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Invited review

Ageing and gut microbes: Perspectives for health maintenance and longevity

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ABSTRACT

The ageing process affects the human gut microbiota phylogenetic composition and its interaction with the immune system. Age-related gut microbiota modifications are associated with immunosenescence and inflamm-ageing in a sort of self-sustaining loop, which allows the placement of gut microbiota unbalances among both the causes and the effects of the inflamm-ageing process. Even if, up to now, the link between gut microbiota and the ageing process is only partially understood, the gut ecosystem shows the potential to become a promising target for strategies able to contribute to the health status of older people. In this context, the consumption of pro/prebiotics may be useful in both prevention and treatment of age-related pathophysiological conditions, such as recovery and promotion of immune functions, i.e. adjuvant effect for influenza vaccine, and prevention and/or alleviation of common "winter diseases", as well as constipation and *Clostridium difficile*-associated diarrhoea. Moreover, being involved in different mechanisms which concur in counteracting inflammation, such as down-regulation of inflammation-associated genes and improvement of colonic mucosa conditions, probiotics have the potentiality to be involved in the promotion of longevity.

Contents

1. Introduction

The health status of elderly people is going to rapidly become an imperative concern, because of the extraordinary rate at which the global population is ageing, as well as the unprecedented longevity that is reached in some parts of the world. For the European member states life expectancy has been calculated to increase from 76.7 years in 2010 to 84.6 in 2060 for men, and from 82.5 in 2010 to 89.1 in 2060 for women [1]. Increasing life span is a direct consequence of the improvement of social–economic–environmental conditions, and implicates a wide-range of drawbacks, such as the increase of global incidence of age-related diseases and disabilities, with a dramatic impact on health care costs [2,3]. The global increase of life expectancy, which regards also other demographic giants, such as China and India, is one of the three major challenges of our times, along with climate change and the energy crisis. For this reason, strategies aimed at preventing or delaying age-related diseases, and maintaining a reasonably good health as long as possible, will be among the major goals for the next decades, from both scientific and social points of view. Among these strategies,

Abbreviations: CDAD, *Clostridium difficile*-associated diarrhoea; CRC, colorectal cancer; DC, dendritic cells; GALT, gut-associated lymphoid tissue; GI, gastrointestinal; IgA, immunoglobulin A; IL, interleukin; LPS, lipopolysaccharide; MAMP, microbial-associated molecular pattern; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NK, natural killer; NLR, NOD-like receptor; PA, polyamine; PRR, pattern recognition receptor; SCFA, short chain fatty acids; s.p.c., secreting plasma cells; TGF, transforming growth factor; TLR, Toll-like receptors; TNF, tumour necrosis factor; Treg, regulatory $CD4^+$ T cells; TSLP, thymic stromal lymphopoietin.

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sensitization to healthy nutrition and lifestyle certainly play a pivotal role, but also the consumption of functional food – defined as "a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects" in accordance to the EU Concerted Action on Functional Food Science in Europe (FUFOSE) [4] – and dietary supplements may represent an additional important approach.

In this review we aim at summarizing the current knowledge of the impact of ageing on the intestinal microbiota composition, with particular attention to some common pathological conditions in the elderly, which can be linked to the age-dependent deterioration of the microbiota–host mutualistic relationship. Following, the potential of probiotics and prebiotics to prevent and/or revert the age-related unbalances in the gut microbial ecosystem will be critically reviewed. The most recent and intriguing studies in animal model about the impact of probiotics on healthy ageing and longevity will be included in the review, in order to provide a wide picture of the potential applications and future perspectives of this field of research.

2. The ageing gut microbiota: overview

Microbes are our life-long companions: they live inside us, the vast majority of them within our gastrointestinal (GI) tract, in a complex and dynamic mutualistic relationship starting from our very first days of life. During delivery and immediately afterwards the infant gut is colonized by microbes of maternal, dietary and environmental origin. Since our early infancy, the interplay with these environmental microbes is pivotal for the development of the intestinal mucosa and the maturation of the human immune system [5]. At weaning, the GI ecosystem is stabilized towards an adult-type phylogenetic architecture [6,7].

The healthy, adult-like GI microbiota has been usually regarded as relatively stable throughout adulthood, until ageing and its related pathophysiological processes start to affect its homeostasis [8,9]. Whilst great interest has been paid to the development of the GI microbiota during infancy and childhood [10], due to its importance for the correct development of immune system and the progression of allergies [11–13], up to few years ago only a small number of studies were focused on the phylogenetic and functional changes that occur in the GI microbiota during ageing, as well as on the impact of these modifications on health and longevity. Only recently, advanced molecular characterization techniques have been applied to the study of the ageing GI microbiota, allowing us to improve our knowledge about the nature and importance of this process.

The current knowledge of the age-related changes in the gut microbiota phylogenetic composition has been reviewed by Biagi et al. [8,14] and Cheng et al. [15]. What emerges from the summarization of the available results is a general agreement in reporting a large inter-individual variability in older subjects [9,16,17]. Furthermore, reduced biodiversity and compromised stability of the intestinal microbiota with respect to younger individuals have often been reported in the elderly [8,20,28]. The comparison of studies performed in subjects having different nationalities highlighted a certain country specificity in how the ageing process impacts on the intestinal microbiota [14,20], possibly related to differences in lifestyle and dietary habits, even if it is not excluded that the use of different DNA extraction procedures and/or molecular characterization techniques may be partly responsible for this observation.

In particular, the effect of age on the dominant components of the gut microbiota, *Firmicutes* and *Bacteroidetes*, is controversial, and results widely vary according to nationality and age of the enrolled subjects [8]. For what concerns *Firmicutes*, members of the *Clostridium* cluster XIVa (a dominant group in the intestinal microbiota, which includes among others the species *Eubacterium rectale*, *Eubacterium hallii*, *Eubacterium ventriosum*, *Clostridium coccoides*, *Clostridium symbiosum*, *Ruminococcus gnavus*, *Ruminococcus obeum* and the genera *Dorea*, *Roseburia*, *Lachnospira*, *Butyrivibrio*) were found to decrease in Japanese, Finnish and Italian elderly and centenarians [9,20–22], whereas an inverse trend was found in German old adults [20]. The species *Faecalibacterium prausnitzii*, belonging to the *Clostridium* cluster IV (which also includes *Clostridium leptum*, *Ruminococcus bromii*, *Ruminococcus callidus*, *Anaerotruncus colihominis*), markedly decreased in Italian elderly and centenarians [9,20], but this result was not confirmed in other European populations [16,20]. However, it is well established that a decline in this important anti-inflammatory *Firmicutes* member of the gut microbiota is typical of frail, hospitalized, antibiotic- and anti-inflammatory-treated elderly [19,23–25]. Conversely, an age-related increase in *Bacteroidetes* was found in German, Austrian, Finnish and Irish elderly [16,19–21], but this was not confirmed in Italian elderly and centenarians [9,20]. Intriguingly, in the case of Irish elderly, *Bacteroidetes* were found to be the dominant phylum instead of *Firmicutes*, which has always been regarded as the most abundant in healthy adults [16].

Even if health-promoting bacteria, such as bifidobacteria, were commonly regarded as decreasing along with ageing [20,26], the most recent studies do not completely support this assumption [9,18,27]. An explanation for this may reside in the remarkable age-related temporal instability shown by *Actinobacteria*, i.e. the phylum that includes the *Bifidobacterium* genus [16,18], even if biases against the *Actinobacteria* phylum, which have been demonstrated to affect both DNA extraction and 16S rRNA amplification steps of 16S rRNA gene-based sequencing protocols [28–30], cannot be excluded.

Much less controversial is the commonly reported age-related increase in facultative anaerobes, including streptococci, staphylococci, enterococci, and enterobacteria [9,18,20,21,31], often classified as "pathobionts", i.e. bacteria present in the healthy gut microbiota in low concentration, which are able to thrive in inflamed conditions, sustaining and nurturing the inflammation itself [32].

Deviations from the healthy-like intestinal microbiota profile similar to those associated with the ageing process have been reported to accompany inflammatory disorders, such as inflammatory bowel diseases and obesity [33–35]. Indeed, the increase in pro-inflammatory pathobionts and the decrease in immune-modulatory species belonging to the *Clostridium* clusters IV and XIVa are hypothesized to be involved in the pro-inflammatory loop that promotes and sustains the inflammatory disorders, determining a disturbance in the host–bacteria equilibrium. In a similar way, the age-related changes in gut microbiota composition here summarized may concur to the complex process that both sustains and is nurtured by the overall inflammatory process typical of the advancing age.

3. Driving forces of the age-related modifications of the gut microbiota

Ageing is characterized by the onset of pathophysiological processes, i.e. the deterioration of the immune system functionality [36,37] and the reduced intestinal motility, which can dramatically compromise the homeostatic equilibrium between microbiota and host [38].

The symbiotic relationship we share with our intestinal microbial counterpart implies the necessity to keep this plethora of microorganisms under a constant surveillance, avoiding an excessive bacterial load on the intestinal mucosal surface. In a healthy

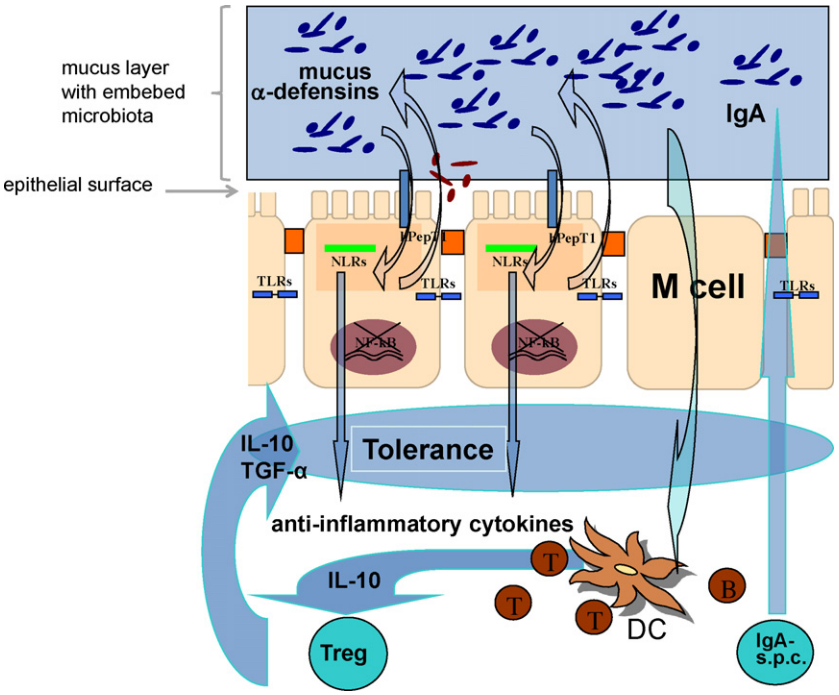

Fig. 1. Simplified model of the immunological mechanisms by which the host immune system keeps the resident microbiota under control in a healthy gastrointestinal ecosystem. Enterocytes actively sample bacterial molecules by apical channels, such as hPepT1 or OCTN2, which are also able to sample bacterial quorum-sensing molecules. Sensing of microorganisms is mediated by pattern recognition receptors (PRR) that specifically recognize microbial macromolecular ligands defined as microbial-associated molecular patterns (MAMP). PRR include two kinds of receptors, Nod-like receptors (NLR), which guard intracellular compartments, and trans-membrane Toll-like receptors (TLR), which scan the basolateral extracellular space. Integration of duration and spatial origin of PRR signals allows the intestinal epithelium to perceive the proximity of resident microorganisms, responding strategically to the bacterial load challenge according to the degree of threat to the epithelial surface. The activation of PRR initiates a downstream mitogen-activated protein kinase (MAPK)- and nuclear factor κB (NF-κB)-dependent response and the intestinal epithelium expresses several antimicrobial effectors, such as small antimicrobial peptides, α-defensins, reactive oxygen species as well as the intestinal mucus, that concur to maintain a bacterial "no man's land" immediately adjacent to the epithelial surface. The NLR response to commensal MAMP induces prevalently immune tolerance signals, such as transforming growth factor β (TGF-β), thymic stromal lymphopoietin (TSLP), and prostaglandin E. Equipped with an endocytotic machinery, M cells continuously transport microbial antigens from the gut lumen into the underlying mucosal lymphoid tissue, where mucosal dendritic cells (DC) present antigen to the native B cells and $CD4^+$ T cells. In the presence of tolerance signals, DC drive the differentiation of specialized regulatory $CD4^+$ T-cells (Treg). Anti-inflammatory Treg promote tolerance to commensal bacteria by the prevention of inflammatory response through the biosynthesis of anti-inflammatory cytokines, such as interleukin (IL)-10 and TGF-α. Simultaneously, the presentation of microbiota antigens to B cells under the control of TGF-β drives their differentiation to IgA secreting plasma cells (s.p.c.). Bacterial strain-specific secretory IgA are synthesized and bound to the layer of mucus coating epithelial surface. By preventing adherence of microorganisms and neutralizing toxins or enzymes, IgA exert an important role in maintaining homeostasis of the symbiotic relationship between host and commensal bacteria, helping to restrict these microrganisms in the intestinal lumen.

GI ecosystem the largest part of the resident microbiota is compartmentalized in the lumen, away from the epithelial surface, and constitutes a complex biofilm embedded in the soft upper portion of the mucus layer [39]. In order to minimize the contact of the bacterial community with the enterocytes, human beings have evolved a well-conceived immune apparatus, where innate and adaptive immune systems cooperate to keep microbiota under control by a "physiological low grade inflammatory status" (Fig. 1) [5]. As front line in the interaction with the intestinal microbiota, enterocytes are the first actors in the microbiota–host immunological cross-talk [40]. Besides the biosynthesis of mucin and antimicrobial compounds, which contribute to the maintenance of a bacterial "no man's land" immediately adjacent to the epithelial surface, enterocytes are specifically equipped to monitor proximity and density of resident microorganisms. Capable to sense microorganisms, enterocytes are strategic to finely tune the immune response of the gut-associated lymphoid tissue (GALT) depending on the perceived degree of threat [41]. In a healthy GI ecosystem, where intestinal microorganisms are compartmentalized in the outer mucus layer, enterocytes induce prevalently immune tolerance signals, maintaining the local dendritic cells in a quiescent status. Such cells present antigens to native $CD4^+$ T cells, driving the differentiation of specialized regulatory $CD4^+$ T cells (Treg), which promote tolerance through the biosynthesis of anti-inflammatory cytokines [5]. However, the simultaneous antigen presentation to B cells drives their differentiation to IgA secreting plasma cells. By preventing adherence of microorganisms and neutralizing toxins or enzymes, IgA are strategic in the restriction of microorganisms to the intestinal lumen.

During the advancement of age, the impairment of the GALT capacity to efficiently synthesize strain-specific secretory IgA, together with the reduced efficiency of the innate immune defences, such as α-defensins, antimicrobial peptides and mucus secretion, may result in the failure to control the resident microbiota, allowing an uncontrolled microbial growth on the enterocyte surface. In this context enterocytes could engage the activation of inflammatory cytokines and chemokines, forcing dendritic cells in the underlying GALT to drive the differentiation of effector T_H1, T_H2 and T_H17 cells that induce a strong pro-inflammatory response [42] (Fig. 2). Indeed, immunosenescence is also accompanied by a chronic, low grade overall inflammatory condition named "inflamm-ageing" [43,44]. Inflammation favours the bloom of pathobionts, a minor component of the healthy intestinal microbiota that in an inflamed GI ecosystem can overtake mutualistic symbionts and support inflammation [45]. For instance, the capacity of *Salmonella enterica* serotype *Thyphimurium* to utilize tetrathionate – a luminal sulphur compound generated during inflammation – as an electron acceptor leads to a growth advantage over the competing microbiota in the lumen of an inflamed GI tract [46]. Creating a self-sustained pro-inflammatory loop that impacts on the entire microbial ecology of the GI tract, pathobionts can thus consolidate a pro-inflammatory status in the gut,

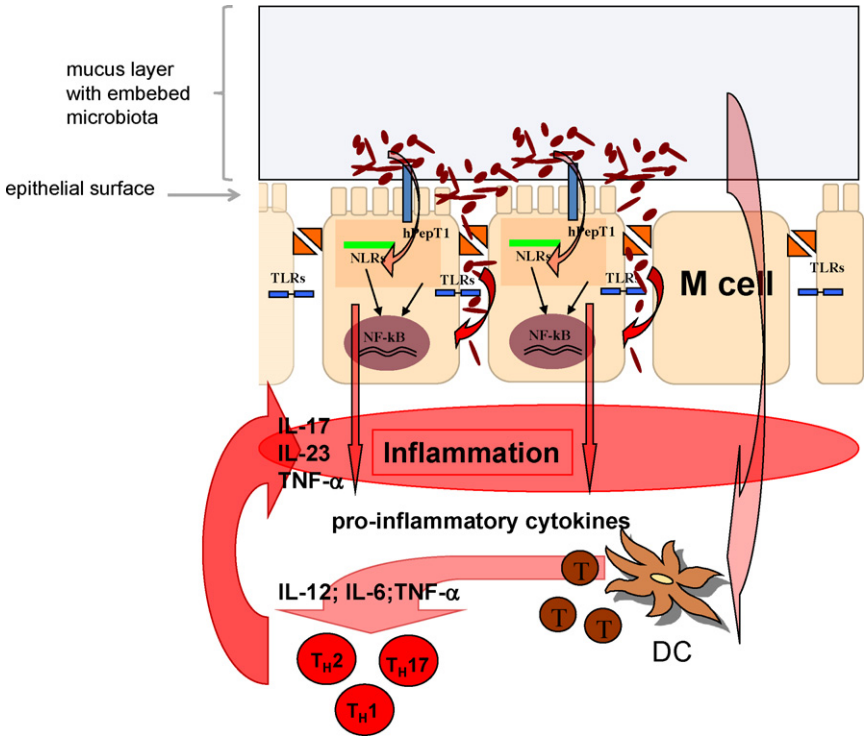

Fig. 2. Immunosenescence dramatically impacts on the homeostatic equilibrium between microbiota and host immune system. The reduced functionality of innate and adaptive immune defences can impair the "first line" effector systems strategic to limit the contact between intestinal microbes and the epithelial surface, such as antimicrobial peptides, α-defensins, reactive oxygen species, intestinal mucus and IgA. This condition may favour bacterial overgrowth on the epithelial surface, resulting in a strong activation of both enterocyte intracellular NLR and basolateral TLR. Engaging the NF-κB-dependent biosynthesis of antimicrobial effectors and inflammatory cytokines and chemokines, such as tumour necrosis factor α (TNF-α), interferon-γ (IFN-γ), IL-6, IL-8 and IL-23, enterocytes respond to the bacterial overgrowth forcing a high inflammatory response. In this pro-inflammatory context dendritic cells drive exclusively the differentiation of effector T_H1, T_H2 and T_H17 cells that perpetuate the strong inflammatory response towards invading microorganisms, consolidating inflammation in the gastrointestinal tract.

contributing to the systemic inflammation and nurturing the process of inflamm-ageing that is detrimental for host longevity.

Besides immunosenescence and inflamm-ageing, diet and lifestyle are other major driving forces of the age-related changes occurring in a non-pathological GI ecosystem. Short- and long-term dietary habits have been shown to have an impact on the gut microbiota composition [47], selecting the microbial groups able to harvest as much energy as possible from the available substrates. Changes in the individual threshold for taste and smell [48], tooth loss and chewing difficulties strongly influence the diet of aged people [49]. This can result in a decreased intake of food containing fibres and proteins, i.e. vegetables and meat, the consumption of which has been strongly correlated to changes in the phylogenetic and functional structure of the gut ecosystem [50,51]. In the recently published study by Claesson et al. [52] it has been shown that the microbiota of elderly people living in long-stay residential care facilities was clearly different from that of the free living elderly, within the same ethnogeographic region. This separation of the microbiota structure was correlated with the different dietary habits of the institutionalized elderly with respect to the community dwelling ones: the less diverse diet of long-term residents was linked to the less diverse faecal microbiota. Differences in dietary habits, as well as in other lifestyle components, may also explain part of the country-related differences regarding the impact of age on the gut microbiota composition.

The poor quality of the diet shown by the majority of the elderly, together with the diminished physical activity with respect to younger people, also causes a reduced intestinal motility, which may end up in constipation, altered bacterial fermentation and metabolite production, and reduced bacterial excretion [8,53], possibly resulting in an excessive "bacterial load challenge" difficult to handle for an immunosenescent host [54,55].

4. Health outcomes of the ageing gut microbiota

As recently hypothesized by Plottel and Blaser [56], from an evolutionary perspective, human symbionts can exert a "clock like function" in the human ageing process. By providing host with metabolic and defence functions, intestinal microbes can improve human fitness in the early life, but may also favour the removal of senescent individuals in the post-reproductive period in order to free resources for younger members of the population. Aside from the provocative nature of this sentence, it is a matter of the fact that changes in the phylogenetic architecture of the gut microbiota that accompany the ageing process also affect those metabolic and physiological functions for which the human metaorganism depends on its microbial counterpart, with several consequences at the functional level.

In particular, certain age-related modifications in the phylogenetic architecture of the gut microbiota can influence the risk of elderly people to develop several types of diseases, through different mechanisms (Table 1). For instance, a dramatically reduced bacterial diversity has been correlated with active *Clostridium difficile*-associated diarrhoea (CDAD), a major nosocomial complication for the elderly in hospitals and long-term facilities, with respect to both healthy people and asymptomatic carriers of the same pathogenic strains [17,57]. Indeed, toxin-producing *C. difficile* rybotypes have also been detected in asymptomatic aged subjects, thus it has been hypothesized that a "healthy-like" commensal microbiota may exert a protective role by preventing potentially pathogenic *C. difficile* from overcoming colonization resistance, proliferating in the colon and producing toxins [17]. This idea of a "predisposing" microbial community with a low biodiversity would explain why the faecal microbiota transplantation, which aims at restoring a protective healthy-like gut

Table 1
Health impact of age-related modifications in gut microbiota composition.

Age-related modifications in gut microbiota composition	Health outcome	Mechanism	References
Reduced biodiversity	Increased risk for CDAD	Reduction of colonization resistance to *C. difficile*	[17,57]
Proliferation of *Enterobacteriaceae*	Stimulation of inflammatory response	Excessive endotoxin production	[55]
	Increased probability to develop metastatic CRC		[66]
Decrease of butyrate-producing bacterial groups	Weakening of the colonic epithelium	Reduction of the protective and trophic function of butyrate on the colonic epithelium	[59–61]
	Stimulation of inflammatory response	Reduction of the anti-inflammatory effect of butyrate	[9,38,64]
	Increased risk for CRC	Reduction of the anti-neoplastic effect of butyrate	[73]
Colonization by toxin-producing *E. coli*, *H. pylori*, *B. fragilis*	Increased risk for CRC	Perturbation of enterocytes cell cycle regulation and growth control, and DNA damage by toxins	[72]

CDAD, *Clostridium difficile*-associated diarrhoea; CRC, colorectal cancer.

microbiota, is proving to be a promising intervention choice for CDAD [58].

The ability of several members of the gut microbial community to produce short chain fatty acids (SCFA, i.e. butyrate, acetate, propionate) [59,60] is an essential feature of a healthy gut ecosystem. Several health-promoting properties have been attributed to these key microbiota metabolites. Studies principally carried out in animal models showed that butyrate, has nutritive, anti-inflammatory, anti-neoplastic properties, and exerts a protective role for the intestinal epithelium, increasing its resistance [61–63]. A lower capacity to produce butyrate in the elderly gut microbiome has been hypothesized by Biagi et al. [9], on the basis of the phylogenetic differences between adults and centenarians, and recently confirmed by Hippe et al. [64], who demonstrated that the elderly had significantly fewer copies of the butyrilCoA:acetateCoA transferase gene compared to younger adults. This functional decline was correlated with decreased amounts of *F. prausnitzii*, *E. hallii*, and bacteria belonging to the *E. rectale*/*Roseburia* group, which are all butyrate-producers and, interestingly, were among the bacterial groups found in lower amounts in centenarians by Biagi et al. [9]. The decrease of anti-inflammatory SCFA-producing bacteria may nurture the inflamm-ageing process in the intestine of aged people. Moreover, the declined butyrate-producing capacity may contribute to the development of degenerative diseases [38] and anorexia [65]. Indeed, an augmented ability to produce SCFA as an additional source of energy for the host has been shown to concur to the obese phenotype, both in mice and in humans [66,67]. Differently, the less efficient SCFA production of the aged gut microbiota may contribute to the onset of malnutrition and sarcopenia in the elderly. However, metagenomic studies on the elderly microbiota are necessary, as it has been done for the obese phenotype, in order to investigate this hypothesis.

SCFA are also a fundamental component of the microbiota–host bio-network to maintain the GI epithelial integrity and, consequently, to ensure a functional epithelial barrier [68]. Butyrate strongly stimulates the release of mucins, the gel-forming protein component of the colonic mucus layer that contributes to the physical separation between microbiota and enterocytes [69,70]. Also, SCFA enhance transepithelial resistance both in vitro and in animal model, with the greatest effect mediated by acetate [71]. In this scenario, the age-related microbiota depletion in SCFA producers may concur in compromising the integrity of the epithelial barrier. Such a weakened gut epithelium allows the passage of whole bacterial cells and their products and disrupts immunological tolerance [68]. In particular, in the context of a weakened gut epithelium, the proliferation of *Enterobacteriaceae* and other Gram-negative bacteria has been proposed to cause an excessive endotoxin challenge for, ending up in an abnormal inflammatory response [55]. Confirming this hypothesis, the increase of *Enterobacteriaceae* in the elderly has been positively correlated with the serum level of two pro-inflammatory cytokines in very old Italian people [9], implying that this overgrowth can be somehow involved in the process of inflamm-ageing.

A pro-inflammatory dysbiosis, together with the decreased butyrate production in the intestine, has also been linked to an increased risk of colorectal cancer (CRC) [72,73]. In particular, metabolic profiling studies of human faecal water extracts demonstrated a profound decrease of SCFA content in CRC [74,75]. The incidence of CRC increases in aged people: about 50% of the western population develop colorectal polyps at the age of 70 and 5% of these polyps progress to cancer [76]. The connection between CRC and human ageing raised the question of how the age-related dysbiosis of the intestinal microbiota can be involved in CRC onset and progression. Colonic bacteria can affect the neoplastic process by induction of mucosal inflammation in the GI tract [72]. In turn, chronic inflammation can support carcinogenesis by inducing gene mutation, inhibiting apoptosis or stimulating angiogenesis and cell proliferation. In particular, NF-κB is emerging as a key factor to provide a mechanistic link between inflammation and CRC [77], and its activation by TLR ligands from intestinal microorganisms has been hypothesized to mediate the intestinal tumour growth under steady-state conditions [78]. Thus, consolidating inflammation in the GI tract, an aged-type intestinal microbiota may support CRC onset and progression.

Furthermore, the impairment of the barrier function of the ageing gut microbiota, together with the immunosenescence-dependent promotion of bacterial overgrowth on the epithelial cell surface, may enhance the risk to develop CRC, favouring persistent GI colonization by toxigenic bacterial strains. Indeed, certain bacterial toxins, such as CagA from *Helicobacter pylori*, toxin from toxigenic *Bacteroides fragilis* strains, and colibactin, cytotoxin necrotizing factor 1 and cytolethal distending toxin from toxigenic *Escherichia coli* strains, can perturb the eukaryotic cellular signaling linked to cell cycle regulation and growth control, or directly damage DNA. All these features are potentially pro-carcinogenic, and persistent GI infection by these toxigenic microorganisms is viewed as a paradigm for bacteria-induced cancer [72]. Besides toxins, also lipopolysaccharides (LPS) – the glycolipid outer membrane constituent of Gram-negative bacteria – have been associated with metastatic colorectal tumour growth [79]. A better understanding of these pro-carcinogenic mechanisms may represent a crucial point in planning preventive and therapeutic strategies for CRC, which may include the modulation of gut microbiota by probiotics, prebiotics and/or antibiotics, with the aim of favouring bacterial species able to exert anti-carcinogenic activity, or create an environment that somehow increases the chances of treatment success.

Also, gut microbiota is known to affect the metabolism of several pharmaceutical agents, with a significant impact on drug efficacy and toxicity [80,81]. The involvement of bacterial enzymatic activities in drug metabolism and assimilation is going to become an important field of interest along the way towards an efficient, personalized medicine, even more significant in the case of the elderly, who are more likely to use multiple drugs and for longer periods than young people. One example of drug–microbiota interaction of particular interest for older people is simvastatin, a commonly prescribed drug that reduces plasma levels of LDL-cholesterol, used to prevent dislipidaemia and coronary heart diseases, the prevalence of which increases with age [82]. It has been recently shown that the metabolism of this molecule can involve gut microbial processes [83]. Moreover, some bacterial-derived secondary bile acids have been associated to the individual variability in the statin-induced cholesterol-lowering effect in humans [84]. It could therefore be hypothesized that part of the variability in the therapeutic response to statin is linked to the variable metabolism of this drug, related in turn to the high inter-individual variability in the gut microbiota composition. In this perspective, the determination of the "structure–activity relationships" of the gut microbiota, and the impact of these relationships on the therapeutic response to drugs, may lead to the design of pharmacological and/or dietary interventions that can improve drug efficacy by altering the phylogenetic/functional architecture of the gut microbiota [84,85].

5. Probiotics and prebiotics during ageing: effectiveness and potentialities

The elderly are often considered "at increased risk" to develop several types of diseases or to get infections, such as influenza, with increased severity and mortality compared to younger people. This is particularly true in elderly care facilities, in which it is likely that infections, after being broken out, spread very easily among residents. For these reasons, prevention of infection is a crucial point in elderly care [86].

Pro/prebiotics are dietary supplements targeting the homeostasis of the intestinal microbial ecosystem, the composition and functionality of which has been associated with the health maintenance of the elderly [52] and can be hypothesized to impact on the host longevity. The use of pro/prebiotics as well as a combination of them (synbiotics) in elderly subjects has been recently reviewed in detail by Tiihonen et al. [87] and Toward et al. [88], from both a preventive and therapeutic point of view.

Pro/prebiotics and synbiotics have been shown to alter the composition of the gut microbiota in older people, especially by inducing an increase in the faecal amount of bifidobacteria and lactobacilli [27,89–91]. However, higher *Bifidobacterium* and/or *Lactobacillus* levels do not represent a health benefit themselves; at best they can be regarded as an indicator of good intestinal health [87], but other parameters need to be considered to determine if a pro/prebiotic treatment can be effective in ameliorating symptoms and/or preventing diseases. Interesting results have been published about the use of probiotics in the prevention and/or treatment of CDAD in the elderly [92], even if a recent meta-analysis pointed out that the only probiotic with reliable efficacy in CDAD is *Saccharomyces boulardii* [93]. Further, pro/prebiotics have been suggested to alleviate constipation in the elderly, especially in those living in elderly care facilities, by increasing defecation frequency, which is the most easily quantifiable health improvement [94–96].

A particularly relevant aspect for elderly care is represented by the immune-modulatory role of probiotics, with the aim to prevent and/or limit the effects of immunosenescence [97]. The ability to positively stimulate the immune system and consequently decrease the risk of infections has often been claimed for pro/prebiotics, but defining and quantifying reduction of disease risk is very hard, especially in apparently healthy people [98]. For several *Bifidobacterium* and *Lactobacillus* probiotic strains positive effects on the immune system of old people have been reported (see Table 2 for the most recent published studies), i.e. increase in NK cell activity, increase in phagocytosis, higher spermine and spermidine levels, which correlate with lower inflammation [8,99–102]. Similar results have been reported also in the case of prebiotic supplementations in the elderly [103,104]. However, only very few studies report observations of practical significance in elderly care, particularly about the adjuvant effect of probiotic treatments to improve vaccine efficacy (see Table 2 for the most recent published studies), such as increase in influenza-specific antibody titre [105,106] or reduced incidence of influenza and fever and higher NK cell and neutrophile activities [107]. *Lactobacillus* species have also been reported to have an intrinsic protective effect against infection, by decreasing not the incidence but the duration of respiratory and GI "winter infections" [108], as well as the duration of fever after viral gastroenteritis [86].

The literature briefly summarized above is quite sparse, and some of the studies had serious limitations in their design, enrolled too few subjects and/or did not completely define the treatment and control groups, making the results hardly comparable [8]. Studies are generally too limited, and show too much variability in terms of age, health status, and lifestyle habits of the enrolled subjects, to allow a realistic conclusion about the potentiality of pro/prebiotics to prevent age-related disorders and counteract immunosenescence. What is emerging to date is that the magnitude of responses and the measurable physiological benefits to pro/prebiotic consumers may vary significantly among individuals [109].

An important comment concerning the use of probiotics on the way towards the personalized medicine has been recently made by Dominguez-Bello et al. [10], who advanced the hypothesis that "the healthy old, rather than the healthy young, are the best donors of probiotic species for old individuals". Indeed, there are several studies in mouse model that report interesting properties of bifidobacteria isolated from the intestine of healthy centenarians, which would support their specific utilization in immunosenescence prevention, such as induction of lymphocyte proliferation, enhancement of NK cell activity and macrophage phagocytosis, enhancement of duodenal villus functionality, inhibition of invasion by pathogens and enhancement of activity of antioxidative enzymes [110–112]. In this scenario, it would be advisable to deepen the studies on the effect of probiotics isolated from healthy elderly in humans, and especially in the elderly showing symptoms of immunosenescence, in order to understand if, and how, it is possible to ameliorate their health status by using "the right species at the right time" [10].

6. Pro/prebiotics and longevity: insights from animal models

Due to the remarkable influence of the gut microbiota in the process of immunosenescence and inflamm-ageing, as well as in preventing the colonization by pathogens and their proliferation and toxin production, it can be hypothesized that gut microbiota homeostasis is able to affect fitness maintenance and, ultimately, the host lifespan [113]. In this perspective, it might be theoretically possible to promote longevity by using microbiota-targeted strategies, such as pro/prebiotics, for long periods and starting earlier in life. However, this is very hard to investigate because longitudinal studies covering the whole lifespan are obviously not possible in humans or, at least, results are not going to be available very soon. To date, insights on the involvement of gut bacteria in longevity derive only from studies in mouse models.

Table 2
Effect of probiotics on gut microbiota, immune system and health of elderly people: 2010–2012 published results.

Subjects (no.)	Age (y)	Probiotic strains	Study design	Period	Effects on gut microbiota	Effects on immune system	Health outcome	References
1072, free living	>70	*L. casei* DN-114001	Multicentric, double blind, placebo controlled	3 m + 1 m follow up	Increase in *L. casei*	ND	Reduction of average duration of common respiratory and gastrointestinal infections	[108]
31, nursing home residents	72–103	*L. acidophilus* NCFM + *L. rhamnosus* HN001	Placebo controlled, cross-over	2 w run in + 4 w probiotic + 4 w placebo	ND	Increase in NK cells cytotoxicity	ND	[100]
77, nursing home residents, frail	84 ± 9	*L. casei* Shirota	open study, placebo controlled	1 m	Increase in *Lactobacillus* and *Bifidobacterium*, decrease in *Enterobacteriaceae*	ND	Decrease in the average duration of norovirus gastroenteritis	[86]
60, nursing home residents	65–85	*L. plantarum* CECT7315/7316	Randomized, double blind, placebo controlled	3 m, influenza vaccine 3 m prior to the trial	ND	Increase in influenza-specific IgA, IgG, IgM	ND	[106]
737, nursing home residents	>65	*L. casei* Shirota	Randomized, double blind, placebo controlled	176 d, influenza vaccine after 21 d	ND	ND	No effect on response to influenza vaccine	[123]
47, free living	65–90	*L. delbrueckii* subsp. *bulgaricus*	Multicentric, double blind, placebo controlled	6 m	ND	Increase in % of NK cells, "immature" T cells, and antimicrobial peptide hBD2; decrease IL8	ND	[102]
27, nursing home resident	>65	*B. longum* BB536	Randomized, double blind, placebo controlled	5 w pre-administration, influenza vaccine after 3 w + 14 w trial	ND	Increase in NK cells activity and neutrophil bactericidal activity	Lower incidence of influenza and fever	[107]

ND, not determined; y, years; m, months; w, weeks; d, days; *L.*, *Lactobacillus*; *B.*, *Bifidobacterium*.

In particular, Matsumoto et al. [114] recently published a study in which 10-month-old mice were supplemented with *Bifidobacterium lactis* for 11 months. After treatment, the animals showed increased longevity compared to the control group. This increased survival seemed to be caused by lower levels of inflammation, especially in the gut. Indeed, the colonic mucosa was in better condition in the *B. lactis*-supplemented group, with increased mucus secretion and better maintenance of tight junctions, and ageing- and inflammation-associated genes were down-regulated, suggesting that *B. lactis* supplementation could be able to counteract inflamm-ageing. Interestingly, the gut microbiota composition of the treated mice resembled to the one found in younger mice, and high faecal concentrations of polyamines (PA) were determined. PA, i.e. putrescine, spermidine and spermine, are compounds with anti-inflammatory and anti-mutagenic properties, required for cell growth and differentiation, and helpful in the maintenance of the mucosal barrier functions. It is known that luminal PA amounts decrease in the elderly, and this has been suggested to be related to senescence [115]. Conversely, the levels of luminal PA increase in pro/prebiotics consumers [116–118]. These findings together lead to the hypothesis that increasing the luminal PA content by means of pro/prebiotics may represent a promising step on the way towards the elaboration of multi-component and personalized strategies to promote a healthier and longer life [119].

7. Conclusions

The ageing process is characterized by a high degree of heterogeneity related to a great number of genetic, epigenetic and stochastic factors. The body itself does not age homogeneously as the different tissues and organs age at different rates, with different characteristics [120,121]. In this scenario it is natural to wonder how and how fast the gut microbiota, frequently referred to as an "additional organ" in terms of metabolic potential [122], will experience the ageing process.

Changes in the gut microbiota composition may be rated as one of numerous age-related physiological processes that, all together, determine "how" a human being will age. Whether and how these changes in gut microbiota phylogenetic and functional architecture are linked to the wellbeing of ageing people, and longevity, is still partly an open question. For instance, for what concerns the intestinal health, one could easily be brought to think that the more the gut microbial community of the elderly resembles that of younger adults, the higher is the probability for that elderly to be relatively healthy. But this may not be completely true: health could also be linked to the ability of the ageing human host to establish a new equilibrium with the aged-type microbial community.

What is certain is that the ageing gut microbiota does show several features that can actively affect not only the health status of old people, i.e. contributing to the onset of pathological conditions known to affect the elderly with a higher incidence than the young adults, such as *C. difficile*-associated diarrhoea and colon cancer, but also their eventual responsiveness to therapies. Targeting the ageing human being as a metaorganism, composed by both human and bacterial cells in a complex and dynamic interplay, could be the right approach for prevention and treatment of diseases, as well as for promotion of healthy ageing and longevity. Strategies may include the manipulation of the gut microbiota composition

through the use of pro/prebiotics. Indeed, despite the limits of the available studies, pro/prebiotics are emerging as a promising approach along the way towards the development of personalized medicine.

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