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

19 Highlights:

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

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, symptoms and severity of age-related diseases. Finally, we discuss how these phenomena could 61 62 influence the susceptibility to COVID-19 infection.

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-72 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).

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

Moreover, inflammaging is crucially involved in the aetiology and progression of age-related diseases, often presented with multimorbidity and may finally lead to organ failure and death (Furman et al., 2019). 98

As immunosenescence proceeds, older people also become more susceptible to infectious diseases and cancer. Indeed, aged people and the oldest-old have an augmented risk for developing and dying from viral infections such as influenza and COVID-19 (Chen et al., 2020). Adults with chronic inflammatory conditions have a heightened risk for developing severe COVID-19 and dying (Huang et al., 2020). The interconnection between immunity and senescence is now receiving unprecedented emphasis during the COVID-19 pandemic, bringing to the fore the critical need to combat 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., 2009), cardiovascular diseases (Olivieri et al., 2008), insulin resistance and diabetes (Paolisso et al., 126 127 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).

The present review aims to summarise recent advancements in immunosenescence. Particular attention is devoted to the intimate relationship between immunosenescence and inflammaging and how these processes impact unsuccessful aging rather than longevity. We also describe the gut microbiota age-related changes as one of the significant triggers of inflammaging and the sex/gender differences in the immune system of the elderly, contributing to the sex/gender disparity in terms of epidemiology, pathophysiology, symptoms and severity of age-related diseases. Finally, we discuss 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 (DAMPs) to induce innate immunity through the NF-kB pathway and the induction of the canonical 155 156 NLRP3 inflammasome (Youm et al., 2013).

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

Neutrophils represent the first line of defence of the innate immune response and kill invading microbes. Neutrophils are recruited to the sites of infection to rapidly carry out their microbicidal activity, which relies on several mechanisms such as phagocytosis, degranulation of antimicrobial 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
- 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

et al., 1986), and ROS production (Fulop et al., 2004). Elderly individuals also display a reduced 166 167 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 pathogenesis of many infectious, inflammatory, and autoimmune diseases, but there is insufficient 173 174 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 maturation stages are present. The CD10^{neg}CD16^{low}CD11b^{low} fraction increased in psoriatic patients, 176 177 and this subset showed the morphology of aged neutrophils, though the lack of CD10 expression is associated with immaturity. The aged neutrophils (CD10^{neg}) are accumulated in the skin and have a 178 179 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).

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- 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).

Interestingly, centenarians show well-preserved neutrophil functions, such as bacterial phagocytosis, chemotaxis and superoxide production, comparable to those of young subjects (Alonso-Fernandez et al., 2008). Moreover, monocyte chemotaxis towards formyl-methionyl-leucyl-phenylalanine (f-MLP), adrenocorticotropic hormone (ACTH), and corticotrophin-releasing hormone (CRH) were 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-kB 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-1β, 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).

Three different monocyte subsets can be individuated based on their phenotype: classical (CD14⁺CD16⁻, which are 90% of circulating monocytes), intermediate (CD14⁺CD16⁺), and nonclassical (CD14^{dim}CD16⁺) monocytes (Hearps et al., 2012). Aging affects the relative distribution of monocyte subsets, with a marked reduction of the classical subset and an increase in the number of intermediate and non-classical monocytes with profound dysregulation in cytokines secretion after 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 M1. 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

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

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

277 (Putcha et al., 2016).

A key role in inflammaging might also be played by single nucleotide polymorphisms (SNPs) in the 278 279 promoter regions of genes encoding for IL-6 and IFN-y. 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 centenarians, contribute to inflammaging. They demonstrated that genetically predisposed 281 282 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-y gene is associated with low IFN-γ production and is positively associated with longevity in male and female centenarians (Lio et 288 289 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 identified. In the subset of CD56^{bright}CD16^{neg/dim} cells, the cells are more immature and secrete 307 cytokines and chemokines, whereas the main NK cell subset CD56^{dim}CD16+ is made up of mature 308 309 NK cells with high cytotoxic capacity after direct contact with tumour or virus-infected target cells (Cooper et al., 2004). Furthermore, a scarce subset of NK cells, devoid of CD56 expression and 310 311 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-Tavernier et al., 2010; Gayoso et al., 2011). Thus, the decline in CD56^{bright} NK cells and the increase 316 in the CD56^{dim}CD57+ subset support that the population of NK cells suffers a process of remodeling 317 with a reduction in the output of more immature CD56bright cells and an accumulation of highly 318 319 differentiated CD56dimCD57+ 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 NK cells, but impairment of NK cell cytotoxicity on a per-cell basis due to the decreased expression 322 323 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-1a, 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 vaccination, the appearance of senescent cells and the higher rates of fungal infection, may beattributable 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 (Mittrücker 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.

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

A decrease in regenerative capacity is one of aging hallmarks and contributes to reducing hematopoietic cells (Lopez-Otin et al., 2013). A good example is an age-related decline in hematopoiesis, causing a diminished production of adaptive immune cells (Lopez-Otin et al., 2013). An increase with age in the frequency of myeloid-biased differentiation at the expense of lymphoid specificity and function is demonstrated in humans (Pang et al., 2011). These changes influence the 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

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

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

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

alertness and favours a better immune response (Pawelec et al., 2012b). The global response to the
many various CMV antigens has been linked to better survival (Bajwa et al., 2017), suggesting that
the increased number of committed memory T cells may not be considered unequivocally detrimental
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 linked to reduced B cells interaction with CD40L⁺ T helper cells because, in older adults, the 503 504 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 proteostasis, such as proteasome activity (Mishto et al., 2006a; Mishto et al., 2006b), are reduced, 548 549 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)-1β, 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

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).

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

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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.

Aging is not uniform, neither across tissues nor among individuals. People at the same chronological age could possess different aging rates due to a unique complex interaction among intrinsic and extrinsic factors (genetic *vs* environment) determining the so-called "biological age" (Hamczyk et al., 2020). Several studies are currently investigating the biomarkers defining biological age (Cohen et al., 2020). However, what is becoming more evident is that the level of inflammaging (tightly 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 diseases641 (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 interventions are the most encouraged. Accordingly, calorie restriction, intermitting fasting, 653 654 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).

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661 4.1 Gut microbiota: at the crossroad among inflammaging, immunosenescence and longevity

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

GM alterations in composition and function occurring during aging and as a consequence of agerelated diseases (Lakshminarayanan et al., 2014), called dysbiosis, could impact on inflammaging due to the continuous stimulation of the IS, which causes immunosenescence (Santoro et al., 2020). Overall, this inflammatory environment contributes to the progression of various pathological conditions in older adults and makes the host more susceptible to dangerous bacteria (Bischoff, 2016). The synergism between GM and immune cells has a remarkable impact on the host's health and 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

somehow to the maintenance of health during the extreme phases of life. Indeed, the GM of the exceptional survivors presented a higher prevalence of *Bifidobacterium*, a long time a known probiotic group of bacteria, as well as higher abundances of subdominant members of the human gut ecosystem that have been explored only recently, such as *Akkermansia* and *Christensenellaceae* (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 glycerophospholipids and sphingolipids and increased excretion of urine specific 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",

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

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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 immune surveillance and leads to serious COVID-19. This reinforces the need to find treatments thatstimulate 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).

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

Finally, it is essential to emphasize the crucial role of inflammaging and immunosenescence in postcovid syndrome or long-covid, one of the major health burdens in the following years. Nearly a third of individuals discharged from hospital after acute COVID-19 need to be re-admitted to hospitals after few months (and more than 1 in 10 died after discharge); two-thirds had increased rates of multiorgan dysfunction and respiratory diseases, diabetes and cardiovascular diseases compared with 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

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

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

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

841 person will age (**Figure 3**).

Moreover, sex and gender strictly impact the IS of the elderly, likely contributing to the sex/gender disparity in terms of epidemiology, pathophysiology, symptoms, and severity of age-related diseases such as autoimmune diseases. There is an intricate interrelationship between immunosenescence and inflammaging able to generate a complex network of adaptive mechanisms that can favor longevity when able to counteract the injuries individuals are exposed lifelong (adaptation) or, on the opposite 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 Campisi, 2014) and likely contributing to the gender disparity in terms of epidemiology, 867 868 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

changes, with marked sex differences (Figure 2). Adult females have more robust innate and adaptive 876 877 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 cells from females produce more IL-10 than do males' T cells (Pietschmann et al., 2003) which may 885 886 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., 2016) and Treg development, thus protecting against autoimmunity. Moreover, the cellular 893 894 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

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

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915 **DECLARATION OF INTEREST**

916 The authors have no conflicts of interest

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1789 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	Progressive loss of SCFA producing bacteria (<i>Faecalibacterium</i> , <i>Roseburia</i> , <i>Coprococcus</i>)	• Augmented capability for glycolysis and SCFA production	Biagi et al., 2010; Wu et al., 2019; Rampelli et al., 2020
		• Decrease in circulating tryptophan concentrations	Collino et al., 2013
		 Specific signature of glycerophospholipids and sphingolipids 	Collino et al., 2013
		• Increased excretion of urine phenylacetylglutamine (PAG) and p-cresol sulfate (PCS)	Montoliu et al., 2014
		• Specific profile of Volatile Organic Compounds (VOCs)	Conte et al., 2020

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

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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

1833

Figure 4: Inflammaging and susceptibility to COVID 19. Aging is characterized by extreme heterogeneity due to the numerous and different exposures to lifelong factors determining each 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
- 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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