mechanism to continuously  
805 down-regulate the inflammatory process and avoid its chronic detrimental effects (Franceschi et al.,  
806 2007; Sayed et al., 2021; Storci et a., 2019).

807 In the era of COVID-19, it is interesting to underline that centenarians showed a remarkable capacity  
808 to recover after coronavirus infection. To this regard, there are anecdotal observations that

809 centenarians and sometimes supercentenarians (people over 110 years old) survived and recovered  
810 after SARS-CoV-2 infection (Abbatecola et al., 2020), as confirmed in a group of centenarians  
811 belonging to "Centenari a Trieste (CaT) study" (Marcon et al., 2020). Centenarians have better  
812 resilience and biological reserves to better cope with inflammaging as they can mount a robust anti-  
813 inflammaging response neutralizing the overall presence of inflammatory processes.  
814 Finally, it is essential to emphasize the crucial role of inflammaging and immunosenescence in post-  
815 covid syndrome or long-covid, one of the major health burdens in the following years. Nearly a third  
816 of individuals discharged from hospital after acute COVID-19 need to be re-admitted to hospitals  
817 after few months (and more than 1 in 10 died after discharge); two-thirds had increased rates of  
818 multiorgan dysfunction and respiratory diseases, diabetes and cardiovascular diseases compared with  
819 the expected risk in the general population (Ayoubkhani et al., 2021).  
820 Consequently, to SARS-CoV-2 infection, the IS underwent complex testing, and the recovery is  
821 highly heterogeneous depending also on the personal background, the severity of disease,  
822 pharmacological treatments and the total capacity of long-term adaptation and resilience.

823

## 824 **6. Conclusions and perspectives**

825 Aging is one of the most intricate and complex biological phenomena and represents the major risk  
826 factor for all age-related diseases, such as infections, cancer, autoimmune disorders, and chronic  
827 inflammatory diseases. A significant characteristic of older people is their heterogeneity regarding  
828 their health status (presence/absence of comorbidities, frailty, cognitive impairment) and their  
829 different capability to mount an immune response to pathogens and vaccines (Franceschi et al., 2017;  
830 Ciabattini et al., 2018)

831 Aging is not uniform among individuals and can be considered a continuum with the extreme  
832 phenotypes represented by diseases and disabilities on one side and healthy aging and longevity on  
833 the other side. Several factors, such as genetics, nutrition, exercise, previous exposure to  
834 microorganisms, sex (biological-related), gender (cultural-related) and human cytomegalovirus  
835 (HCMV) status, can influence immunosenescence. Many age-related changes in IS have been  
836 described, and most of them have been considered harmful and causes many age-related diseases.

837 Changes occur in both the innate and the adaptive IS, but not with the same extent or the same  
838 consequences. Therefore, it could be conceptualized that the age-related immune changes may be a  
839 mix of adaptation/resilience and maladaptation, closely related to the immunobiography (Franceschi  
840 et al. 2017a; Fulop et al., 2018).The balance between these two processes will establish how the  
841 person will age (**Figure 3**).

842 Moreover, sex and gender strictly impact the IS of the elderly, likely contributing to the sex/gender  
843 disparity in terms of epidemiology, pathophysiology, symptoms, and severity of age-related diseases  
844 such as autoimmune diseases. There is an intricate interrelationship between immunosenescence and  
845 inflammaging able to generate a complex network of adaptive mechanisms that can favor longevity  
846 when able to counteract the injuries individuals are exposed lifelong (adaptation) or, on the opposite  
847 side, increase the susceptibility to diseases when inadequate (maladaptation) (Figure 3).  
848 The study of this interconnection is now becoming of particular interest during the COVID-19  
849 pandemic, bringing to the fore the critical need to combat immunosenescence and inflammaging and  
850 improve older people's immune function and resilience. Future studies are necessary to elucidate these  
851 interactions and increase targets for new interventions to decrease the deleterious effects of aging and  
852 use the beneficial effects for a better health span in the elderly. The gut microbiota dysbiosis occurring  
853 during aging plays an essential role in modulating inflammaging, and information gathered from the  
854 studies on centenarians could represent a valuable health-promoting strategy to encourage treatments  
855 with longevity-associated probiotics. The rapid advancement of knowledge and technologies to study  
856 the IS and the integration of omics such as genetic/epigenetic/metabolic and environmental factors  
857 (nutrition and physical activity) will pave the way to improve the insight on the beneficial effects of  
858 immunosenescence and inflammaging as processes triggered to adapt and counteract aging also at  
859 personal level.

860  
861

#### 862 **BOX 1 –Inflammaging: also a matter of sex and gender?**

863 Although women experienced almost five years of advantage in life expectancy, these are years of  
864 diseases and disability (Ostan et al., 2016; Gemmati et al., 2019), showing a higher prevalence of  
865 multimorbidity patterns than men (Abad-Díez et al. 2014). Inflammaging represents the common hub  
866 shared by the majority of the age-related diseases (ARDs) (Furman et al., 2019; Franceschi and  
867 Campisi, 2014) and likely contributing to the gender disparity in terms of epidemiology,  
868 pathophysiology, symptoms and severity of diseases (Franceschi et al., 2018a; Ostan et al., 2016). A  
869 complex interplay modulates the lifelong balance between inflammaging and anti-inflammaging  
870 among sex (genetics, epigenetics and hormones) and gender (environmental, lifestyle and socio-  
871 cultural) factors, profoundly affects aging trajectories and ARDs risk (Franceschi et al., 2007;  
872 Franceschi et al., 2018a). Differences between males and females in inflammaging have been reported  
873 with contradictory results likely due to the variety of factors modulating inflammaging in different  
874 experimental settings and human populations (Yang and Kozloski, 2011, Newman et al., 2016, Milan-  
875 Mattos et al., 2019, Di Benedetto et al., 2019, Marquez et al., 2020). During life, the IS evolves and

876 changes, with marked sex differences (**Figure 2**). Adult females have more robust innate and adaptive  
877 immune responses (Oertelt-Prigione, 2012) with higher CD4<sup>+</sup> T cell counts and CD4<sup>+</sup>:CD8<sup>+</sup> ratios  
878 than males (Klein & Flanagan 2016). Transcriptional data confirmed this pattern in women while  
879 suggesting a higher expression of genes related to myeloid cells in men (Bongen et al., 2019). The  
880 number and activity of B and T cells in aged males rapidly decline compared to females (Goetzl et  
881 al., 2010; Marquez et al., 2020). Age-related differences between the two sexes increase after age 65,  
882 with older men having higher monocytes activity and inflammation (Marquez et al., 2020). At  
883 variance, during menopause, females have higher pro-inflammatory markers such as CRP and GM-  
884 CSF compared with males (Furman et al., 2014). However, some evidence shows that, with age, T  
885 cells from females produce more IL-10 than do males' T cells (Pietschmann et al., 2003) which may  
886 neutralize the adverse effects of inflammaging with age. Although these effects may contribute to an  
887 improved humoral response in women, at the same time, they can favour the appearance of  
888 autoreactive clones (Sakiani et al., 2013). Females are indeed 2-10-fold more prone to a series of  
889 disabling autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus  
890 erythematosus, systemic rheumatoid arthritis, myasthenia gravies, Hashimoto's thyroiditis and  
891 Sjogren's syndrome (Keestra et al., 2021). Female hormones decrease the expression of autoimmune  
892 regulator gene (AIRE) by triggering the negative selection of self-reactive T-cells (Bakhru et al.,  
893 2016) and Treg development, thus protecting against autoimmunity. Moreover, the cellular  
894 mosaicism resulting from the random inactivation of X chromosome loci in all mammal cells from  
895 females is likely to create a unique functional plasticity within female immune cells (Youness et al.,  
896 2021; Yu et al., 2021) and thus be involved in the aetiology of female autoimmune diseases. The  
897 better immune females' response is also evident after vaccinations when women reveal higher  
898 immunoglobulins and seroconversion and lower disease (Flanagan et al., 2017). Finally, it is  
899 important to stress that sex/gender differences in the IS cell number and function are not the sole  
900 contributor to the sex dimorphism in inflammaging, resulting from the sum of the age-related local,  
901 chronic inflammatory processes of multiple organs/systems.

902

903

904 **AUTHOR CONTRIBUTIONS**

905 DM and AS contributed to the concept, writing and critical discussion of the manuscript. EB  
906 contributed to the critical discussion of the manuscript and the revision of the literature. All authors  
907 reviewed and/or edited the manuscript before submission.

908

909 **FUNDING**

910 This work has been partially supported by: the Roberto and Cornelia Pallotti legacy for cancer  
911 research and the JPI-HDHL-Metadis, "EURODIET" project (ID: 1164; 2020-2023) to AS; by the  
912 Fondazione CR Firenze (2019) n. 24213-(2018.1015) and Project of Excellence "Gender Medicine"  
913 2020, Department of Experimental and Clinical Biomedical Sciences "Mario Serio to DM.

914

915 **DECLARATION OF INTEREST**

916 The authors have no conflicts of interest

917 **References:**

918 Website: <https://www.who.int/news-room/fact-sheets/detail/ageing-and-health>

919 Abad-Díez, J.M., Calderón-Larrañaga, A., Poncel-Falcó, A., Poblador-Plou, B., Calderón-Meza,  
920 J.M., Sicras-Mainar, A., Clerencia-Sierra, M., Prados-Torres, A., 2014. Age and gender differences  
921 in the prevalence and patterns of multimorbidity in the older population. *BMC Geriatr.* 14,75.  
922 <https://doi.org/10.1186/1471-2318-14-75>.

923 Abbatecola, A.M., Antonelli-Incalzi, R., 2020. Editorial: COVID-19 spiraling of frailty in older  
924 Italian patients. *J Nutr Health Aging.* 24(5), 453–5. <https://doi.org/10.1007/s12603-020-1357-9>.

925 Ademokun, A., Wu, Y.C., Dunn-Walters, D., 2010. The ageing B cell population: composition and  
926 function. *Biogerontology.* 11, 125–137. <https://doi.org/10.1007/s10522-009-9256-9>.

927 Akbar, A.N., Henson, S.M., Lanna, A., 2016. Senescence of T lymphocytes: implications for  
928 enhancing human immunity. *Trends Immunol* 37(12), 866–76.  
929 <https://doi.org/10.1016/j.it.2016.09.002>.

930 Alberti, S., Cevenini, E., Ostan, R., Capri, M., Salvioli, S., Bucci, L., Ginaldi, L., DeMartinis, M.,  
931 Franceschi, C., Monti, D., 2006. Age-dependent modifications of type 1 and type 2 cytokines within  
932 virgin and memory CD4+ T cells in humans. *Mech. Ageing Dev.* 127 (6), 560–566.  
933 <https://doi.org/10.1016/j.mad.2006.01.014>.

934 Alonso-Fernandez, P., Puerto, M., Mate, I., Ribera, J.M., de la Fuente, M., 2008. Neutrophils of  
935 centenarians show function levels similar to those of young adults. *J. Am. Geriatr. Soc.* 56, 2244–  
936 2251. <https://doi.org/10.1111/j.1532-5415.2008.02018.x>.

937 Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D., Zychlinsky, A., 2012. Neutrophil function: from  
938 mechanisms to disease. *Annu Rev Immunol* 30, 459–489. <https://doi.org/10.1146/annurev-immunol-020711-074942>.

939

- 940 Andrasfay, T., Goldman, N., 2021. Reductions in 2020 US life expectancy due to COVID-19 and the  
941 disproportionate impact on the Black and Latino populations. *Proc Natl Acad Sci U S A.* 118(5),  
942 e2014746118. <https://doi.org/10.1101/2020.07.12.20148387>.
- 943 Appay, V., Sauce, D., 2014. Naive T cells: the crux of cellular immune aging? *Exp Gerontol.* 54, 90–  
944 3. <https://doi.org/10.1016/j.exger.2014.01.003>.
- 945 Ayoubkhani, D., Khunti, K., Nafilyan, V., Maddox, T., Humberstone, B., Diamond, I., Banerjee, A.,  
946 2021. Post-covid syndrome in individuals admitted to hospital with covid-19: retrospective cohort  
947 study. *BMJ.* 372, n693. <https://doi.org/10.1136/bmj.n693>.
- 948 Bajwa, M., Vita, S., Vescovini, R., Larsen, M., Sansoni, P., Terrazzini, N., Caserta, S., Thomas, D.,  
949 Davies, K.A., Smith, H., Kern, F., 2017. CMV-specific T-cell responses at older ages: broad  
950 responses with a large central memory component may be key to long-term survival. *J Infect Dis.*  
951 215(8), 1212–20. <https://doi.org/10.1093/infdis/jix080>.
- 952 Bakhru, P., Su, M.A., 2016. Estrogen turns down “the AIRE”. *J. Clin. Invest.* 126, 1239–1241.  
953 <https://doi.org/10.1172/JCI86800>.
- 954 Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.J., Pulendran, B., Palucka, K.,  
955 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18, 767–811.  
956 <https://doi.org/10.1146/annurev.immunol.18.1.767>.
- 957 Barandouzi, Z.A., Starkweather, A.R., Henderson, W.A., Gyamfi, A., Cong, X.S., 2020. Altered  
958 composition of gut microbiota in depression: A systematic review. *Front Psychiatry.* 11, 541.  
959 <https://doi.org/10.3389/fpsy.2020.00541>.
- 960 Barbé-Tuana, F., Funchal, G., Schmitz, C.R.R., Maurmann, R.M., Bauer, M.E., 2020. The interplay  
961 between immunosenescence and age-related diseases. *Semin Immunopathol.* 42(5), 545–557.  
962 <https://doi.org/10.1007/s00281-020-00806-z>.
- 963 Basile, G., Paffumi, I., D’Angelo, A.G., Figliomeni, P., Cucinotta, M.D., Pace, E., Ferraro, M., Saitta,  
964 S., Lasco, A., Gangemi, S., 2012. Healthy centenarians show high levels of circulating interleukin-  
965 22 (IL-22). *Arch Gerontol Geriatr.* 54, 459–461. <https://doi.org/10.1016/j.archger.2011.05.004>.
- 966 Batista, M.A., Calvo-Fortes, F., Silveira-Nunes, G., Camatta, G.C., Speziali, E., Turroni, S., Teixeira-  
967 Carvalho, A., Martins-Filho, O.A., Neretti, N., Maioli, T.U., Santos, R.R., Brigidi, P., Franceschi, C.,  
968 Faria, A.M.C., 2020. Inflammaging in Endemic Areas for Infectious Diseases. *Front Immunol.* 11,  
969 579972. <https://doi.org/10.3389/fimmu.2020.579972>.
- 970 Bektas, A., Schurman, S.H., Sen, R., Ferrucci, L., 2017. Human T cell immunosenescence and  
971 inflammation in aging. *J Leukoc Biol* 102, 977–988. <https://doi.org/10.1189/jlb.3RI0716-335R>.
- 972 Biagi, E., Nylund, L., Candela, M., Ostan, R., bucci, L., Pini, E., Nikkila, J., Monti, D., Satokari, R.,  
973 Franceschi, C., Brigidi, P., De Vos, W., 2010. Through aging, and beyond: gut microbiota and  
974 inflammatory status in seniors and centenarians. *PloS One.* 5(5), e10667.  
975 <https://doi.org/10.1371/journal.pone.0010667>.
- 976 Biagi, E., Candela, M., Fairweather-Tait, S., Franceschi, C., Brigidi, P., 2012. Aging of the human  
977 metaorganism: the microbial counterpart. *Age (Dordr).* 34(1), 247–267.  
978 <https://doi.org/10.1007/s11357-011-9217-5>.



- 979 Biagi, E., Candela, M., Turrioni, S., Garagnani, P., Franceschi, C., Brigidi, P., 2013. Aging and gut  
980 microbes: perspectives for health maintenance and longevity. *Pharmacol. Res.* 69(1), 11–20.  
981 <https://doi.org/10.1016/j.phrs.2012.10.005>.
- 982 Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turrioni, S., Consolandi, C.,  
983 Quercia, S., Scurti, M., Monti, D., Capri, M., Brigidi, P., Candela, M., 2016. Gut microbiota and  
984 extreme longevity. *Curr. Biol.* 26(11), 1480–1485. <https://doi.org/10.1016/j.cub.2016.04.016>.
- 985 Biagi, E., Rampelli, S., Turrioni, S., Quercia, S., Candela, M., Brigidi, P., 2017. The gut microbiota  
986 of centenarians: signatures of longevity in the gut microbiota profile. *Mech. of Ageing Dev.* 165(Pt  
987 B), 180–184. <https://doi.org/10.1016/j.mad.2016.12.013>.
- 988 Biagi, E., Santoro, A., 2021. A trait of longevity: the microbiota of centenarians (2021), in:  
989 “Comprehensive Gut Microbiota”; edited Elsevier [https://doi.org/10.1016/B978-0-12-819265-](https://doi.org/10.1016/B978-0-12-819265-8.00052-8)  
990 [8.00052-8](https://doi.org/10.1016/B978-0-12-819265-8.00052-8) in press
- 991 Bischoff, S.C., 2016. Microbiota and aging. *Curr. Opin. Clin. Nutr. Metab. Care.* 19, 26–30.  
992 <https://doi.org/10.1097/MCO.0000000000000242>.
- 993 Bonafè, M., Olivieri, F., Cavallone, L., Giovagnetti, S., Mayegiani, F., Cardelli, M., Pieri, C., Marra,  
994 M., Antonicelli, R., Lisa, R., Rizzo, M.R., paolisso, G., Monti, D., Franceschi, C., 2001. A gender-  
995 dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. *Eur. J.*  
996 *Immunol.* 31, 2357–61. [https://doi.org/10.1002/1521-4141\(200108\)31:8](https://doi.org/10.1002/1521-4141(200108)31:8<2357::aid-immu2357>3.0.co;2-x)  
997 [#60;2357::aid-immu2357](https://doi.org/10.1002/1521-4141(200108)31:8<2357::aid-immu2357>3.0.co;2-x)  
#62;3.0.co;2-x.
- 998 Bongen, E., Lucian, H., Khatri, A., Fragiadakis, G.K., Bjornson, Z.B., Nolan, G.P., Utz, P.J., Khatri,  
999 P., 2019. Sex Differences in the Blood Transcriptome Identify Robust Changes in Immune Cell  
1000 Proportions with Aging and Influenza Infection. *Cell Rep.* 29(7), 1961-1973.e4.  
1001 <https://doi.org/10.1016/j.celrep.2019.10.019>.
- 1002 Brubaker, A. L., Rendon, J.L., Ramirez, L., Choudhry, M. A. and Kovacs, E. J., 2013. Reduced  
1003 neutrophil chemotaxis and infiltration contributes to delayed resolution of cutaneous wound infection  
1004 with advanced age. *J. Immunol.* 190, 1746–1757. <https://doi.org/10.4049/jimmunol.1201213>.
- 1005 Bulati, M., Buffa, S., Candore, G., Caruso, C., Dunn-Walters, D.K., Pellicanò, M., Wu, Y.C., Colonna  
1006 Romano, G., 2011. B cells and immunosenescence: a focus on IgG+IgD–CD27– (DN) B cells in  
1007 aged humans. *Ageing Res Rev.* 10, 274–84. <https://doi.org/10.1016/j.arr.2010.12.002>.
- 1008 Butcher, S., Chahal, H., Savey, E., Killampalli, V.V., Alpar, E.K., Lord, J.M., 2001. Functional  
1009 Decline in Human Neutrophils with Age. *ScientificWorldJournal.* 1, 67.  
1010 <https://doi.org/10.1100/TSW.2001.105>.
- 1011 Calder, P.C., Ahluwalia, N., Brouns, F., Buetler, T., Clement, K., Cunningham, K., Esposito, K.,  
1012 Jönsson, L.S., Kolb, H., Lansink, M., Marcos, A., Margioris, A., Matusheski, N., Nordmann, H.,  
1013 O’Brien, J., Pugliese, G., Rizkalla, S., Schalkwijk, C., Tuomilehto, J., Wärnberg, J., Watzl, B.,  
1014 Winklhofer-Roob, B.M., 2011. Dietary factors and low-grade inflammation in relation to overweight  
1015 and obesity. *Br J Nutr.* 106, S5–78. <https://doi.org/10.1017/S0007114511005460>.
- 1016 Callender, L.A., Carroll, E.C., Beal, R.W.J., Chambers, E.S., Nourshargh, S., Akbar, A.N., Henson,  
1017 S.M., 2018. Human CD8(+) EMRA T cells display a senescence-associated secretory phenotype  
1018 regulated by p38 MAPK. *Aging Cell.* 17(1), e12675. <https://doi.org/10.1111/ace1.12675>.

- 1019 Campisi, J., d'Adda di Fagagna, F., 2007. Cellular senescence: when bad things happen to good cells.  
1020 *Nat. Rev. Mol. Cell. Biol.* 8(9), 729-40. <https://doi.org/10.1038/nrm2233>.
- 1021 Campisi, J., Robert, L., 2014. Cell senescence: role in aging and age-related diseases. *Interdiscip.*  
1022 *Top. Gerontol.* 39, 45–61. <https://doi.org/10.1159/000358899>.
- 1023 Carissimo, G., Xu, W., Kwok, I., Abdad, M. Y., Chan, Y. H., Fong, S. W., Puan, K. J., Lee, C. Y. P.,  
1024 Yeo, N. K. W., Amrun, S. N., Chee, R. S. L., How, W., Chan, S., Fan, B. E., Andiappan, A. K., Lee,  
1025 B., Röttschke, O., Young, B. E., Leo, Y. S., Lye, D.C., Renia, L., Ng, L.G., Larbi, A., Ng, L. F.,  
1026 2020. Whole blood immunophenotyping uncovers immature neutrophil-to-VD2 T-cell ratio as an  
1027 early marker for severe COVID-19. *Nature Communications*, 11(1), 1–12.  
1028 <https://doi.org/10.1038/s41467-020-19080-6>
- 1029 Cevenini, E., Caruso, C., Candore, G., Capri, M., Nuzzo, D., Duro, G., Rizzo, C., Colonna-Romano,  
1030 G., Lio, D., Di Carlo, D., Palmas, M.G., Scurti, M., Pini, E., Franceschi, C., Vasto, S., 2010. Age-  
1031 related inflammation: the contribution of different organs, tissues and systems. How to face it for  
1032 therapeutic approaches. *Curr. Pharm. Des.* 16(6), 609–618.  
1033 <https://doi.org/10.2174/138161210790883840>.
- 1034 Cevenini, E., Monti, D., Franceschi, C., 2013. Inflamm-aging. *Curr. Opin. Clin. Nutr. Metab. Care*  
1035 16 (1), 14–20. <https://doi.org/10.1097/MCO.0b013e32835ada13>.
- 1036 Chang, C. J., Lin, T. L., Tsai, Y. L., Wu, T.R., Lai, W.F., Lu, C.C., lai, H.C., 2019. Next generation  
1037 probiotics in disease amelioration. *J. Food Drug Anal.* 27(3), 615–622.  
1038 <https://doi.org/10.1016/j.jfda.2018.12.011>.
- 1039 Chen, Y., Klein, S.L., Garibaldi, B.T., Li, H., Wu, C., Osevala, N.M., Li, T., Margolick, J.B., Pawelec,  
1040 G., Leng, S.X., 2021. Aging in COVID-19: Vulnerability, immunity and intervention. *Ageing Res.*  
1041 *Rev.* 65, 101205. <https://doi.org/10.1016/j.arr.2020.101205>.
- 1042 Chow, R. D., Majety, M., & Chen, S., 2021. The aging transcriptome and cellular landscape of the  
1043 human lung in relation to SARS-CoV-2. *Nature Communications*, 12 (1).  
1044 <https://doi.org/10.1038/s41467-020-20323-9>
- 1045 Ciabattini, A., Nardini, C., Santoro, F., Garagnani, P., Franceschi, C., Medaglini, D., 2018.  
1046 Vaccination in the elderly: the challenge of immune changes with aging. *Semin. Immunol.* 40, 83–  
1047 94. <https://doi.org/10.1016/j.smim.2018.10.010>.
- 1048 Ciabattini, A., Garagnani, P., Santoro, F., Rappuoli, R., Franceschi, C., Medaglini, D., 2020. Shelter  
1049 from the cytokine storm: pitfalls and prospects in the development of SARS-CoV-2 vaccines for an  
1050 elderly population. *Semin. Immunopathol.* 42(5), 619-634. <https://doi.org/10.1007/s00281-020-00821-0>.
- 1052 Claesson, M.J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H., Flannery, E., Marchesi,  
1053 J.R., Falush, D., Dinan, T., Fitzgerald, G., Stanton, C., van Sinderen, D., O'Connor, M., Harnedy, N.,  
1054 O'Connor, K., Henry, C., O'Mahony, D., FitzGerald, A.P., Shanahan, F., Twomey, C., Hill, C., Ross,  
1055 R.P., O'Toole, P.W., 2011. Composition, variability, and temporal stability of the intestinal  
1056 microbiota of the elderly. *PNAS*, 108 Suppl 1, 4586-91. <https://doi.org/10.1073/pnas.1000097107>.
- 1057 Claesson, M.J., Jeffery, I.B., Conde, S., Power, S.E., O'Connor, E.M., Cusack, S., Harris, H.M.B.,  
1058 Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G.F., Deane, J., O'Connor, M.,

- 1059 Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., Stanton, C.,  
1060 Marchesi, J.R., Fitzgerald, A.P., Shanahan, F., Hill, C., Ross, R.P., O'Toole, P.W., 2012. Gut  
1061 microbiota composition correlates with diet and health in the elderly. *Nature* 488, 178–184.  
1062 <https://doi.org/10.1038/nature11319>.
- 1063 Cohen, A.A., Kennedy, B.K., Anglas, U., Bronikowski, A.M., Deelen, J., Dufour, F., Ferbeyre, G.,  
1064 Ferrucci, L., Franceschi, C., Frasca, D., Friguet, B., Gaudreau, P., Gladyshev, V.N., Gonos, E.S.,  
1065 Gorbunova, V., Gut, P., Ivanchenko, M., Legault, V., Lemaitre, J.F., Lontis, T., Liu, G.H., Liu, M.,  
1066 Maier, A.B., Nobrega, O.T., Olde Rikkert, M.G.M., Pawelec, G., Rheault, S., Senior, A.M., Simm,  
1067 A., Soo, S., Traa, A., Ukraintseva, S., Vanhaelen, Q., Van Raamsdonk, J.M., Witkowski, J.M.,  
1068 Yashin, A.I., Ziman, R., Fulop, T., 2020. Lack of consensus on an aging biology paradigm? A global  
1069 survey reveals an agreement to disagree, and the need for an interdisciplinary framework. *Mech.*  
1070 *Ageing Dev.* 191, 111316. <https://doi.org/10.1016/j.mad.2020.111316>.
- 1071 Collino, S., Montoliu, I., Martin, F.P., Scherer, M., Mari, D., Salvioli, S., Bucci, L., Ostan, R., Monti,  
1072 D., Biagi, E., Brigidi, P., Franceschi, C., Rezzi, S., 2013. Metabolic signatures of extreme longevity  
1073 in northern Italian centenarians reveal a complex remodeling of lipids, amino acids, and gut  
1074 microbiota metabolism. *PLoS ONE*. 8, e56564. <https://doi.org/10.1371/journal.pone.0056564>.
- 1075 Colonna-Romano, G., Bulati, M., Aquino, A., Scialabba, G., Candore, G., Lio, D., Motta, M.,  
1076 Malaguarnera, M., Caruso, C., 2003. B cells in the aged: CD27, CD5, and CD40 expression. *Mech.*  
1077 *Ageing Dev.* 124, 389–93. [https://doi.org/10.1016/s0047-6374\(03\)00013-7](https://doi.org/10.1016/s0047-6374(03)00013-7).
- 1078 Colonna-Romano, G., Bulati, M., Aquino, A., Pellicano, M., Vitello, S., Lio, D., Candore, G., Caruso,  
1079 C., 2009. A double-negative (IgD-CD27-) B cell population is increased in the peripheral blood of  
1080 older adults. *Mech. Ageing Dev.* 130, 681–690. <https://doi.org/10.1016/j.mad.2009.08.003>.
- 1081 Conte, M., Ostan, R., Fabbri, C., Santoro, A., Guidarelli, G., Vitale, G., Mari, D., Sevini, F., Capri,  
1082 M., Sandri, M., Monti, D., Franceschi, C., Salvioli, S., 2019. Human Aging and Longevity Are  
1083 Characterized by High Levels of Mitokines. *J. Gerontol. A. Biol. Sci. Med. Sci.* 74(5), 600-607.  
1084 <https://doi.org/10.1093/gerona/gly153>.
- 1085 Conte, M., Conte, G., Martucci, M., Monti, D., Casarosa, L., Serra, A., Mele, M., Franceschi, C.,  
1086 Salvioli, S., 2020. The smell of longevity: a combination of Volatile Organic Compounds (VOCs)  
1087 can discriminate centenarians and their offspring from age-matched subjects and young controls.  
1088 *Geroscience.* 42(1), 201-216. <https://doi.org/10.1007/s11357-019-00143-6>.
- 1089 Costantini, A., Viola, N., Berretta, A., Galeazzi, R., Matakchione, G., Sabbatinelli, J., Storci, G., De  
1090 Matteis, S., Butini, L., Rippo, M. R., Procopio, A. D., Caraceni, D., Antonicelli, R., Olivieri, F., &  
1091 Bonafè, M., 2018. Age-related M1/M2 phenotype changes in circulating monocytes from  
1092 healthy/unhealthy individuals. *Aging*, 10(6), 1268–1280. <https://doi.org/10.18632/aging.101465>
- 1093 Cooper, M. A., Fehniger, T. A., Fuchs, A., Colonna, M. and Caligiuri, M. A., 2004. NK cell and DC  
1094 interactions. *Trends. Immunol.* 25, 47–52. <https://doi.org/10.1016/j.it.2003.10.012>.
- 1095 Coppé, J.P., Patil, C.K., Rodier, F., Sun, Y., Munoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y.,  
1096 Campisi, J., 2008. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions  
1097 of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* 6, 2853-2868.  
1098 <https://doi.org/10.1371/journal.pbio.0060301>.

- 1099 Cossarizza, A., Ortolani, C., Paganelli, R., Barbieri, D., Monti, D., Sansoni, P., Fagiolo, U.,  
1100 Castellani, G., Bersani, F., Londei, M., Franceschi, C., 1996. CD45 isoforms expression on CD4+  
1101 and CD8+ T cells throughout life, from newborns to centenarians: implications for T cell memory.  
1102 *Mech. Ageing Dev.* 86(3),173-95. [https://doi.org/10.1016/0047-6374\(95\)01691-0](https://doi.org/10.1016/0047-6374(95)01691-0).
- 1103 Cossarizza, A., Ortolani, C., Monti, D., Franceschi, C., 1997. Cytometric analysis of  
1104 immunosenescence. *Cytometry.* 27, 297-313. [https://doi.org/10.1002/\(sici\)1097-0320\(19970401\)27:4<297::aid-cyto1>3.0.co;2-a](https://doi.org/10.1002/(sici)1097-0320(19970401)27:4<297::aid-cyto1>3.0.co;2-a).
- 1106 Covarrubias, A. J., Kale, A., Perrone, R., Lopez-Dominguez, J. A., Pisco, A. O., Kasler, H. G.,  
1107 Schmidt, M. S., Heckenbach, I., Kwok, R., Wiley, C. D., Wong, H. S., Gibbs, E., Iyer, S. S., Basisty,  
1108 N., Wu, Q., Kim, I. J., Silva, E., Vitangcol, K., Shin, K. O., Lee, Y.M., Riley, R., Ben-Sahra, I., Ott,  
1109 M., Schilling, B., Scheibye-Knudsen, M., Ishihara, K., Quake, S.R., Newman, J., Brenner, C.,  
1110 Campisi, J., Verdin, E., 2020. Senescent cells promote tissue NAD+ decline during ageing via the  
1111 activation of CD38+ macrophages. *Nature Metabolism*, 2(11), 1265–1283.  
1112 <https://doi.org/10.1038/s42255-020-00305-3>
- 1113 Dall’Olio, F., Vanhooren, V., Chen, C. C., Slagboom, P. E., Wuhrer, M., & Franceschi, C., 2013. N-  
1114 glycomic biomarkers of biological aging and longevity: a link with inflammaging. *Ageing Res. Rev.*,  
1115 12(2), 685–698. <https://doi.org/10.1016/j.arr.2012.02.002>.
- 1116 Das, A., Ranganathan, V., Umar, D., Thukral, S., George, A., Rath, S., Bal, V., 2017.  
1117 Effector/memory CD4 T cells making either Th1 or Th2 cytokines commonly co-express T-bet and  
1118 GATA-3. *PLoSOne.* 12(10), e0185932. <https://doi.org/10.1371/journal.pone.0185932>.
- 1119 De Martinis, M., Franceschi, C., Monti, D., Ginaldi, L., 2005. Inflamm-aging and lifelong antigenic  
1120 load as major determinants of ageing rate and longevity. *FEBS. Lett.* 579, 2035–2039.  
1121 <https://doi.org/10.1016/j.febslet.2005.02.055>.
- 1122 Deelen, J., Kettunen, J., Fischer, K., van der Spek, A., Trompet, S., Kastenmuller, G., Boyd, A.,  
1123 Zierer, J., van den Akker, E.B., Ala-Korpela, M., Amin, N., Demirkan, A., Ghanbari, M., van Heemst,  
1124 D., Ikram, M.A., van Klinken, J.B., Mooijaart, S.P., Peters, A., Salomaa, V., Sattar, N., Spector, T.D.,  
1125 Tiemeier, H., Verhoeven, A., Waldenberger, M., Wurtz, P., Davey Smith, G., Metspalu, A., Perola,  
1126 M., Menni, C., Geleijnse, J.M., Drenos, F., Beekman, M., Jukema, J.W., van Duijn, C.M., Slagboom,  
1127 P.E., 2019. A metabolic profile of all-cause mortality risk identified in an observational study of  
1128 44,168 individuals. *Nat. Commun.* 10, 3346. <https://doi.org/10.1038/s41467-019-11311-9>.
- 1129 Derhovanessian, E., Maier, A.B., Hähnel, K., Zelba, H., de Craen, A.J., Roelofs, H., Slagboom E.P.,  
1130 Westendorp, R.G.J., Pawelec, G., 2013. Lower proportion of naïve peripheral CD8+ T cells and an  
1131 unopposed proinflammatory response to human Cytomegalovirus proteins in vitro are associated with  
1132 longer survival in very elderly people. *Age (Dordr).* 35(4), 1387–99. <https://doi.org/10.1007/s11357-012-9425-7>.
- 1134 Di Benedetto, S., Gaetjen, M., Müller, L., 2019. The Modulatory Effect of Gender and  
1135 Cytomegalovirus- Seropositivity on Circulating Inflammatory Factors and Cognitive Performance in  
1136 Elderly Individuals. *Int. J. Mol. Sci.* 20(4), 990. <https://doi.org/10.3390/ijms20040990>.
- 1137 Douek, D.C., Koup, R.A., 2000. Evidence for thymic function in the elderly. *Vaccine.* 16, 1638-41.  
1138 [https://doi.org/10.1016/s0264-410x\(99\)00499-5](https://doi.org/10.1016/s0264-410x(99)00499-5).

- 1139 Evert, J., Lawler, E., Bogan, H., Perls, T., 2003. Morbidity profiles of centenarians: survivors,  
1140 delayers, and escapers. *J. Gerontol. A. Biol. Sci. Med. Sci.* 58 (3), 232–237.  
1141 <https://doi.org/10.1093/gerona/58.3.m232>.
- 1142 Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D.,  
1143 Franceschi, C., Paganelli, R., 1993. Increased cytokine production in mononuclear cells of healthy  
1144 elderly people. *Eur. J. Immunol.* 23, 2375–2378. <https://doi.org/10.1002/eji.1830230950>.
- 1145 Fagnoni, F.F., Vescovini, R., Mazzola, M., Bologna, G., Nigro, E., Lavagetto, G., Franceschi, C.,  
1146 Passeri, M., Sansoni, P., 1996. Expansion of cytotoxic CD8+ CD28– T cells in healthy ageing people,  
1147 including centenarians. *Immunology.* 88(4), 501–507. <https://doi.org/10.1046/j.1365-2567.1996.d01-689.x>.
- 1149 Fecteau, J. F., Cote, G. and Neron, S., 2006. A new memory CD27-IgG+ B cell population in  
1150 peripheral blood expressing VH genes with low frequency of somatic mutation. *J. Immunol.* 177,  
1151 3728–3736. <https://doi.org/10.4049/jimmunol.177.6.3728>.
- 1152 Ferreira, C., Viana, S.D., Reis, F., 2020. Is Gut Microbiota Dysbiosis a Predictor of Increased  
1153 Susceptibility to Poor Outcome of COVID-19 Patients? An Update. *Microorganisms.* 9(1), 53.  
1154 <https://doi.org/10.3390/microorganisms9010053>.
- 1155 Ferrucci, L., Fabbri, E., 2018. Inflammageing: chronic inflammation in ageing, cardiovascular  
1156 disease, and frailty. *Nat. Rev. Cardiol.* 15(9), 505–522. <https://doi.org/10.1038/s41569-018-0064-2>.
- 1157 Flanagan, K. L., Fink, A. L., Plebanski, M. & Klein, S. L., 2017. Sex and Gender Differences in the  
1158 Outcomes of Vaccination over the Life Course. *Annu. Rev. Cell Dev. Biol.* 33, 577–599.  
1159 <https://doi.org/10.1146/annurev-cellbio-100616-060718>.
- 1160 Franceschi, C., Monti, D., Barbieri, D., Grassilli, E., Troiano, L., Salvioli, S., Negro, P., Capri, M.,  
1161 Guido, M., Azzi, R., Sansoni, P., Paganelli, R., Fagiolo, U., Baggio, G., Donazzan, S., Mariotti, S.,  
1162 D’Addato, S., Gaddi, A., Ortolani, C., Cossarizza, A., 1995a. Immunosenescence in Humans:  
1163 Deterioration or Remodelling? *Int. Rev. Immunol.* 12,57–74.  
1164 <https://doi.org/10.3109/08830189509056702>.
- 1165 Franceschi, C., Monti, D., Sansoni, P., Cossarizza, A., 1995b. The immunology of exceptional  
1166 individuals: the lesson of centenarians. *Immunol. Today.* 16 (1), 12–16. [https://doi.org/10.1016/0167-5699\(95\)80064-6](https://doi.org/10.1016/0167-5699(95)80064-6).
- 1168 Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G.,  
1169 2000a. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.*  
1170 908, 244–254. <https://doi.org/10.1111/j.1749-6632.2000.tb06651.x>.
- 1171 Franceschi, C., Bonafè, M., Valensin, S., 2000b. Human immunosenescence: the prevailing of innate  
1172 immunity, the failing of clonotypic immunity, and the filling of immunological space.  
1173 *Vaccine.* 18(16), 1717–20. [https://doi.org/10.1016/s0264-410x\(99\)00513-7](https://doi.org/10.1016/s0264-410x(99)00513-7).
- 1174 Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L.,  
1175 Celani, L., Scurti, M., Cevenini, E., Cstellani, G.C., Salvioli, S., 2007. Inflammaging and anti-  
1176 inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech.*  
1177 *Ageing Dev.* 128, 92–105. <https://doi.org/10.1016/j.mad.2006.11.016>.

- 1178 Franceschi, C., Grignolio, A., 2010. Immunosenescence within an evolutionary perspective, in:  
1179 Grignolio A, (Eds.), *Immunology Today: Three Historical Perspectives under Three Theoretical*  
1180 *Horizons*. Bononia University Press, Bologna, pp. 79–99.
- 1181 Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential  
1182 contribution to age-associated diseases. *J. Gerontol. A. Biol. Sci. Med. Sci.* 69 Suppl 1, S4-S9.  
1183 <https://doi.org/10.1093/gerona/glu057>.
- 1184 Franceschi, C., Salvioli, S., Garagnani, P., de Eguileor, M., Monti, D., Capri, M., 2017a.  
1185 Immunobiography and the Heterogeneity of Immune Responses in the Elderly: A Focus on  
1186 Inflammaging and Trained Immunity. *Front. Immunol.* 8, 982.  
1187 <https://doi.org/10.3389/fimmu.2017.00982>.
- 1188 Franceschi, C., Garagnani, P., Vitale, G., Capri, M., Salvioli, S., 2017b. Inflammaging and 'Garb-  
1189 aging'. *Trends. Endocrinol. Metab.* 28(3), 199-212. <https://doi.org/10.1016/j.tem.2016.09.005>.
- 1190 Franceschi, C., Passarino, G., Mari, D., Monti D., 2017c. Centenarians as a 21st century healthy aging  
1191 model: A legacy of humanity and the need for a world-wide consortium (WWC100+). *Mech. Ageing*  
1192 *Dev.* 165(Pt B), 55-58. <https://doi.org/10.1016/j.mad.2017.06.002>.
- 1193 Franceschi, C., 2017d. Healthy ageing in 2016: Obesity in geroscience - is cellular senescence the  
1194 culprit? *Nat. Rev. Endocrinol.* 13(2), 76-78. <https://doi.org/10.1038/nrendo.2016.213>.
- 1195 Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri,  
1196 M., Salvioli, S., 2018a. The Continuum of Aging and Age-Related Diseases: Common Mechanisms  
1197 but Different Rates. *Front. Med.* 5, 61. <https://doi.org/10.3389/fmed.2018.00061>.
- 1198 Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., Santoro, A., 2018b. Inflammaging: a new  
1199 immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14(10), 576-590.  
1200 <https://doi.org/10.1038/s41574-018-0059-4>.
- 1201 Franceschi, C., Ostan, R., Santoro, A., 2018c. Nutrition and Inflammation: Are Centenarians Similar  
1202 to Individuals on Calorie-Restricted Diets? *Annu. Rev. Nutr.* 38, 329-356.  
1203 <https://doi.org/10.1146/annurev-nutr-082117-051637>.
- 1204 Fransen, F., van Beek, A.A., Borghuis, T., El Aidy, S., Hugenholtz, F., van der-Gaast-de Jongh, C.,  
1205 Savelkoul, H.F.J., De Jonge, M.I., Boekschoten, M.V., Smidt, H., Faas, M.M., de Vos, P., 2017. Aged  
1206 gut microbiota contributes to systemical inflammaging after transfer to germ-free mice. *Front.*  
1207 *Immunol.* 8, 1385. <https://doi.org/10.3389/fimmu.2017.01385>.
- 1208 Frasca, D., Diaz, A., Romero, M., Landin, A. M. and Blomberg, B. B., 2014. High TNF-alpha levels  
1209 in resting B cells negatively correlate with their response. *Exp. Gerontol.* 54, 116–122.  
1210 <https://doi.org/10.1016/j.exger.2014.01.004>.
- 1211 Frasca, D., Diaz, A., Romero, M., Blomberg, B.B., 2016. The generation of memory B cells is  
1212 maintained, but the antibody response is not, in the elderly after repeated influenza immunizations.  
1213 *Vaccine.* 34, 2834–40. <https://doi.org/10.1016/j.vaccine.2016.04.023>.
- 1214 Frasca, D., Diaz, A., Romero, M., Garcia, D., Blomberg, B.B., 2020. B Cell Immunosenescence.  
1215 *Annu. Rev. Cell Dev. Biol.* 36, 551-574. <https://doi.org/10.1146/annurev-cellbio-011620-034148>.

- 1216 Frasca, D., Reidy, L., Cray, C., Diaz, A., Romero, M., Kahl, K., Blomberg, B.B., 2021. Influence of  
1217 obesity on serum levels of SARS-CoV-2-specific antibodies in COVID-19 patients. *PLoS One*. 16(3),  
1218 e0245424. <https://doi.org/10.1371/journal.pone.0245424>.
- 1219 Fulop, T., Larbi, A., Douziech, N., Fortin, C., Guérard, K.P., Lesur, O., Khalil, A., Dupuis, G., 2004.  
1220 Signal transduction and functional changes in neutrophils with aging. *Aging Cell*. 3(4), 217-26.  
1221 <https://doi.org/10.1111/j.1474-9728.2004.00110.x>.
- 1222 Fulop, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E.H., Cohen, A.A., Witkowski, J.M., Franceschi,  
1223 C., 2018. Immunosenescence and inflamm-aging as two sides of the same coin: Friends or Foes?  
1224 *Front. Immunol.* 8, 1960. <https://doi.org/10.3389/fimmu.2017.01960>.
- 1225 Fulop, T., Larbi, A., Hirokawa, K., Cohen, A.A., Witkowski, J.M., 2020. Immunosenescence is both  
1226 functional/adaptive and dysfunctional/maladaptive. *Semin. Immunopathol.* 42(5), 521-536.  
1227 <https://doi.org/10.1007/s00281-020-00818-9>.
- 1228 Furman, D., Hejblum, B.P., Simon, N., Jovic, V., Dekker, C.L., Thiébaud, R., Tibshirani, R.J., Davis,  
1229 M.M., 2014. Systems analysis of sex differences reveals an immunosuppressive role for testosterone  
1230 in the response to influenza vaccination. *Proc. Natl. Acad. Sci.* 111, 869–874. <https://doi.org/10.1073/pnas.1321060111>.
- 1232 Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy,  
1233 D.W., Fasano, A., Miller, G.W., Miller, A.H., Mantovani, A., Weyand, C.M., Barzilai, N., Goronzy,  
1234 J.J., Rando, T.A., Effros, R.B., Lucia, A., Kleinstreuer, N., Slavich, G.M., 2019. Chronic  
1235 inflammation in the etiology of disease across the life span. *Nat. Med.* 25, 1822–1832.  
1236 <https://doi.org/10.1038/s41591-019-0675-0>.
- 1237 Gallenga, C.E., Parmeggiani, F., Costagliola, C., Sebastiani, A., Gallenga, P.E., 2014. Inflammaging:  
1238 should this term be suitable for age related macular degeneration too? *Inflamm. Res.* 63, 105–107.  
1239 <https://doi.org/10.1007/s00011-013-0684-2>.
- 1240 Gangemi, S., Basile, G., Monti, D., Merendino, R.A., Di Pasquale, G., Bisignano, U., Nicita-Mauro,  
1241 V., Franceschi, C., 2005. Age-related modifications in circulating IL-15 levels in humans. *Mediators  
1242 Inflamm.* 2005(4), 245–247. <https://doi.org/10.1155/MI.2005.245>.
- 1243 Garagnani, P., Marquis, J., Delledonne, M., Pirazzini, C., Marasco, E., Kwiatkowska, K.M., Iannuzzi,  
1244 V., Bacalini, M.G., Valsesia, A., Carayol, J., Raymond, F., Ferrarini, A., Xumerle, L., Collino, S.,  
1245 Mari, D., Arosio, B., Casati, M., Ferri, E., Monti, D., Nacmias, B., Sorbi, S., Luiselli, D., Pettener,  
1246 D., Castellani, G., Sala, C., Passarino, G., De Rango, F., D'Aquila, P., Bertamini, L., Martinelli, N.,  
1247 Girelli, D., Olivieri, O., Giuliani, C., Descombes, P., Franceschi, C., 2021. Whole-genome  
1248 sequencing analysis of semi-supercentenarians. *Elife*. 10, e57849.  
1249 <https://doi.org/10.7554/eLife.57849>.
- 1250 Gayoso, I., Sanchez-Correa, B., Campos, C., Alonso, C., Pera, A., Casado, J. G., Morgado, S.,  
1251 tarazona, R., Solana, R., 2011. Immunosenescence of human natural killer cells. *J. Inn. Immun.* 3,  
1252 337–343. <https://doi.org/10.1159/000328005>.
- 1253 Geerlings, S. Y., Kostopoulos, I., de Vos, W. M., Belzer, C., 2018. Akkermansia muciniphila in the  
1254 human gastrointestinal tract: when, where, and how? *Microorganisms*. 6(3), 75.  
1255 <https://doi.org/10.3390/microorganisms6030075>.

- 1256 Gemmati, D., Varani, K., Bramanti, B., Piva, R., Bonaccorsi, G., Trentini, A., Manfrinato, M.C.,  
1257 Tisato, V., Carè, A., Bellini, T., 2019. "Bridging the Gap" Everything that Could Have Been Avoided  
1258 If We Had Applied Gender Medicine, Pharmacogenetics and Personalized Medicine in the Gender-  
1259 Omics and Sex-Omics Era. *Int. J. Mol. Sci.* 21(1), 296. <https://doi.org/10.3390/ijms21010296>.
- 1260 Gemmati, D., Bramanti, B., Serino, M.L., Secchiero, P., Zauli, G., Tisato, V., 2020. COVID-19 and  
1261 Individual Genetic Susceptibility/Receptivity: Role of ACE1/ACE2 Genes, Immunity, Inflammation  
1262 and Coagulation. Might the Double X-chromosome in Females Be Protective against SARS-CoV-2  
1263 Compared to the Single X-Chromosome in Males? *Int. J. Mol. Sci.* 21(10),3474.  
1264 <https://doi.org/10.3390/ijms21103474>.
- 1265 Genedani, S., Filafferro, M., Carone, C., Ostan, R., Bucci, L., Cevenini, E., Franceschi, C., Monti, D.,  
1266 2008. Influence of f-MLP, ACTH(1-24) and CRH on in vitro chemotaxis of monocytes from  
1267 centenarians. *Neuroimmunomodulation.* 15(4-6), 285-289. <https://doi.org/10.1159/000156472>.
- 1268 Gerli, R., Monti, D., Bistoni, O., Mazzone, A.M., Peri, G., Cossarizza, A., Di Gioacchino, M.,  
1269 Cesarotti, M.E., Doni, A., Mantovani, A., Franceschi, C., Paganelli, R., 2000. Chemokines, sTNF-Rs  
1270 and sCD30 serum levels in healthy aged people and centenarians. *Mech. Ageing Dev.* 121(1-3), 37-  
1271 46. [https://doi.org/10.1016/s0047-6374\(00\)00195-0](https://doi.org/10.1016/s0047-6374(00)00195-0).
- 1272 Ghosh, T.S., Rampelli, S., Jeffery, I.B., Santoro, A., Neto, M., Capri, M., Giampieri, E., Jennings,  
1273 A., Candela, M., Turrone, S., Zoetendal, E.G., Hermes, G.D.A., Elodie, C., Meunier, N., Brugere,  
1274 C.M., Pujos-Guillot, E., Berendsen, A.M., De Groot, L.C.P.G.M., Feskens, E.J.M., Kaluza, J.,  
1275 Pietruszka, B., Bielak, M.J., Comte, B., Maijo-Ferre, M., Nicoletti, C., De Vos, W.M., Fairweather-  
1276 Tait, S., Cassidy, A., Brigidi, P., Franceschi, C., O'Toole, P.W., 2020. Mediterranean diet intervention  
1277 alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE  
1278 1-year dietary intervention across five European countries. *Gut.* 69(7), 1218-1228.  
1279 <https://doi.org/10.1136/gutjnl-2019-319654>.
- 1280 Ginaldi, L., De Martinis, M., Monti, D., Franceschi, C., 2005. Chronic antigenic load and apoptosis  
1281 in immunosenescence. *Trends Immunol.* 26(2), 79-84. <https://doi.org/10.1016/j.it.2004.11.005>.
- 1282 Goetzl, E. J., Huang, M.C., Kon, J., Patel, K., Schwartz, J.B., Fast, K., Ferrucci, L., Madara, K., Taub,  
1283 D.D., Longo, D.L., 2010. Gender specificity of altered human immune cytokine profiles in aging.  
1284 *FASEB J.* 24(9), 3580-3589. <https://doi.org/10.1096/fj.10-160911>.
- 1285 Goronzy, J.J., Weyand, C.M., 2012. Immune aging and autoimmunity. *Cell. Mol. Life Sci.* 69, 1615-  
1286 1623. <https://doi.org/10.1007/s00018-012-0970-0>.
- 1287 Goronzy, J.J., Fang, F., Cavanagh, M.M., Qi, Q., Weyand, C.M., 2015. Naive T cell maintenance and  
1288 function in human aging. *J. Immunol.* 194, 4073-4080. <https://doi.org/10.4049/jimmunol.1500046>.
- 1289 Hadrup, S.R., Strindhall, J., Kollgaard, T., Seremet, T., Johansson, B., Pawelec, G., Thor Straten, P.,  
1290 Wikby, A., 2006. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage  
1291 predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in  
1292 the very elderly. *J. Immunol.* 176, 2645-2653. <https://doi.org/10.4049/jimmunol.176.4.2645>.
- 1293 Hamann, L., Szwed, M., Mossakowska, M., Chudek, J., Puzianowska-Kuznicka, M., 2020. First  
1294 evidence for STING SNP R293Q being protective regarding obesity-associated cardiovascular  
1295 disease in age-advanced subjects - a cohort study. *Immun. Ageing.* 17, 7.  
1296 <https://doi.org/10.1186/s12979-020-00176-y>.



- 1297 Hamczyk, M.R., Nevado, R.M., Baretino, A., Fuster, V., Andres, V., 2020. Biological Versus  
1298 Chronological Aging: JACC Focus Seminar. *J. Am. Coll. Cardiol.* 75, 919-930.  
1299 <https://doi.org/10.1016/j.jacc.2019.11.062>.
- 1300 Harper S. The Impact of the Covid-19 Pandemic on Global Population Ageing. *J Popul Ageing*.  
1301 2021 May 22:1-6. doi: 10.1007/s12062-021-09330-w. Epub ahead of print. PMID: 34055101;  
1302 PMCID: PMC8140566.
- 1303 Hazeldine, J., Lord, J.M., 2013. The impact of ageing on natural killer cell function and potential  
1304 consequences for health in older adults. *Ageing Res. Rev.* 12(4),1069-1078.  
1305 <https://doi.org/10.1016/j.arr.2013.04.003>.
- 1306 Hearps, A.C., Martin, G. E., Angelovich, T. A., Cheng, W. J., Maisa, A., Landay, A. L., Jaworowski,  
1307 A., Crowe, S.M., 2012. Aging is associated with chronic innate immune activation and dysregulation  
1308 of monocyte phenotype and function. *Aging Cell.* 11, 867–875. <https://doi.org/10.1111/j.1474-9726.2012.00851.x>.
- 1310 Horvath, S., Pirazzini, C., Bacalini, M.G., Gentilini, D., Di Blasio, A.M., Delledonne, M., Mari, D.,  
1311 Arosio, B., Monti, D., Passarino, G., De Rango, F., D’Aquila, P., Giuliani, C., Marasco, E., Collino,  
1312 S., Descombes, P., Garagnani, P., Franceschi, C., 2015. Decreased epigenetic age of PBMCs from  
1313 Italian semi- supercentenarians and their offspring. *Aging (Albany NY).* 7,1159–70.  
1314 <https://doi.org/10.18632/aging.100861>.
- 1315 Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z.,  
1316 Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie,  
1317 J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., Cao, B., 2020. Clinical features of patients infected  
1318 with 2019 novel coronavirus in Wuhan, China. *Lancet.* 395, 497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5).
- 1320 Huang, W.C., Chen, Y.H., Chuang, H.L., Chiu, C.C., Huang, C.C., 2019. Investigation of the effects  
1321 of microbiota on exercise physiological adaption, performance, and energy utilization using a  
1322 gnotobiotic animal model. *Front. Microbiol.* 10, 1906. <https://doi.org/10.3389/fmicb.2019.01906>.
- 1323 Jeljeli, M., Riccio, L.G.C., Doridot, L., Chêne, C., Nicco, C., Chouzenoux, S., Deletang, Q., Allanore,  
1324 Y., Kavian, N., Batteux, F., 2019. Trained immunity modulates inflammation-induced fibrosis. *Nat.*  
1325 *Commun.* 10(1), 5670. <https://doi.org/10.1038/s41467-019-13636-x>.
- 1326 Jennings, A., Cashman, K.D., Gillings, R., Cassidy, A., Tang, J., Fraser, W., Dowling, K.G., Hull,  
1327 G.L.J., Berendsen, A.A.M., de Groot, L.C.P.G.M., Pietruszka, B., Wierzbicka, E., Ostan, R.,  
1328 Bazzocchi, A., Battista, G., Caumon, E., Meunier, N., Malpuech-Brugère, C., Franceschi, C., Santoro,  
1329 A., Fairweather-Tait, S.J., 2018. A Mediterranean-like dietary pattern with vitamin D3 (10 µg/d)  
1330 supplements reduced the rate of bone loss in older Europeans with osteoporosis at baseline: results of  
1331 a 1-y randomized controlled trial. *Am. J. Clin. Nutr.* 108(3), 633-640.  
1332 <https://doi.org/10.1093/ajcn/nqy122>.
- 1333 Jennings, A., Berendsen, A.M., de Groot, L.C.P.G.M., Feskens, E.J.M., Brzozowska, A., Sicinska,  
1334 E., Pietruszka, B., Meunier, N., Caumon, E., Malpuech-Brugère, C., Santoro, A., Ostan, R.,  
1335 Franceschi, C., Gillings, R., O' Neill, C.M., Fairweather-Tait, S.J., Minihane, A.M., Cassidy, A.,  
1336 2019. Mediterranean-Style Diet Improves Systolic Blood Pressure and Arterial Stiffness in Older  
1337 Adults. *Hypertension.* 73(3), 578-586. <https://doi.org/10.1161/HYPERTENSIONAHA.118.12259>.

- 1338 Jing, Y., Shaheen, E., Drake, R. R., Chen, N., Gravenstein, S., Deng, Y., 2009. Aging is associated  
1339 with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic  
1340 cells are relatively unaltered in human peripheral blood. *Hum. Immunol.* 70, 777–784.  
1341 <https://doi.org/10.1016/j.humimm.2009.07.005>.
- 1342 Jurberg, A. D., Cotta-de-Almeida, V., Temerozo, J. R., Savino, W., Bou-Habib, D. C., Riederer, I.,  
1343 2018. Neuroendocrine Control of Macrophage Development and Function. *Front. Immunol.* 9, 1440.  
1344 <https://doi.org/10.3389/fimmu.2018.01440>.
- 1345 Kashtanova, D. A., Klimenko, N. S., Strazhesko, I. D., Starikova, E.V., Glushchenko, O.E., Gudkov,  
1346 D.A., Tkacheva, O.N., 2020. A cross-sectional study of the gut microbiota composition in Moscow  
1347 long-livers. *Microorganisms.* 8(8), 1162. <https://doi.org/10.3390/microorganisms8081162>.
- 1348 Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L., Gordon, J.I., 2011. Human nutrition, the gut  
1349 microbiome and the immune system. *Nature.* 474, 327–336. <https://doi.org/10.1038/nature10213>.
- 1350 Kestra, S.M., Male, V., Salali, G.D., 2021. Out of balance: the role of evolutionary mismatches in  
1351 the sex disparity in autoimmune disease. *Med Hypotheses.* 151,110558.  
1352 <https://doi.org/10.1016/j.mehy.2021.110558>.
- 1353 Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C.,  
1354 Lithgow, G.J., Morimoto, R.I., Pessin, J.E., Rando, T.A., Richardson, A., Schadt, E.E., Wyss-Coray,  
1355 T., Sierra, F., 2014. Geroscience: Linking Aging to Chronic Disease. *Cell.* 159,709–713.  
1356 <https://doi.org/10.1016/j.cell.2014.10.039>.
- 1357 Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. *Nat. Rev. Immunol.* 16(10),  
1358 626-638. <https://doi.org/10.1038/nri.2016.90>.
- 1359 Kong, F., Hua, Y., Zeng, B., Ning., R., Li, Y., Zhao, J., 2016. Gut microbiota signatures of longevity.  
1360 *Curr. Biol.* 26(18), R832–R833. <https://doi.org/10.1016/j.cub.2016.08.015>.
- 1361 Lago, R., Gómez, R., Lago, F., Gómez-Reino, J., Gualillo, O., 2008. Leptin beyond body weight  
1362 regulation—current concepts concerning its role in immune function and inflammation. *Cell.*  
1363 *Immunol.* 252, 139–145. <https://doi.org/10.1016/j.cellimm.2007.09.004>.
- 1364 Lakshminarayanan, B., Stanton, C., O’Toole, P.W., Ross, R.P., 2014. Compositional dynamics of the  
1365 human intestinal microbiota with aging: implications for health. *J. Nutr. Health Aging.* 18,773–786.  
1366 <https://doi.org/10.1007/s12603-014-0549-6>.
- 1367 Larbi, A., Fulop, T., 2014. From “truly naïve” to “exhausted senescent” T cells: when markers predict  
1368 functionality. *Cytometry A.* 85(1), 25–35. <https://doi.org/10.1002/cyto.a.22351>.
- 1369 Le Garff-Tavernier, M., Beziat, V., Decocq, J., Siguret, V., Gandjbakhch, F., Pautas, E., Debré, P.,  
1370 Merle-Beral, H., Vieillard, V., 2010. Human NK cells display major phenotypic and functional  
1371 changes over the lifespan. *Aging Cell.* 9, 527–535. <https://doi.org/10.1111/j.1474-9726.2010.00584.x>.
- 1373 Lee, S.H., Yun, Y., Kim, S.J., Lee, E.J., Chang, Y., Ryu, S., Shin, H., Kim, H.L., Kim, H.N., Lee,  
1374 J.H., 2018. Association between cigarette smoking status and composition of gut microbiota:  
1375 population-based cross-sectional study. *J. Clin. Med.* 7, 282. <https://doi.org/10.3390/jcm7090282>.

- 1376 Lehallier, B., Gate, D., Schaum, N., Nanasi, T., Lee, S.E., Yousef, H., Moran Losada, P., Berdnik,  
1377 D., Keller, A., Verghese, J., Sathyan, S., Franceschi, C., Milman, S., Barzilai, N., Wyss-Coray, T.,  
1378 2019. Undulating changes in human plasma proteome profiles across the lifespan. *Nat. Med.* 25,  
1379 1843-1850. <https://doi.org/10.1038/s41591-019-0673-2>.
- 1380 Lio, D., Scola, L., Crivello, A., Bonafè, M., Franceschi, C., Olivieri, F., Colonna-Romano, G.,  
1381 Candore, G., Caruso, C., 2002. Allele frequencies of +874T→A single nucleotide polymorphism at  
1382 the first intron of interferon- $\gamma$  gene in a group of Italian centenarians. *Exp. Gerontol.* 37,315–19.  
1383 [https://doi.org/10.1016/s0531-5565\(01\)00198-x](https://doi.org/10.1016/s0531-5565(01)00198-x).
- 1384 López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging.  
1385 *Cell.* 153(6), 1194-217. <https://doi.org/10.1016/j.cell.2013.05.039>.
- 1386 Maggio, M., Guralnik, J.M., Longo, D.L., Ferrucci, L., 2006. Interleukin-6 in aging and chronic  
1387 disease: a magnificent pathway. *J. Gerontol. A. Biol. Sci. Med. Sci.* 61, 575–584.  
1388 <https://doi.org/10.1093/gerona/61.6.575>.
- 1389 Marcon, G., Tettamanti, M., Capacci, G., Fontanel, G., Spanò, M., Nobili, A., Forloni, G., Franceschi,  
1390 C., 2020. COVID-19 mortality in Lombardy: the vulnerability of the oldest old and the resilience of  
1391 male centenarians. *Aging.* 12(15), 15186–15195. <https://doi.org/10.18632/aging.103872>.
- 1392 Mardinoglu, A., Shoaie, S., Bergentall, M., Ghaffari, P., Zhang, C., Larsson, E., Bäckhed, F., Nielsen,  
1393 J., 2015. The gut microbiota modulates host amino acid and glutathione metabolism in mice. *Mol.*  
1394 *Syst. Biol.*, 11, 834. <https://doi.org/10.15252/msb.20156487>.
- 1395 Mariani, E., Meneghetti, A., Neri, S., Ravaglia, G., Forti, P., Cattini, L. and Facchini, A., 2002.  
1396 Chemokine production by natural killer cells from nonagenarians. *Eur. J. Immunol.* 32, 1524–1529.  
1397 [https://doi.org/10.1002/1521-4141\(200206\)32:6<1524::AID-IMMU1524>3.0.CO;2-E](https://doi.org/10.1002/1521-4141(200206)32:6<1524::AID-IMMU1524>3.0.CO;2-E).
- 1398 Marois, G., Muttarak, R., Scherbov, S., 2020. Assessing the potential impact of COVID-19 on life  
1399 expectancy. *PLoS ONE* 15(9), e0238678. <https://doi.org/10.1371/journal.pone.0238678>.
- 1400 Márquez, E. J., Chung, C.H., marches, R., Rossi, R.J., Nehar-Belaid, D., Eroglu, A., Mellert, D.J.,  
1401 kuchel, G.A., Banchereau, J., Ucar, D., 2020. Sexual-dimorphism in human immune system aging.  
1402 *Nat. Commun.* 11, 751. <https://doi.org/10.1038/s41467-020-14396-9>.
- 1403 Marseglia, A., Xu, W., Fratiglioni, L., Fabbri, C., Berendsen, A.A.M., Bialecka-Debek, A., Jennings,  
1404 A., Gillings, R., Meunier, N., Caumon, E., Fairweather-Tait, S., Pietruszka, B., De Groot,  
1405 L.C.P.G.M., Santoro, A., Franceschi, C., 2018. Effect of the NU-AGE Diet on Cognitive Functioning  
1406 in Older Adults: A Randomized Controlled Trial. *Front. Physiol.* 9, 349.  
1407 <https://doi.org/10.3389/fphys.2018.00349>.
- 1408 Martinez-Guryn, K., Hubert, N., Frazier, K., Urlass, S., Musch, M.W., Ojeda, P., Pierre, J.F.,  
1409 Miyoshi, J., Sontag, T., Cham, C., Reardon, C., Leone, V., Chang, E.B., 2018. Small intestine  
1410 microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. *Cell. Host.*  
1411 *Microbe.* 23, 458-469. <https://doi.org/10.1016/j.chom.2018.03.011>.
- 1412 Martucci, M., Ostan, R., Biondi, F., Bellavista, E., Fabbri, C., Bertarelli, C., Salvioli, S., Capri, M.,  
1413 Franceschi, C., Santoro, A., 2017. Mediterranean diet and inflammaging within the hormesis  
1414 paradigm. *Nutr. Rev.* 75(6), 442-455. <https://doi.org/10.1093/nutrit/nux013>.

- 1415 McLaughlin, M.E., Kao, R., Liener, I.E., Hoidal, J.R., 1986. A quantitative in vitro assay of  
1416 polymorphonuclear leukocyte migration through human amnion membrane utilizing <sup>111</sup>In-oxine. *J.*  
1417 *Immunol. Methods.* 95(1), 89-98. [https://doi.org/10.1016/0022-1759\(86\)90321-2](https://doi.org/10.1016/0022-1759(86)90321-2).
- 1418 Meazza, C., Vitale, G., Pagani, S., Castaldi, D., Ogliari, G., Mari, D., Laarej, K., Tinelli, C., Bozzola,  
1419 M., 2011. Common adipokine features of neonates and centenarians. *J. Pediatr. Endocrinol. Metab.*  
1420 24(11-12):953-957. <https://doi.org/10.1515/jpem.2011.373>.
- 1421 Merz, M.P., Turner, J.D., 2021. Is early life adversity a trigger towards inflammaging? *Exp.*  
1422 *Gerontol.* 150, 111377. <https://doi.org/10.1016/j.exger.2021.111377>.
- 1423 Metcalf, T.U., Wilkinson, P.A., Cameron, M.J., Ghneim, K., Chiang, C., Wertheimer, A.M., Hiscott,  
1424 J.B., Nikolich-Zugich, J., Haddad, E.K., 2017. Human Monocyte Subsets Are Transcriptionally and  
1425 Functionally Altered in Aging in Response to Pattern Recognition Receptor Agonists. *J. Immunol.*  
1426 199(4),1405-1417. <https://doi.org/10.4049/jimmunol.1700148>.
- 1427 Milan-Mattos, J. C., Anibal, F.F., Perseguini, N.M., Minatel, V., Rehder-Santos, P., Castro, C.A.,  
1428 Vasilceac, F.A., Mattiello, S.M., Faccioli, L.H., Catai, A.M., 2019. Effects of natural aging and  
1429 gender on pro-inflammatory markers. *Braz. J. Med. Biol. Res.* 52, e8392.  
1430 <https://doi.org/10.1590/1414-431X20198392>.
- 1431 Mishto, M., Santoro, A., Bellavista, E., Sessions, R., Textoris-Taube, K., Dal Piaz, F., Carrard, G.,  
1432 Forti, K., Salvioli, S., Friguet, B., Kloetzel, P.M., Rivett, A.J., Franceschi, C., 2006a. A structural  
1433 model of 20S immunoproteasomes: effect of LMP2 codon 60 polymorphism on expression, activity,  
1434 intracellular localisation and insight into the regulatory mechanisms. *Biol. Chem.* 387(4), 417-29.  
1435 <https://doi.org/10.1515/BC.2006.056>.
- 1436 Mishto, M., Bellavista, E., Santoro, A., Stolzing, A., Ligorio, C., Nacmias, B., Spazzafumo, L.,  
1437 Chiappelli, M., Licastro, F., Sorbi, S., Pession, A., Ohm, T., Grune, T., Franceschi, C., 2006b.  
1438 Immunoproteasome and LMP2 polymorphism in aged and Alzheimer's disease brains. *Neurobiol.*  
1439 *Aging.* 27(1), 54-66. <https://doi.org/10.1016/j.neurobiolaging.2004.12.004>.
- 1440 Mittrücker, H.W., Visekruna, A., Huber, M., 2014. Heterogeneity in the differentiation and function  
1441 of CD8<sup>+</sup> T cells. *Arch. Immunol. Ther. Exp.* 62(6), 449–58. <https://doi.org/10.1007/s00005-014-0293-y>.  
1442
- 1443 Modi C, Böhm V, Ferraro S, Stein G, Seljak U. Estimating COVID-19 mortality in Italy early in the  
1444 COVID-19 pandemic. *Nat Commun.* 2021 May 12;12(1):2729. doi: 10.1038/s41467-021-22944-0.  
1445 PMID: 33980836; PMCID: PMC8115692.
- 1446 Monti, D., Salvioli, S., Capri, M., Malorni, W., Straface, E., Cossarizza, A., Botti, B., Piacentini, M.,  
1447 Baggio, G., Barbi, C., Valensin, S., Bonafè, M., Franceschi, C., 2000. Decreased susceptibility to  
1448 oxidative stress-induced apoptosis of peripheral blood mononuclear cells from healthy elderly and  
1449 centenarians. *Mech. Ageing Dev.* 121(1-3), 239-50. [https://doi.org/10.1016/s0047-6374\(00\)00220-7](https://doi.org/10.1016/s0047-6374(00)00220-7).
- 1450 Monti, D., Ostan, R., Borelli, V., Castellani, G., Franceschi, C., 2017. Inflammaging and human  
1451 longevity in the omics era. *Mech. Ageing Dev.* 165(Pt B), 129-138.  
1452 <https://doi.org/10.1016/j.mad.2016.12.008>.
- 1453 Montoliu, I., Scherer, M., Beguelin, F., DaSilva, L., Mari, D., Salvioli, S., Martin, F.P., Capri, M.,  
1454 Bucci, L., Ostan, R., Garagnani, P., Monti, D., Biagi, E., Brigidi, P., Kussmann, M., Rezzi, S.,

- 1455 Franceschi, C., Collino, S., 2014. Serum profiling of healthy aging identifies phospho- and  
1456 sphingolipid species as markers of human longevity. *Aging (Albany NY)*. 6(1), 9-25.  
1457 <https://doi.org/10.18632/aging.100630>.
- 1458 Morrisette-Thomas, V., Cohen, A.A., Fülöp, T., Riesco, É., Legault, V., Li, Q., Milot, E., Dusseault-  
1459 Bélanger, F., Ferrucci, L., 2014. Inflamm-aging does not simply reflect increases in pro-inflammatory  
1460 markers. *Mech. Ageing Dev.* 139, 49-57. <https://doi.org/10.1016/j.mad.2014.06.005>.
- 1461 Nasi, M., Troiano, L., Lugli, E., Pinti, M., Ferraresi, R., Monterastelli, E., Mussi, C., Salvioli, G.,  
1462 Franceschi, C., Cossarizza, A., 2006. Thymic output and functionality of the IL-7/IL-7 receptor  
1463 system in centenarians: implications for the neo-lymphogenesis at the limit of human life. *Aging Cell*.  
1464 5, 167-175. <https://doi.org/10.1111/j.1474-9726.2006.00204.x>.
- 1465 Netea, M.G., van der Meer, J.W., 2017. Trained Immunity: An Ancient Way of Remembering. *Cell*  
1466 *Host Microbe*. 21(3), 297-300. <https://doi.org/10.1016/j.chom.2017.02.003>.
- 1467 Newman, A.B., Sanders, J.L., Kizer, J.R., Boudreau, R.M., Odden, M.C., Zeki Al Hazzouri, A.,  
1468 Arnold, A.M., 2016. Trajectories of function and biomarkers with age: the CHS All Stars Study. *Int.*  
1469 *J. Epidemiol.* 45(4), 1135-1145. <https://doi.org/10.1093/ije/dyw092>.
- 1470 Nikolich-Zugich, J., Goodrum, F., Knox, K., Smithey, M.J., 2017. Known unknowns: how might the  
1471 persistent herpesvirome shape immunity and aging? *Curr. Opin. Immunol.* 48, 23–30.  
1472 <https://doi.org/10.1016/j.coi.2017.07.011>.
- 1473 O'Toole, P. W., Marchesi, J. R., Hill, C., 2017. Next-generation probiotics: the spectrum from  
1474 probiotics to live biotherapeutics. *Nat. Microbiol.* 2, 17057.  
1475 <https://doi.org/10.1038/nmicrobiol.2017.57>.
- 1476 Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J.Z., Abe, F., Osawa, R.,  
1477 2016. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-  
1478 sectional study. *BMC. Microbiol.* 16, 90. <https://doi.org/10.1186/s12866-016-0708-5>.
- 1479 Oertelt-Prigione, S., 2012. Immunology and the menstrual cycle. *Autoimm. Rev.* 11(6-7), A486-492.  
1480 <https://doi.org/10.1016/j.autrev.2011.11.023>.
- 1481 Oki, K., Toyama, M., Banno, T., Chonan, O., Benno, Y., Watanabe, K., 2016. Comprehensive  
1482 analysis of the fecal microbiota of healthy Japanese adults reveals a new bacterial lineage associated  
1483 with a phenotype characterized by a high frequency of bowel movements and a lean body type. *BMC*  
1484 *Microbiol.* 16(1), 284. <https://doi.org/10.1186/s12866-016-0898-x>.
- 1485 Olivieri, F., Spazzafumo, L., Antonicelli, R., Marchegiani, F., Cardelli, M., Sirolla, C., Galeazzi, R.,  
1486 Giovagnetti, S., Mocchegiani, E., Franceschi, C., 2008. Combination of biomarkers to predict  
1487 mortality in elderly patients with myocardial infarction. *Mech. Ageing Dev.* 129 (4), 231–237.  
1488 <https://doi.org/10.1016/j.mad.2008.01.002>.
- 1489 Olivieri, F., Rippo, M.R., Monsurrò, V., Salvioli, S., Capri, M., Procopio, A.D., Franceschi, C., 2013.  
1490 MicroRNAs linking inflamm-aging, cellular senescence and cancer. *Ageing Res. Rev.* 12(4), 1056-  
1491 1068. <https://doi.org/10.1016/j.arr.2013.05.001>.

- 1492 Ortmann, W., Kolaczowska, E., 2018. Age is the work of art? Impact of neutrophil and organism  
1493 age on neutrophil extracellular trap formation. *Cell Tissue Res.* 371, 473–488.  
1494 <https://doi.org/10.1007/s00441-017-2751-4>.
- 1495 Ostan, R., Bucci, L., Capri, M., Salvioli, S., Scurti, M., Pini, E., Monti, D., Franceschi, C., 2008.  
1496 Immunosenescence and immunogenetics of human longevity. *Neuroimmunomodulation.* 15 (4–6),  
1497 224–240. <https://doi.org/10.1159/000156466>.
- 1498 Ostan, R., Bucci, L., Cevenini, E., Palmas, M.G., Pini, E., Scurti, M., Vescovini, R., Caruso, C., Mari,  
1499 D., Vitale, G., Franceschi, C., Monti, D., 2013. Metabolic syndrome in the offspring of centenarians:  
1500 focus on prevalence, components, and adipokines. *Age (Dordr).* 35(5), 1995–2007.  
1501 <https://doi.org/10.1007/s11357-012-9483-x>.
- 1502 Ostan, R., Monti, D., Guerresi, P., Bussolotto, M., Franceschi, C., Baggio, G., 2016. Gender, aging  
1503 and longevity in humans: an update of an intriguing/neglected scenario paving the way to a gender-  
1504 specific medicine. *Clin. Sci. (Lond).* 130(19), 1711–1725. <https://doi.org/10.1042/CS20160004>.
- 1505 Palmer, D.B., 2013. The effect of age on thymic function. *Front. Immunol.* 4, 316.  
1506 <https://doi.org/10.3389/fimmu.2013.00316>.
- 1507 Panda, A., Arjona, A., Sapey, E., Bai, F., Fikrig, E., Montgomery, R.R., Lord, J.M., Shaw, A.C.,  
1508 2009. Human innate immunosenescence: causes and consequences for immunity in old age. *Trends.*  
1509 *Immunol.*30(7), 325–333. <https://doi.org/10.1016/j.it.2009.05.004>.
- 1510 Panda, A., Qian, F., Mohanty, S., van Duin, D., Newman, F.K., Zhang, L., Chen, S., Towle, V.,  
1511 Belshe, R.B., Fikrig, E., Allore, H.G., Montgomery, R.R., Shaw, A.C., 2010. Age-associated decrease  
1512 in TLR function in primary human dendritic cells predicts influenza vaccine response. *J. Immunol.*  
1513 184(5), 2518–27. <https://doi.org/10.4049/jimmunol.0901022>.
- 1514 Pang, W.W., Price, E.A., Sahoo, D., Beerman, I., Maloney, W.J., Rossi, D.J., Schrier, S.L.,  
1515 Weissman, I.L., 2011. Human bone marrow hematopoietic stem cells are increased in frequency and  
1516 myeloid-biased with age. *Proc. Natl. Acad. Sci. U. S. A.* 108, 20012–20017.  
1517 <https://doi.org/10.1073/pnas.1116110108>.
- 1518 Paolisso, G., Barbieri, M., Bonafè, M., Franceschi, C., 2000. Metabolic age modelling: the lesson  
1519 from centenarians. *Eur. J. Clin. Invest.* 30(10), 888–94. <https://doi.org/10.1046/j.1365-2362.2000.00729.x>.
- 1521 Paolisso, G., Barbieri, M., Rizzo, M.R., Carella, C., Rotondi, M., Bonafè, M., Franceschi, C., Rose,  
1522 G., De Benedictis, G., 2001. Low insulin resistance and preserved beta-cell function contribute to  
1523 human longevity but are not associated with TH-INS genes. *Exp. Gerontol.* 37 (1), 149–156.  
1524 [https://doi.org/10.1016/s0531-5565\(01\)00148-6](https://doi.org/10.1016/s0531-5565(01)00148-6).
- 1525 Passeri, G., Pini, G., Troiano, L., Vescovini, R., Sansoni, P., Passeri, M., Guerresi, P., Delsignore, R.,  
1526 Pedrazzoni, M., Franceschi, C., 2003. Low vitamin D status, high bone turnover, and bone fractures  
1527 in centenarians. *J. Clin. Endocrinol. Metab.* 88 (11), 5109–5115. <https://doi.org/10.1210/jc.2003-030515>.
- 1529 Pawelec, G., Hirokawa, K., Fülöp, T., 2001a. Altered T cell signalling in ageing. *Mech. Ageing Dev.*  
1530 122(14), 1613–37. <https://doi.org/10.1016/s0047>.

- 1531 Pawelec, G., Müller, L., Wagner, W., 2001b. MHC class II-restricted tumor antigens and CD4+ T  
1532 cells play a role in hematological malignancies as well as solid tumors. *Trends. Immunol.* 22(8), 422-  
1533 3. [https://doi.org/10.1016/s1471-4906\(01\)01987-1](https://doi.org/10.1016/s1471-4906(01)01987-1).
- 1534 Pawelec, G., Ouyang, Q., Wagner, W., Biol, D., Wikby, A., 2003. Pathways to a robust immune  
1535 response in the elderly. *Immunol. Allergy Clin. N. Am.* 23 (1), 1–13. [https://doi.org/10.1016/s0889-8561\(02\)00075-9](https://doi.org/10.1016/s0889-8561(02)00075-9).  
1536
- 1537 Pawelec, G., Koch, S., Franceschi, C., Wikby, A., 2006. Human immunosenescence: does it have an  
1538 infectious component? *Ann. N. Y. Acad. Sci.* 1067, 56–65. <https://doi.org/10.1196/annals.1354.009>.
- 1539 Pawelec, G., 2012a. Hallmarks of human “immunosenescence”: adaptation or dysregulation? *Immun.*  
1540 *Ageing.* 9, 15. <https://doi.org/10.1186/1742-4933-9-15>.
- 1541 Pawelec, G., McElhaney, J.E., Aiello, A.E., Derhovanessian, E., 2012b. The impact of CMV infection  
1542 on survival in older humans. *Curr. Opin. Immunol.* 24, 507–511.  
1543 <https://doi.org/10.1016/j.coi.2012.04.002>.
- 1544 Pawelec, G., 2017. Immunosenescence and cancer. *Biogerontol.* 18, 717–721.  
1545 <https://doi.org/10.1007/s10522-017-9682-z>.
- 1546 Pawelec, G., 2018. Age and immunity: What is “immunosenescence”? *Exp. Gerontol.* 105, 4–9.  
1547 <https://doi.org/10.1016/j.exger.2017.10.024>.
- 1548 Pawelec, G., Bronikowski, A., Cunnane, S.C., Ferrucci, L., Franceschi, C., Fülöp, T., Gaudreau, P.,  
1549 Gladyshev, V.N., Gonos, E.S., Gorbunova, V., Kennedy, B.K., Larbi, A., Lemaître, J.F., Liu, G.H.,  
1550 Maier, A.B., Morais, J.A., Nóbrega, O.T., Moskalev, A., Rikkert, M.O., Seluanov, A., Senior, A.M.,  
1551 Ukraintseva, S., Vanhaelen, Q., Witkowski, J., Cohen, A.A., 2020a. The conundrum of human  
1552 immune system "senescence". *Mech. Ageing Dev.* 192, 111357.  
1553 <https://doi.org/10.1016/j.mad.2020.111357>.
- 1554 Pawelec, G., 2020b. The human immunosenescence phenotype: does it exist? *Semin. Immunopathol.*  
1555 42(5), 537-544. <https://doi.org/10.1007/s00281-020-00810-3>.
- 1556 Perez-Cabezas, B., Naranjo-Gomez, M., Fernandez, M. A., Grifols, J. R., Pujol-Borrell, R. and  
1557 Borrás, F. E., 2007. Reduced numbers of plasmacytoid dendritic cells in aged blood donors. *Exp.*  
1558 *Gerontol.* 42, 1033–1038. <https://doi.org/10.1016/j.exger.2007.05.010>.
- 1559 Perretti, M., D'Acquisto, F., 2006. Novel aspects of annexin 1 and glucocorticoid biology: intersection  
1560 with nitric oxide and the lipoxin receptor. *Inflamm. Allergy Drug Targets.* 5(2), 107-114.  
1561 <https://doi.org/10.2174/187152806776383170>.
- 1562 Pietschmann, P., Gollob, E., Brosch, S., Hahn, P., Kudlacek, S., Willheim, M., Woloszczuk, W.,  
1563 Peterlik, M., Tragl, K.H., 2003. The effect of age and gender on cytokine production by human  
1564 peripheral blood mononuclear cells and markers of bone metabolism. *Exp. Gerontol.* 38, 1119–1127.  
1565 [https://doi.org/10.1016/s0531-5565\(03\)00189-x](https://doi.org/10.1016/s0531-5565(03)00189-x).
- 1566 Pinti, M., Cevenini, E., Nasi, M., De Biasi, S., Salvioli, S., Monti, D., Benatti, S., Gibellini, L.,  
1567 Cotichini, R., Stazi, M.A., Trenti, T., Franceschi, C., Cossarizza, A., 2014. Circulating mitochondrial  
1568 DNA increases with age and is a familiar trait: Implications for "inflamm-aging". *Eur. J. Immunol.*  
1569 44(5), 1552-1562. <https://doi.org/10.1002/eji.201343921>.

- 1570 Puchta, A., Naidoo, A., Verschoor, C.P., Loukov, D., Thevaranjan, N., Mandur, T.S., Nguyen, P.S.,  
1571 Jordana, M., Loeb, M., Xing, Z., Kobzik, L., Larché, M.J., Bowdish, D.M., 2016. TNF drives  
1572 monocyte dysfunction with age and results in impaired anti-pneumococcal immunity. *PLOS Pathog.*  
1573 12(1), e1005368. <https://doi.org/10.1371/journal.ppat.1005368>.
- 1574 Qi, Q., Liu, Y., Cheng, Y., Glanville, J., Zhang, D., Lee, J.Y., Olshen, R.A., Weyand, C.M., Boyd,  
1575 S.D., Goronzy, J.J., 2014. Diversity and clonal selection in the human T-cell repertoire. *Proc. Natl.*  
1576 *Acad. Sci. U. S. A.* 111, 13139–13144. <https://doi.org/10.1073/pnas.1409155111>.
- 1577 Qian, F., Wang, X.M., Zhang, L., Chen, S., Piecychna, M., Allore, H., Bockenstedt, L., Malawista,  
1578 S., Bucala, R., Shaw, A.C., Fikrig, E., Montgomery, R.R., 2012. Age-associated elevation in TLR5  
1579 leads to increased inflammatory responses in the elderly. *Aging Cell.* 11, 104–110.  
1580 <https://doi.org/10.1111/j.1474-9726.2011.00759.x>.
- 1581 Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N.,  
1582 Levenez, F., Yamada, T., Mende, D.R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H.,  
1583 Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.M., Hansen, T., Le Paslier, D.,  
1584 Linneberg, A., Nielsen, H.B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu,  
1585 C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H., Wang, J., Brunak, S., Doré,  
1586 J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenback, J., Meta HIT Consortium,  
1587 Bork, P., Ehrlich, S.D., Wang, J., 2010. A human gut microbial gene catalogue established by  
1588 metagenomic sequencing. *Nature*, 464, 59–65. <https://doi.org/10.1038/nature08821>.
- 1589 Rampelli, S., Soverini, M., D'Amico, F., Barone, M., Tavella, T., Monti, D., Capri, M., Astolfi, A.,  
1590 Brigidi, P., Biagi, E., Franceschi, C., Turroni, S., Candela, M., 2020. Shotgun metagenomics of gut  
1591 microbiota in humans with up to extreme longevity and the increasing role of xenobiotic degradation.  
1592 *mSystems.* 5(2), e00124–20. <https://doi.org/10.1128/mSystems.00124-20>.
- 1593 Rodriguez-Rosales, Y. A., Langereis, J. D., Gorris, M. A. J., van den Reek, J. M. P. A., Fasse, E.,  
1594 Netea, M. G., de Vries, I. J. M., Gomez-Muñoz, L., van Cranenbroek, B., Körber, A., Sonderman,  
1595 W., Joosten, I., de Jong, E. M. G. J., & Koenen, H. J. P. M., 2021. Immunomodulatory aged  
1596 neutrophils are augmented in blood and skin of psoriasis patients. *Journal of Allergy and Clinical*  
1597 *Immunology*, 1–11. <https://doi.org/10.1016/j.jaci.2021.02.041>
- 1598 Sadighi Akha, A.A., 2018. Aging and the immune system: An overview. *J. Immunol. Methods.*  
1599 463,21–26. <https://doi.org/10.1016/j.jim.2018.08.005>.
- 1600 Sakiani, S., Olsen, N.J., Kovacs, W.J., 2013. Gonadalsteroids and humoral immunity. *Nat. Rev.*  
1601 *Endocrinol.* 9, 56–62. <https://doi.org/10.1038/nrendo.2012.206>.
- 1602 Salvioli, S., Capri, M., Bucci, L., Lanni, C., Racchi, M., Uberti, D., Memo, M., Mari, D., Govoni, S.,  
1603 Franceschi, C., 2009. Why do centenarians escape or postpone cancer? The role of IGF-1,  
1604 inflammation and p53. *Cancer Immunol. Immunother.* 58, 1909–1917.  
1605 <https://doi.org/10.1007/s00262-008-0639-6>
- 1606 Salvioli, S., Monti, D., Lanzarini, C., Conte, M., Pirazzini, C., Bacalini, M.G., Garagnani, P., Giuliani,  
1607 C., Fontanesi, E., Ostan, R., Bucci, L., Sevini, F., Yani, S.L., Barbieri, A., Lomartire, L., Borelli, V.,  
1608 Vianello, D., Bellavista, E., Martucci, M., Cevenini, E., Pini, E., Scurti, M., Biondi, F., Santoro, A.,  
1609 Capri, M., Franceschi, C., 2013. Immune system, cell senescence, aging and longevity—inflammm-  
1610 aging reappraised. *Curr. Pharm. Des.* 19(9),1675–1679.  
1611 <https://doi.org/10.2174/138161213805219531>.



- 1612 Sansoni, P., Brianti, V., Fagnoni, F., Snelli, G., Marcato, A., Passeri, G., Monti, D., Cossarizza, A.,  
 1613 Franceschi, C., 1992. NK cell activity and T-lymphocyte proliferation in healthy centenarians. *Ann.*  
 1614 *N. Y. Acad. Sci.* 663, 505-507. <https://doi.org/10.1111/j.1749-6632.1992.tb38717.x>.
- 1615 Sansoni, P., Vescovini, R., Fagnoni, F., Biasini, C., Zanni, F., Zanlari, L., Telera, A., Lucchini, G.,  
 1616 Passeri, G., Monti, D., Franceschi, C., Passeri, M., 2008. The immune system in extreme longevity.  
 1617 *Exp Gerontol.* 43, 61–65. <https://doi.org/10.1016/j.exger.2007.06.008>.
- 1618 Sansoni, P., Vescovini, R., Fagnoni, F.F., Akbar, A., Arens, R., Chiu, Y.L., Cičin-Šain, L., Dechanet-  
 1619 Merville, J., Derhovanessian, E., Ferrando-Martinez, S., Franceschi, C., Frasca, D., Fulöp, T.,  
 1620 Furman, D., Gkrania-Klotsas, E., Goodrum, F., Grubeck-Loebenstien, B., Hurme, M., Kern, F.,  
 1621 Lillieri, D., López-Botet, M., Maier, A.B., Marandu, T., Marchant, A., Matheï, C., Moss, P.,  
 1622 Muntasell, A., Remmerswaal, E.B., Riddell, N.E., Rothe, K., Sauce, D., Shin, E.C., Simanek, A.M.,  
 1623 Smithey, M.J., Söderberg-Nauclér, C., Solana, R., Thomas, P.G., van Lier, R., Pawelec, G., Nikolich-  
 1624 Zugich, J., 2014. New advances in CMV and immunosenescence. *Exp. Gerontol.* 55, 54-62.  
 1625 <https://doi.org/10.1016/j.exger.2014.03.020>.
- 1626 Santoro, A., Bazzocchi, A., Guidarelli, G., Ostan, R., Giampieri, E., Mercatelli, D., Scurti, M.,  
 1627 Berendsen, A., Surala, O., Jennings, A., Meunier, N., Caumon, E., Gillings, R., Kadi, F., Capel, F.,  
 1628 Cashman, K.D., Pietruszka, B., Feskens, E.J.M., De Groot, L.C.P.G.M., Battista, G., Salvioli, S.,  
 1629 Franceschi, C., 2018a. A Cross-Sectional Analysis of Body Composition Among Healthy Elderly  
 1630 From the European NU-AGE Study: Sex and Country Specific Features. *Front. Physiol.* 9, 1693.  
 1631 <https://doi.org/10.3389/fphys.2018.01693>.
- 1632 Santoro, A., Ostan, R., Candela, M., Biagi, E., Brigidi, P., Capri, M., Franceschi, C., 2018b. Gut  
 1633 microbiota changes in the extreme decades of human life: a focus on centenarians. *Cell. Mol. Life*  
 1634 *Sci.* 75(1), 129–148. <https://doi.org/10.1007/s00018-017-2674-y>.
- 1635 Santoro, A., Martucci, M., Conte, M., Capri, M., Franceschi, C., Salvioli, S., 2020a. Inflammaging,  
 1636 hormesis and the rationale for anti-aging strategies. *Ageing Res. Rev.* 64, 101142.  
 1637 <https://doi.org/10.1016/j.arr.2020.101142>.
- 1638 Santoro, A., Zhao, J., Wu, L., Carru, C., Biagi, E., Franceschi, C., 2020b. Microbiomes other than the  
 1639 gut: inflammaging and age-related diseases. *Semin. Immunopathol.* 42(5), 589-605.  
 1640 <https://doi.org/10.1007/s00281-020-00814-z>.
- 1641 Sayed, N., Huang, Y., Nguyen, K., Krejciova-Rajaniemi, Z., Grawe, A.P., Gao, T., Tibshirani, R.,  
 1642 Hastie, T., Alpert, A., Cui, L., Kuznetsova, T., Rosenberg-Hasson, Y., Ostan, R., Monti, D., Lehallier,  
 1643 B., Shen-Orr, S.S., Maecker, H.T., Dekker, C.L., Wyss-Coray, T., Franceschi, C., Jovic, V., Haddad,  
 1644 F., Montoya, J.G., Wu, J.C., Davis, M.M., Furman, D., 2021. Deep Learning Identifies an  
 1645 Inflammatory Clock which Predicts Multimorbidity, Immunosenescence, Frailty and Cardiovascular  
 1646 Aging in Humans. *Nature Aging* in press
- 1647 Schmitt, V., Rink, L., Uciechowski, P., 2013. The Th17/Treg balance is disturbed during aging. *Exp.*  
 1648 *Gerontol.* 48, 1379–1386. <https://doi.org/10.1016/j.exger.2013.09.003>.
- 1649 Sergio, G., 2008. Exploring the complex relations between inflammation and aging (inflamm-aging):  
 1650 Anti-inflamm-aging remodelling of inflamm- aging, from robustness to frailty. *Inflamm. Res.* 57,  
 1651 558–563. <https://doi.org/10.1007/s00011-008-7243-2>.

- 1652 Shanley, D.P., Aw, D., Manley, N.R., Palmer, D.B., 2009. An evolutionary perspective on the  
1653 mechanisms of immunosenescence. *Trends. Immunol.* 30, 374–381.  
1654 <https://doi.org/10.1016/j.it.2009.05.001>.
- 1655 Shaw, A.C., Panda, A., Joshi, S.R., Qian, F., Allore, H.G., Montgomery, R.R., 2011. Dysregulation  
1656 of human Toll-like receptor function in aging. *Ageing Res. Rev.* 10(3), 346-53.  
1657 <https://doi.org/10.1016/j.arr.2010.10.007>.
- 1658 Simell, B., Vuorela, A., Ekström, N., Palmu, A., Reunanen, A., Meri, S., Käyhty, H., Väkeväinen,  
1659 M., 2011. Aging reduces the functionality of anti-pneumococcal antibodies and the killing of  
1660 *Streptococcus pneumoniae* by neutrophil phagocytosis. *Vaccine.* 29(10), 1929-1934.  
1661 <https://doi.org/10.1016/j.vaccine.2010.12.121>.
- 1662 Sizzano, F., Collino, S., Cominetti, O., Monti, D., Garagnani, P., Ostan, R., Pirazzini, C., Bacalini,  
1663 M.G., Mari, D., Passarino, G., Franceschi, C., Palini, A., 2018. Evaluation of Lymphocyte Response  
1664 to the Induced Oxidative Stress in a Cohort of Ageing Subjects, including Semisupercentenarians and  
1665 Their Offspring. *Mediators Inflamm.* 2018, 7109312. <https://doi.org/10.1155/2018/7109312>.
- 1666 Smith, R.P., Easson, C., Lyle, S.M., Kapoor, R., Donnelly, C.P., Davidson, E.J., Parikh, E., Lopez,  
1667 J.V., Tartar, J.L., 2019. Gut microbiome diversity is associated with sleep physiology in humans.  
1668 *PLoS One.* 14, e0222394. <https://doi.org/10.1371/journal.pone.0222394>.
- 1669 Sochocka, M., Diniz, B.S., Leszek, J., 2017. Inflammatory Response in the CNS: Friend or Foe? *Mol.*  
1670 *Neurobiol.* 54(10), 8071-8089. <https://doi.org/10.1007/s12035-016-0297-1>.
- 1671 Solana, R., Tarazona, R., Aiello, A.E., Akbar, A.N., Appay, V., Beswick, M., Bosch, J.A., Campos,  
1672 C., Cantisán, S., Cicin-Sain, L., Derhovanessian, E., Ferrando-Martínez, S., Frasca, D., Fulöp, T.,  
1673 Govind, S., Grubeck-Loebenstein, B., Hill, A., Hurme, M., Kern, F., Larbi, A., López-Botet, M.,  
1674 Maier, A.B., McElhaney, J.E., Moss, P., Naumova, E., Nikolich-Zugich, J., Pera, A., Rector, J.L.,  
1675 Riddel, N., Sanchez-Correa, B., Sansoni, P., Sauce, D., van Lier, R., Wang, G.C., Wills, M.R.,  
1676 Zieliński, M., Pawelwc, G., 2012a. CMV and immunosenescence: from basics to clinics. *Immun.*  
1677 *Ageing.* 9(1), 23. <https://doi.org/doi:10.1186/1742-4933-9-23>.
- 1678 Solana, R., Tarazona, R., Gayoso, I., Lesur, O., Dupuis, G., Fulop, T., 2012. Innate  
1679 immunosenescence: effect of aging on cells and receptors of the innate immune system in humans.  
1680 *Semin. Immunol.* 24(5), 331-341. <https://doi.org/10.1016/j.smim.2012.04.008>.
- 1681 Song, P., An, J., Zou, M.H., 2020. Immune Clearance of Senescent Cells to Combat Ageing and  
1682 Chronic Diseases. *Cells.* 9(3), 671. <https://doi.org/10.3390/cells9030671>.
- 1683 Song, S., Lam, E.W., Tchkonina, T., Kirkland, J.L., Sun, Y., 2020. Senescent Cells: Emerging Targets  
1684 for Human Aging and Age-Related Diseases. *Trends. Biochem. Sci.* 45, 578-592.  
1685 <https://doi.org/10.1016/j.tibs.2020.03.008>.
- 1686 Soto-Martin, E.C., Warnke, I., Farquharson, F.M., Christodoulou, M., Horgan, G., Derrien, M.,  
1687 Faurie, J.M., Flint, H.J., Duncan, S.H., Louis, P., 2020. Vitamin biosynthesis by human gut butyrate-  
1688 producing bacteria and cross-feeding in synthetic microbial communities. *mBio.* 11, e00886- e00920.  
1689 <https://doi.org/10.1128/mBio.00886-20>.
- 1690 Spazzafumo, L., Olivieri, F., Abbatecola, A.M., Castellani, G., Monti, D., Lisa, R., Galeazzi, R.,  
1691 Sirolla, C., Testa, R., Ostan, R., Scurti, M., Caruso, C., Vasto, S., Vescovini, R., Ogliari, G., Mari,

- 1692 D., Lattanzio, F., Franceschi, C., 2013. Remodelling of biological parameters during human aging:  
1693 evidence for complex regulation in longevity and in type 2 diabetes. *Age (Dordr)*. 35, 419–429.  
1694 <https://doi.org/10.1007/s11357-011-9348-8>.
- 1695 Spits, H., Bernink, J.H., Lanier, L., 2016. NK cells and type 1 innate lymphoid cells: partners in host  
1696 defense. *Nat. Immunol.* 17, 758–764. <https://doi.org/10.1038/ni.3482>.
- 1697 Steinmann, G.G., Klaus, B., Muller-Hermelink, H.K., 1985. The involution of the ageing human  
1698 thymic epithelium is independent of puberty. A morphometric study. *Scand. J. Immunol.* 22, 563–  
1699 575. <https://doi.org/10.1111/j.1365-3083.1985.tb01916.x>.
- 1700 Storci, G., De Carolis, S., Papi, A., Bacalini, M.G., Gensous, N., Marasco, E., Tesei, A., Fabbri, F.,  
1701 Arienti, C., Zanoni, M., Sarnelli, A., Santi, S., Olivieri, F., Mensà, E., Latini, S., Ferracin, M., Salvioli,  
1702 S., Garagnani, P., Franceschi, C., Bonafè, M., 2019. Genomic stability, anti-inflammatory phenotype,  
1703 and up-regulation of the RNaseH2 in cells from centenarians. *Cell Death Differ.* 26, 1845–58.  
1704 <https://doi.org/10.1038/s41418-018-0255-8>.
- 1705 Stout, R.D. and Suttles, J., 2005. Immunosenescence and macrophage functional plasticity:  
1706 dysregulation of macrophage function by age- associated microenvironmental changes. *Immunol.*  
1707 *Rev.* 205, 60–71. <https://doi.org/10.1038/s41418-018-0255-8>.
- 1708 Straub, R.H., Mocchegiani, E., (eds) (2004) *The Neuroendocrine Immune Network in Ageing*  
1709 (*Neuroimmune Biology*, Vol. 4), Elsevier
- 1710 Strindhall, J., Nilsson, B.O., Lofgren, S., Ernerudh, J., Pawelec, G., Johansson, B., Wikby, A., 2007.  
1711 No immune risk profile among individuals who reach 100 years of age: findings from the Swedish  
1712 NONA immune longitudinal study. *Exp. Gerontol.* 42 (8), 753–761.  
1713 <https://doi.org/10.1016/j.exger.2007.05.001>.
- 1714 Sun, J., Xu, J., Ling, Y., Wang, F., Gong, T., Yang, C., Ye, S., Ye, K., Wei, D., Song, Z., Chen, D.,  
1715 Liu, J., 2019. Fecal microbiota transplantation alleviated Alzheimer’s disease-like pathogenesis in  
1716 APP/PS1 transgenic mice. *Transl. Psychiatry.* 9, 189. <https://doi.org/10.1038/s41398-019-0525-3>.
- 1717 Tavella, T., Rampelli, S., Guidarelli, G., Bazzocchi, A., Gasperini, C., Pujos-Guillot, E., Comte, B.,  
1718 Barone, M., Biagi, E., Candela, M., Nicoletti, C., Kadi, F., Battista, G., Salvioli, S., O’Toole, P.W.,  
1719 Franceschi, C., Brigidi, P., Turrioni, S., Santoro, A., 2021. Elevated gut microbiome abundance of  
1720 Christensenellaceae, Porphyromonadaceae and Rikenellaceae is associated with reduced visceral  
1721 adipose tissue and healthier metabolic profile in Italian elderly. *Gut Microbes.* 13(1), 1-19.  
1722 <https://doi.org/10.1080/19490976.2021.1880221>.
- 1723 Trim, W.V., Walhin, J.P., Koumanov, F., Bouloumié, A., Lindsay, M.A., Chen, Y.C., Travers, R.L.,  
1724 Turner, J.E., Thompson, D., 2021. Divergent immunometabolic changes in adipose tissue and skeletal  
1725 muscle with ageing in healthy humans. *J. Physiol.* <https://doi.org/10.1113/JP280977>.
- 1726 Tu, W., Rao, S., 2016. Mechanisms underlying T cell immunosenescence: aging and  
1727 Cytomegalovirus infection. *Front Microbiol* 7, 2111. <https://doi.org/10.3389/fmicb.2016.02111>.
- 1728 Tuikhar, N., Keisam, S., Labala, R. K., Imrat, Ramakrishnan, P., Arunkumar, M.C., Ahmed, G.,  
1729 Biagi, E., Jeyaram, K., 2019. Comparative analysis of the gut microbiota in centenarians and young  
1730 adults shows a common signature across genotypically non-related populations. *Mech. Ageing Dev.*  
1731 179, 23–35. <https://doi.org/10.1016/j.mad.2019.02.001>.

- 1732 van Duin, D., Mohanty, S., Thomas, V., Ginter, S., Montgomery, R. R., Fikrig, E., Allore, H. G.,  
1733 Medzhitov, R., Shaw, A.C., 2007. Age-associated defect in human TLR-1/2 function. *J. Immunol.*  
1734 178, 970–975. <https://doi.org/10.4049/jimmunol.178.2.970>.
- 1735 Vasudev, A., Ying, C.T., Ayyadhury, S., Puan, K.J., Andiappan, A.K, Nyunt, M.S., Shadan, N.B.,  
1736 Mustafa, S., Low, I., Rotzschke, O., Fulop, T., Ng, T.P., Larbi, A., 2014.  $\gamma/\delta$  T cell subsets in human  
1737 aging using the classical  $\alpha/\beta$  T cell model. *J. Leukoc. Biol.* 96(4), 647-655.  
1738 <https://doi.org/10.1189/jlb.5A1213-650RR>.
- 1739 Vescovini, R., Telera, A., Fagnoni, F.F., Biasini, C., Medici, M.C., Valcavi, P., di Pede, P., Lucchini,  
1740 G., Zanlari, L., Passeri, G., Zanni, F., Chezzi, C., Franceschi, C., Sansoni, P., 2004. Different  
1741 contribution of EBV and CMV infections in very long-term carriers to age- related alterations  
1742 of CD8+ T cells. *Exp. Gerontol.* 39, 1233–1243. <https://doi.org/10.1016/j.exger.2004.04.004>.
- 1743 Vescovini, R., Biasini, C., Fagnoni, F.F., Telera, A.R., Zanlari, L., Pedrazzoni, M., Bucci, L., Monti,  
1744 D., Medici, M.C., Chezzi, C., Franceschi, C., Sansoni, P., 2007. Massive load of functional effector  
1745 CD4+ and CD8+ T cells against cytomegalovirus in very old subjects. *J. Immunol.* 179(6), 4283-  
1746 4291. <https://doi.org/10.4049/jimmunol.179.6.4283>.
- 1747 Vitale, G., Salvioli, S., Franceschi, C., 2013. Oxidative stress and the ageing endocrine system. *Nat.*  
1748 *Rev. Endocrinol.* 9(4), 228-240. <https://doi.org/10.1038/nrendo.2013.29>.
- 1749 Vorobjeva, N.V., Chernyak, B.V., 2020. NETosis: Molecular Mechanisms, Role in Physiology and  
1750 Pathology. *Biochemistry (Mosc).* 85, 1178–1190. <https://doi.org/10.1134/S0006297920100065>.
- 1751 Weltevrede, M., Eilers, R., de Melker, H.E., van Baarle, D., 2016. Cytomegalovirus persistence and  
1752 T-cell immunosenescence in people aged fifty and older: A systematic review. *Exp. Gerontol.* 77, 87-  
1753 95. <https://doi.org/10.1016/j.exger.2016.02.005>.
- 1754 Wenisch, C., Patruta, S., Daxböck, F., Krause, R., Hörl, W., 2000. Effect of age on human neutrophil  
1755 function. *J. Leukoc. Biol.* 67(1), 40-45. <https://doi.org/10.1002/jlb.67.1.40>.
- 1756 Wikby, A., Nilsson, B.O., Forsey, R., Thompson, J., Strindhall, J., Lofgren, S., Ernerudh, J., Pawelec,  
1757 G., Ferguson, F., Johansson, B., 2006. The immune risk phenotype is associated with IL-6 in the  
1758 terminal decline stage: findings from the Swedish NONA immune longitudinal study of very late life  
1759 functioning. *Mech. Ageing Dev.* 127 (8), 695–704. <https://doi.org/10.1016/j.mad.2006.04.003>.
- 1760 Wu, L., Zeng, T., Zinellu, A., Rubino, S., Kelvin, D.J., Carru, C., 2019. A cross-sectional study of  
1761 compositional and functional profiles of gut microbiota in sardinian centenarians. *mSystems.* 4(4),  
1762 e00325-19. <https://doi.org/10.1128/mSystems.00325-19>.
- 1763 Yang, Y., Kozloski, M., 2011. Sex differences in age trajectories of physiological dysregulation:  
1764 Inflammation, metabolic syndrome, and allostatic load. *J. Gerontol. A. Biol. Sci. Med. Sci.* 66(5),  
1765 493–500. <https://doi.org/10.1093/gerona/qlr003>.
- 1766 Youm, Y.H., Grant, R.W., McCabe, L.R., Albarado, D.C., Nguyen, K.Y., Ravussin, A., Pistell, P.,  
1767 Newman, S., Carter, R., Laque, A., Munzberg, H., Rosen, C.J., Ingram, D.K., Salbaum, J.M., Dixit,  
1768 V.D., 2013. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional  
1769 decline in aging. *Cell Metab.* 18, 519–532. <https://doi.org/10.1016/j.cmet.2013.09.010>.

- 1770 Youness, A., Miquel, C.H., Guéry, J.C., 2021. Escape from X Chromosome Inactivation and the  
1771 Female Predominance in Autoimmune Diseases. *Int. J. Mol. Sci.* 22(3), 1114.  
1772 <https://doi.org/10.3390/ijms22031114>.
- 1773 Yu, B., Qi, Y., Li, R., Shi, Q., Satpathy, A.T., Chang, H.Y., 2021. B cell-specific XIST complex  
1774 enforces X-inactivation and restrains atypical B cells. *Cell.* 184(7), 1790-1803.e17.  
1775 <https://doi.org/10.1016/j.cell.2021.02.015>.
- 1776 Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L.,  
1777 Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., Cao, B., 2020. Clinical course and risk  
1778 factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort  
1779 study. *Lancet.* 395(10229), 1054–1062. [https://doi.org/10.1016/S0140-6736\(20\)30566-3](https://doi.org/10.1016/S0140-6736(20)30566-3).
- 1780 Zhu, J., Yamane, H., Paul, W.E., 2010. Differentiation of effector CD4 T cell populations (\*). *Annu.*  
1781 *Rev. Immunol.* 28, 445-489. <https://doi.org/10.1146/annurev-immunol-030409-101212>.
- 

1782

1783

1784

1785

1786

1787

1788

**Table 1: Age-related changes in GM and its metabolites in Italian Elderly and Centenarians**

	<b>Elderly</b>	<b>Centenarians</b>	<b>References</b>
<b>Biodiversity</b>	Decreased biodiversity	Increased biodiversity	Biagi et al., 2016
<b>Composition</b>	<b>Increase of pathobionts</b> ( <i>Enterobacteriaceae</i> and <i>Desulfovibrionaceae</i> )	<b>Increase of probiotic bacteria</b> <i>Bifidobacterium</i> and higher abundance of <i>Akkermansia</i> and <i>Christensenellaceae</i>	Biagi et al., 2010, 2012, 2013, 2016
<b>Adaptation</b>	<b>Maladaptation</b>	<b>Good adaptation</b>	Biagi et al., 2016
<b>Function</b>	<ul style="list-style-type: none"> <li>Progressive loss of SCFA producing bacteria (<i>Faecalibacterium</i>, <i>Roseburia</i>, <i>Coprococcus</i>)</li> </ul>	<ul style="list-style-type: none"> <li>Augmented capability for glycolysis and SCFA production</li> </ul>	Biagi et al., 2010; Wu et al., 2019; Rampelli et al., 2020
		<ul style="list-style-type: none"> <li>Decrease in circulating tryptophan concentrations</li> </ul>	Collino et al., 2013
		<ul style="list-style-type: none"> <li>Specific signature of glycerophospholipids and sphingolipids</li> </ul>	Collino et al., 2013
		<ul style="list-style-type: none"> <li>Increased excretion of urine phenylacetylglutamine (PAG) and p-cresol sulfite (PCS)</li> </ul>	Montoliu et al., 2014
		<ul style="list-style-type: none"> <li>Specific profile of Volatile Organic Compounds (VOCs) in urine and feces</li> </ul>	Conte et al., 2020

1790

1791

1792

1793

1794

1795

1796

1797

1798

1799

1800

1801

1802

1803 **FIGURE LEGENDS**

1804

1805 **Figure 1: Age-related changes in innate and adaptive immunity and their contribution to**  
1806 **inflammaging.** Crucial components of the innate IS such as neutrophils, NK, monocytes,  
1807 macrophages and dendritic cells undergo profound modifications with age. Also, the function of T  
1808 and B cells in adaptive immunity changes in the elderly. Both age-related changes in innate and  
1809 adaptive IS trigger the increase of inflammatory mediators that together with other modifications such  
1810 as increase of cell debris and damaged-associated molecular patterns (DAMPs), senescent cells,  
1811 inflamma-miRs, coagulation pathway components, Agalactosylated N-glycans, metaflammation and  
1812 decrease of disposal capability of proteasome and autophagy, gut microbiota dysbiosis and impaired  
1813 regulation of complement contribute to inflammaging. Created with BioRender.com

1814

1815 **Figure 2: Sex differences in innate and adaptive immunity throughout the life course.** A series  
1816 of immunological components differ between human females and males across the course of life.  
1817 Besides genes and hormones, environmental factors can modulate the functioning of the immune  
1818 system differentially between males and females. Compared to women, men experience a faster  
1819 progression to immunosenescence highlighted by changes in immune cells and inflammatory  
1820 mediators. **Abbreviations:** TLR, Toll-like receptor; TNF, tumour necrosis factor; T<sub>reg</sub>, regulatory T  
1821 cells, IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; DC, dendritic cells; pDC,  
1822 plasmacytoid dendritic cells; NK, Natural Killer cells; ILC, innate lymphoid cells; Th, T helper  
1823 lymphocytes. Created with BioRender.com

1824

1825 **Figure 3: Adaptation or maladaptation to lifelong pro- and anti-inflammatory stimuli leads to**  
1826 **longevity or diseases.** The pro- and anti-inflammatory stimuli that our organism is exposed to  
1827 lifelong combined with a healthy or unhealthy lifestyle (nutrition and physical activity) and gut  
1828 microbiota affect the IS remodeling triggering an adaptive or a maladaptive response. Excessive  
1829 stimulation of pro-inflammatory pathways and an ineffective anti-inflammatory response constitutes  
1830 a driving force for developing age-related diseases and disabilities. Instead, achieving successful  
1831 aging and longevity is determined by a lower predisposition to mount inflammatory response  
1832 combined with an efficient anti-inflammatory network. Created with BioRender.com

1833

1834 **Figure 4: Inflammaging and susceptibility to COVID 19.** Aging is characterized by extreme  
1835 heterogeneity due to the numerous and different exposures to lifelong factors determining each  
1836 individual's different immune responses. The different remodeling and adaptive reaction of the

1837 immune system triggered by inflammaging could explain the different susceptibility to COVID-19  
1838 among aged people. The adaptive anti-inflammatory response triggered by a mild inflammaging  
1839 could reduce the susceptibility to COVID-19 or the disease severity. A poor remodeling and the  
1840 consequent maladaptation of the immune system, triggered by a high inflammaging, could increase  
1841 the risk of SARS-CoV-2 infection and the severity of the disease. Created with BioRender.com

1842

1843