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

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

2

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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 (≥ 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- κ 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^{neg}CD16^{low}CD11b^{low} 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^{neg}) 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- κ 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- α production, particularly in response to engagement of TLR1/2 and an
219 increased release of TNF- α 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- α , IL-6
228 and IL-1 β , 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⁺ 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 α , IFN- γ , IL-1 β , 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 α 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- α , 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- γ . 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- γ gene is associated with low
288 IFN- γ 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- γ (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^{bright}CD16^{neg/dim} cells, the cells are more immature and secrete
308 cytokines and chemokines, whereas the main NK cell subset CD56^{dim}CD16⁺ 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^{bright} NK cells and the increase
317 in the CD56^{dim}CD57⁺ subset support that the population of NK cells suffers a process of remodeling
318 with a reduction in the output of more immature CD56^{bright} cells and an accumulation of highly
319 differentiated CD56^{dim}CD57⁺ 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 α , 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- α/β 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- α , 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⁺ and CD8⁺T cell populations with different functions (Das et al., 2017). CD4⁺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⁺ 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⁺ 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⁺
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⁺ cells. However, a well
393 detectable number of TREC⁺ 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⁺ T cell pool in humans in healthy aging, but less so in respect of naïve CD8⁺ 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⁺ T cells than their
404 CD4⁺ 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⁺ and CD8⁺ 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⁺ T cell reactivity is correlated with increasing numbers of late differentiated CD28⁻CD8⁺ 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⁺CD27⁻CD28⁻ 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⁺ T cells that, occasionally, were strikingly expanded.
454 Moreover, most anti-CMV CD8⁺ T cells did not bear the CD28 molecule, thus supporting the
455 hypothesis that the age-related expansion of CD28⁻ 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⁺ and, to a lesser extent, CD4⁺ 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⁺
466 and CD4⁺ 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⁻ 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⁺ 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⁺ 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- α , typical of inflammaging, can cause
509 human unstimulated B cells from elderly individuals to release significantly higher levels of TNF- α
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⁺CD27⁻], IgM memory [IgD⁺CD27⁺], switched memory [IgD⁻CD27⁺],
513 and late/exhausted memory [IgD⁻CD27⁻] (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 β , IL-6 and Tumor Necrosis Factor (TNF)- α
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 (≥ 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⁺ T cell counts and CD4⁺:CD8⁺ 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

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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
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908

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914

915 **DECLARATION OF INTEREST**

916 The authors have no conflicts of interest

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

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

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1803 **FIGURE LEGENDS**

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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

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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_{reg}, 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

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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

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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

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