Intestinal barrier functions in ageing

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Abstract

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Keywords	intestinal epithelium, aging, intestinal barrier, intestinal permeability, inflammation, gastrointestinal tract

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Intestinal epithelial barrier functions in ageing

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Abstract

The intestinal epithelial barrier protects the mucosa of the gastrointestinal (GI)-tract and plays a key role in maintaining the host homeostasis. It encompasses several elements that include the intestinal epithelium and biochemical and immunological products, such as the mucus layer, antimicrobial peptides (AMPs) and secretory immunologlobulin A (sIgA). These components are interlinked with the large microbial community inhabiting the gut to form a highly sophisticated biological system that plays an important role on many aspects of human health both locally and systemically. Like any other organ and tissue, the intestinal epithelial barrier is affected by the ageing process. New insights have surfaced showing that critical functions, including intestinal stem cell regeneration and regulation of the intestinal crypt homeostasis, barrier integrity, production of regulatory cytokines, and epithelial innate immunity to pathogenic antigens change across life. Here we review the age-associated changes of the various components of the intestinal epithelial barrier and we highlight the necessity to elucidate further the mechanisms underlying these changes. Expanding our knowledge in this area is a goal of high medical relevance and it will help to define intervention strategies to ameliorate the quality of life of the ever-expanding elderly population.

1. Introduction

Mucosal surfaces cover a vast area of our body, among these the mucosa lining the GI-tract represents the primary and largest area of contact (~300m^2) with environmental factors and antigens. The intestinal epithelial barrier must tackle the daunting task to prevent the penetration of macromolecules and potentially pathogenic microorganisms, while ensuring nutrients absorption and the monitoring of the luminal contents *via* a variety of mechanisms for both microbe recognition and antigen-sampling (Turner, 2009; France and Turner, 2017). These complex and at times seemingly opposing functions require the presence of highly specialized cells and structures strategically located along the GI-tract acting in synergy to maintain barrier integrity. The intestinal epithelial barrier is formed by several highly integrated physical (the epithelium), biochemical (mucus, anti-microbial peptides, AMP) and immunological (sIgA) elements. In the past years, a large body of evidence has emerged showing that the integrity of the intestinal epithelial barrier is critical for health and disease (Choi et al. 2017). Defects in barrier function may lead to chronic immune activation contributing to local and systemic diseases including coeliac disease, colorectal cancer, inflammatory bowel disease (IBD) and metabolic disorders such as obesity and diabetes (Fasano et al. 2000; He et al. 2017; Suenaert et al. 2002; Grivennikov et al. 2012; Araujo et al. 2017). Importantly, the loss of barrier integrity appeared to have detrimental consequences far beyond the gut. A malfunctioning intestinal epithelial barrier is thought to contribute to the pathogenesis of disturbances of the central nervous system (CNS) ranging from Parkinson's (Schwiertz et al. 2018; Clairembault et al. 2015), Alzheimer's (Köhler et al. 2016), Multiple sclerosis (Canara-Lemarroy et al. 2018) and depression (Maes et al. 2013; Stevens et al. 2018). Elegant experiments in *Drosophila* (Rera et al. 2012; Gervais and Bardin 2017) have shown that alterations of barrier integrity in the gut are strictly associated with age-associated metabolic and inflammatory pattern and they represent a reliable marker of "impending" death.

In spite of its central role in maintaining health, our knowledge of the basic events underlying the physiological modification of the epithelial barrier in late life is still rather scarce. Recently,

experiments involving the use of *in vitro* developed intestinal organoids, *ex-vivo* human intestinal tissues and laboratory animals brought to the surface new information that shed some light on the ageing process in the gut. However, much still remains to be done in this area. The identification of the age-associated modifications of the various components of the intestinal epithelial barrier, and the underlying mechanisms, is a much needed step required to design effective intervention strategies to address an array of late life-related disturbances that have a significant impact on health and well-being.

2. The intestinal crypt: where it all begins

2.1-Introduction to the intestinal stem cell niche

The intestinal epithelium encounters daily a vast amount of material ranging from semi-digested food to microbes. The latter could be either part of the large microbial community, the microbiota, physiologically inhabiting the gut or pathogens that can reach the intestine *via* contaminated food or other sources. Thus, the intestinal epithelium faces a hostile environment that leads to constant loss and damage of the epithelial cells. Throughout the intestinal tract, the continuous renewal of the epithelium relies on the presence of intestinal epithelial stem cells (IESCs) located in a specialized region of the intestinal crypt (Seishima and Barker, 2019). The organization of the intestinal epithelium varies according to the different geographical areas of the gut that are characterized by distinct functional requirements. Therefore, the architecture of the intestinal crypt that houses a dedicated population of stem and progenitor cells that self-renew to maintain epithelial function throughout life differs between the small and large intestine. In the small intestine, each intestinal villus is encircled by at least six crypts of Lieberkühn (Fig. 1A), while in the colon the mucosal layer is arranged into crypts individually connected to the flat luminal surface *via* a circular opening (Fig. 1B). During the past few years, the intestinal niche environment and some of the critical factors underlying IESC proliferation and crypt homeostasis in the small

intestine have been identified and key regulatory events dissected in detail (Greicius and Virshup, 2019; Tan and Barker, 2014). The base of the crypt harbours the IESCs that are located at the +4 position starting from the bottom of the crypt and above the Paneth cells or as crypt base columnar cells (CBC) interspersed between the Paneth cells. Stem cells give origin to rapidly dividing transit-amplifying (TA) cells that differentiate into enterocytes (absorptive lineage) or enteroendocrine cells, goblet cells, tuft cells and Paneth cells (all belonging to secretory lineages). The stem cell niche environment also comprises additional cell types that include fibroblasts, myofibroblasts, smooth muscle cells, neural cells, endothelial cells, lymphocytes and monocytes (Fig.1). These cells provide secreted factors, such as the wingless-related integration site 3 (Wnt3), epidermal growth factor (EGF) and bone morphogenetic protein (BMP) signaling factors, such as Noggin, Gremlin and chordin that control the proliferation of intestinal epithelial cells.

2.2- Effect of ageing on the IESC.

The use of *Drosophila* midgut, that closely mimics its mammalian counterpart (Gervais and Bardin 2017) and the identification of the IESC-specific marker leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) in mice (Barker et al. 2007) have helped to shed some light on how ageing modifies the intestinal stem cell. Experiments in the *Drosophila* midgut section (Guo et al. 2016) showed that the number of IESCs, identified by the expression of the transcription factor escargot (Esg) and the Notch ligand delta (D1) (esg^+/Dl^+ cells) (Ohlstein and Spradling, 2006) increased in ageing. However, this event was paralleled by a decline in function (Biteau et al, 2008; Choi et al 2008) and it has been argued that the increase in IESC numbers was only apparent and due to an accumulation of mis-differentiated IESC cells retaining specific markers. It is possible that environmental factors, such as bacterial infections played a significant role in the increase of IESC in ageing flies. This hypothesis is supported by the activation of environment-mediated stress responses signalling pathways including the c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK)38 (MAPK38) and

platelet derived growth factor/vascular endothelial growth factor (PDGF/VEGF) (Buchon et al 2009, Biteau et al. 2008, Choi et al. 2008, Hochmuth et al. 2011; Park et al. 2009). In particular, the elevated JNK activity in IESC during ageing might play a role in the loss of intestinal homeostasis. Indeed, a finely tuned balance between stress signalling and processes that regulate IESC cell proliferation and differentiation, including the interaction between JNK and Notch signalling is required to avoid accumulation of mis-differentiated cells. Additional studies in *Drosophila* also revealed that increased proliferation level of IESC was also linked to accumulation of damaged proteins and DNA (Park et al. 2012; Na et al. 2013; Park et al. 2014). These latter events are likely to be brought about by age-associated defects in DNA damage repair response (DDR) a key pathway for stem cell protection (Kenyon and Gerson, 2007; Behrens et al. 2014; Park, 2015). Recently, the generation of genetically engineered flies lacking specific DDR-associated genes, such as *Mre11, Rad50, Nbs, ATM, ATR, Chk1/2* informed on the role of defective DDR on IESC. Lack of DDR-associated genes led to early ageing as shown by IESC hyper-proliferation, increased centrosome amplification and overall affecting fly's survival (Park et al. 2018). Interesting, sex differences have been reported in IESC proliferation and function in ageing (Regan et al. 2016). The gut of ageing female flies appeared heavily damaged by excessive IESC proliferation, with disruption of the intestinal epithelium occurring as early as 2-3 weeks of age. The progressive morphological abnormalities of the intestinal epithelial cells (IECs) eventually led to formation of tumours. In contrast, ageing males did show reduced levels of IESC proliferation that ensured absence or a delay in the onset of gut abnormalities. However, the low IESC proliferation rate made the males more vulnerable than females to intestinal infections. Thus, in spite of being detrimental to barrier integrity in ageing, the high proliferative capacity of IESC protected the female flies from environmental stress in late life.

In mammalian, a large panel of experiments concurred that, similarly to what observed in *Drosophila* the ageing process altered the function and regenerative capacity of IESC. The identification of the Lgr5 as IESC-specific marker (Barker et al. 2007) and the subsequent

development of *in vitro* grown intestinal organoids (Sato et al. 2009; Sato et al. 2013) made it possible to untangle regulatory events taking place at the level of the intestinal epithelial niche. In contrast to what previously suggested (Heller 1990; Xiao et al. 2001; Potten et al. 2001), the mitotic index declined significantly in ageing (Nalappareddy et al. 2017). This was also confirmed by the observation that IESC from mice yielded fewer and less complex intestinal organoids compared to their young counterparts (Moorefiled et al. 2017). In addition, consistent with reduced mitotic index, specific cell cycle regulators (i.e. CDNK1C) were reduced in aged mice (Nalappareddy et al. 2017). Parallel experiments conducted in aged Sox9-EGFP reporter mice also showed increased levels of apoptosis in the crypt as shown by increased expression of apoptosis-promoting genes such as *p53, Sirt7, Max, Bak1* and *Bax* in active IESC (Moorefiled et al. 2017). Overall, these age-associated alterations of the IESC proliferative and survival capacity have important consequences for intestinal crypt homeostasis, epithelium formation and the overall architecture of the gut. Ageing is indeed associated with modifications of the intestinal architecture, including increase in villus height (Martin et al. 1998; Holt et al. 1984; Corazza, 1998). *In vivo* experiments in double transgenic TRE-*Omomyc;actin-rt*TA mice that exhibit decreased cell proliferation in several tissues, including the intestine, coupled with mathematical modelling approach have shown that cell proliferation within the crypt is the principal driving force for IECs migration along the villi (Parker et al. 2017). Indeed, the blocking of IESC proliferation in the crypt *via* intra-peritoneal administration of the cytosine arabinoside (Ara-C) inhibited the progress of IECs along the crypt-villus axis. The reduced mitotic rate coupled with increased level of apoptosis of active IESC in ageing (Nalappareddy et al. 2017) potentially contributed to the different architecture of the ageing gut by reducing the speed at which epithelial cells migrated along the crypt/villus axis.

Most important is the notion that the ability to repair an experimentally induced damage to the intestinal crypt also diminished in ageing mice (Choi et al. 2018). This was due to the reduced intra-crypt motion of IESC. The latter event sets in rapidly in the young intestine after the

application of the insult and it allows maintaining the optimal cell organization and spatial distribution of IESC within the crypt. Ageing did not affect the number of Lrg5^{+} crypt as well as the total number of Lrg5^{+} cells (Choi et al. 2018); however, an age-associated decline of the Wnt signalling has been reported (Nalappareddy et al. 2017). Experiments in *Lgr5-eGFP-IRES-CreER*^{T2} and *Rosa26*^{YFP} confetti mice and intestinal organoids showed a decline in the expression of Wnt in IESC, Paneth cells and the niche-associated mesenchymal cells. Although the mechanisms underlying the down regulation of Wnt signalling in ageing remain to be determined, the importance of the loss of Wnt signals (i.e. Wnt3) was confirmed by the observation that adding exogenous Wnt3a to organoid cultures restored a young-like regenerative potential.

Furthermore, similarly to all tissue-specific stem cells, the regenerative capacity of the ageing IESC is reduced by telomerase attrition. Reduction of telomere length occurs at cell division due to the inability to copy the very ends of chromosomes and it represents one of the key drivers of ageing (Hao et al. 2005). Ultimately, age-related loss of telomeric DNA triggers chronic DDR leading to cell death, apoptosis and an accelerated ageing process (Rudolph et al. 1999; Newgard et al. 2013; Steenstrup et al. 2017). Recently, the possibility to generate adult tissues with telomeres longer than normal in the absence of genetic modifications gave rise to the idea of using a similar approach to rejuvenate tissue in regenerative medicine. The presence of a longer telomere delayed the onset of the detrimental effects of age-associated telomere attrition and maintained a young-like regenerative capacity of IESC for an extended period of life (Varela et al. 2016). Telomere integrity is also under the control of the epigenetics dynamics of the telomeric/subtelomeric area. Experiments in telomerase-deficient mice (*G3Terc*$^{-/-}$) showed that the deletion of the growth arrest and DNA damage-inducible protein 45 alpha (Gadd45a), critical for epigenetic gene activation *via* repair-mediated DNA demethylation, markedly reduced the DDR and improved the function and regenerative capability of IESCs and in so doing extended the life span of *G3Terc*$^{-/-}$ mice (Diao et al. 2018).

The accumulation of somatic mutations across life also has a negative impact on IESC function. A genome-wide mutation pattern analysis in individuals whose age ranged between 3 and 87 years determined that IESC, both in the colon and small intestine accumulated somatic mutation at a much higher rate compared to a less mitotically active tissue such as the liver (Blokzijl et al. 2016). The same study also reported a low inter-individual variation in mutation in individuals with very different life styles. The latter observation is important and it suggested the incidental effect of exposure to environmental factors has a minimal effect on the mutations in IESC throughout life.

Finally, the constant host-microbe interaction also plays a role in IESC proliferation. A combination of *in vivo* and *ex-vivo* studies in mice showed that the intestinal microbes promoted monocyte-stem cell interactions; in turn, monocytes *via* both cytokine secretion and cell-to-cell interaction promoted crypt cell proliferation to help maintain gut homeostasis (Jeffery et al. 2017; Skoczek et al. 2014). Thus, it is plausible to hypothesize that changes of the gut microbiome occurring in ageing might alter the pattern of microbe-derived signals and ultimately contribute to the altered IESC proliferative capacity.

3. Cellular, biochemical and immunological intestinal barriers.

3.1 The intestinal epithelium

The epithelial cells emerging from the crypt are organized in a single layer that forms a highly selective barrier whose one of the main tasks is to prevent access to macromolecules and microbes while allowing a continuous influx of water, ions and nutrients. The integrity of the barrier is afforded, in first place by the rather impermeable plasma membrane of the IECs and by inter-cellular junctional complexes that seal the paracellular space (Buckley and Turner, 2018). At the apical domain of the IEC, at the base of the microvilli the plasma membranes of adjacent cells are intimately connected (Fig. 2). The tight junctions (TJs) are the most apical of the junctional complexes. These are formed by a large array of interlinked proteins that include transmembrane and membrane proteins and signalling molecules. TJs display a very

complex composition that include more than 40 different proteins (Furuse, 2010). This large variety ensures that TJs, in addition to creating a formidable barrier contribute to establish cell polarity (Cereijido et al. 1998) and participate in signalling, transcriptional regulation, cell cycle (Hernandez et al. 2007; Matter and Balda, 2003, Zihini et al. 2014, Van Itallie and Anderson, 2006; Tsukita et al. 2008) and vesicles trafficking (Yeaman et al. 2004). The principal TJs proteins are claudins, zonula occludens 1 and 2 (ZO1/2), occludin and F□actin. Just below the TJs complexes the proteins E□cadherin, α□catenin 1, β□catenin, catenin δ1 form the adherens junction that plays a significant role in the assembly and function of TJs. TJs and adherens junction are supported by a perijunctional ring of actin and myosin. Beneath the adherens junction, an additional structure, the desmosome participates in the sealing of the paracellular space by strengthening the adhesion bonds between adjacent IECs. Similarly to TJs and adherens junctions, desmosomes encompass numerous interacting proteins that include desmoglein, desmocollin, desmoplakin and keratin filaments (Tariq et al. 2015, Spindler et al. 2015, Schmidt et al. 1994, Garrod et al. 2008, Ungewiß et al. 2017).

In the past few years, information on how the integrity of the intestinal barrier changes in ageing humans and laboratory animals, has emerged. Studies in rodents and non-human primates reported that intestinal permeability increased with age and in some case, alteration of the expression of TJ components was observed. Pioneering studies carried out in the 80s reported reduced levels of excretion of the tracer polyethylene glycol (PEG) 400 in the urine of aged rats compared to their young counterparts, thus suggesting an increased permeability to large macromolecules (Hollander et al. 1985; Hollander and Tarnawski, 1985). The same authors also observed that the increasing intestinal permeability is a continuous process that progresses as the rat ages (Ma et al. 1992). More recent studies have used colonic biopsies from non-human primates (baboons) to tackle the issue of permeability in ageing (Tran and Greenwood-Van Meerveld, 2013). Aged baboons showed reduced expression of critical TJ proteins such as ZO-1, occludin and junctional adhesion molecule-A (JAMA-1) and increased permeability to macromolecules, such as horseradish peroxidase (HRP, approx. 44 kDa). In

the terminal ileum of the human small intestine, the TJs expression and intestinal permeability displayed a rather different pattern (Man et al. 2015). The expression of zonula occludens-1 (ZO-1), occludin and JAMA-1 did not vary in healthy elderly (67-77 years old) and overall permeability to macromolecules was not affected. However, both humans and baboons expressed higher levels of claudin-2. The level of claudin-2 showed a trend towards an increase in colonic biopsies of baboons (Tran and Greenwood-Van Meerveld, 2013) whereas the increase was significantly more pronounced in biopsies from the terminal ileum of ageing humans (Man et al. 2015). In the human ileum, the up-regulation of claudin-2 led to a significant decline of transepithelial electric resistance (TEER); a measure of the ionic gradient across the intestinal barrier. An additional study in a small number of female vervet monkeys suggested that healthy ageing is not associated to a dramatic structural alteration of the colon; however, it is accompanied by functional deficit of the intestinal barrier assessed by evaluating markers of microbial translocation (Wilson et al. 2018). In the latter report, however, the qualitative and quantitative analyses of the various TJ components were not carried out. Furthermore, the levels of serum zonulin, an indirect marker of intestinal permeability were higher in healthy elderly (≥70 years-old) compared to healthy young individuals (18–30 years-old) (Qi et al, 2017). Of interest, higher levels of serum zonulin were negatively correlated with skeletal muscle strength and habitual physical activity, two indices of physical frailty.

Furthermore, seminal new information on the biological relevance of the integrity of the intestinal epithelium in ageing has emerged from studies in *Drosophila*. The cellular junctions found in flies include spot adherens junctions (SAJs), the zonula adherens (ZA), pleated and smooth septate junctions (SJs), gap junctions, and hemiadherens junctions (HAJs) (Tepass et al. 2001; Izumi and Furuse, 2014). Similarities between *Drosophila* and vertebrate TJs have been observed (Willot et al. 1993; Furuse and Tsukita, 2006). However, up to date studies monitoring the effect of ageing on individual components of the TJ complexes in *Drosophila* are lacking. Nonetheless, elegant experiments that utilized a non-invasive assay to determine intestinal integrity demonstrated that systemic metabolic alterations, increased levels of

inflammation and impending death could be predicted in individual flies based solely on the loss of barrier integrity (Rera et al. 2012).

3.2-The mucus layer

The mucosal surface of the GI-tract is covered by a layer of mucus that plays a critical role in protecting the intestinal epithelium (Johansson and Hansson, 2011). Mucus is secreted by goblet cells whose number increases along the proximal-distal axis of the gut peaking in the colon (MacDermott et al. 1974; Johansson 2012). Mucus contains several major components, including mucins (Pelaseyed et al. 2014) that are characterized by mucin domains heavily O-glycosylated. The structures of the O-glycans present in mucin are diverse and complex, consisting predominantly of core 1-4 mucin-type O-glycans containing α- and β- linked N-acetyl-galactosamine, galactose and N-acetyl-glucosamine (Bennet, 2012; Bergstrom and Xia 2013; Brockhausen, 2009; Tailford et al. 2015). Within the mucus layer, two well-separated areas could be identified. First, a firm inner layer, mostly bacteria-free is in intimate contact with the intestinal epithelium. Second, an outer less firm layer provides a lubricated surface for the progression of the luminal contents and a nutritional substrate for certain species of commensal microbes (Johansson et al. 2011; Johansson et al. 2008). The critical role of the mucus layer has been long neglected; ultimately though, a clear-cut evidence of its importance in the protection of the host is provided by the observation that mucus-deficient mice (*Muc2*$^{-/-}$) spontaneously developed severe colitis around 4 weeks of age (Van der Sluis et al. 2006).

Very little is known on the structure and function of mucus in ageing but in the past few years some information has surfaced. It would appear that the effects of the ageing process on the production of mucus varied according to the geographical location in the gut. The thickness of the gastric and duodenal mucus layer did not change with age in normal, healthy individuals (Newton et al. 2000). This suggested that the mechanical protection afforded by the mucus layer, at least in these two locations, is not affected by ageing. In addition, the ileum of aged mice showed a slight increase in the number of goblet cells/villus that displayed larger mucin granules indicative of an increase in mucus abundance (Tremblay et al. 2017). Also, others

observed that the overall number of goblet cells in the ileal Peyer's patches (PPs) of ageing mice remained unchanged (Kobayashi et al. 2013). In other areas of the GI-tract the effects of ageing on the mucus layer are more pronounced. In both wild type mice and transgenic *Ercc1−/Δ7* mouse model of accelerated ageing, the thickness of the colonic mucus layer decreased compared to their young counterparts (van Beek et al. 2016). The same report demonstrated that the mucus thickness could be restored in ageing by supplementation with a specific lactobacillus strain, thus reinforcing the notion of a direct link between intestinal microbes and goblet cells activity. Also, it appeared that the age-associated reduction of the thickness of the mucus layer in mice is influenced by the sex of the host with males being significantly more affected by the ageing process compared to females (Elderman et al. 2017). Of significance, the thinning of the mucus in ageing was paralleled by changes on the microbiota composition and immunity (Sovran et al. 2019).

The effects of ageing on the chemical composition of the mucus, and in particular its glycosylation pattern is another important aspect that remains to be thoroughly addressed. Microbes adhere to mucins O-glycans *via* mucus-binding protein (MUB), a large multi-repeat cell-surface adhesins found in bacteria inhabiting the GI tract. Yet, glycans embedded in the mucus layer represent a nutritional substrate for a variety of gut microbes (Gusils et al. 2004; Johansson 2011). Taken together these observations imply that glycans-mediated interaction/binding between the mucus layer and gut bacteria is a key step in the selection and maintenance of the local microbiota. To this end, it is worth reporting that the adhesion of certain microbial species to mucus declined in ageing (Ouwehand et al. 1999; He et al. 2001a; He et al. 2001b). This notion is of potential significance. First, change in protein glycosylation is an important factor in ageing (Krištić et al. 2014; Miura and Endo, 2016). Second, one could speculate that age-associated changes in the glycosylation pattern of mucins might be a factor contributing to the alterations of the microbiota profile observed in the elderly (O'Toole and Jeffery, 2015). Furthermore, the phospholipid composition of the mucus layer also rapidly changes during the very early stages of life (Okuyama et al. 1998); however, up to date no information is available on this aspect of the mucus chemistry during ageing.

More recently, it has become apparent that the role of the mucus layer extended beyond its protective role and it included distinct regulatory properties that contributed to shape the immunological properties of anti-inflammatory intestinal DCs (Shan et al. 2013).

Overall, these observations pointed to a pivotal role exerted by the mucus layer in the protection of the intestinal epithelium and in shaping both the immunological microenvironment and optimal habitat for a healthy microbiota. Changes of the mucus thickness and chemical structure during ageing have the potential to alter the intestinal environment with important consequences on the microbial community and inflammation of the gut.

3.3- Anti-microbial peptides (AMPs)

Antimicrobial peptides (AMPs) represent the first line of defence to combat infections at the host-microbe interface in the gut (Maróti et al. 2011). They are small (six to 100 amino acids), cationic and amphipathic peptides that display broad-spectrum activity against bacteria, fungi, parasites and viruses (Peters et al. 2010). Their role though, is not confined to fighting off pathogens, they also contribute to the regulation of intestinal homeostasis by controlling the abundance and profile of the gut microbiota (Bevins et al. 2011). AMPs disrupt bacterial membranes and they can be toxic to mammalian cells as well; for this reason their expression must be kept under the strict control of both transcriptional and post-translational mechanisms. Information has been collected in the past few years on age-associated changes of the production of AMPs in mice and more recently in *Drosophila*. In ageing humans the production of AMPs by peripheral mononuclear blood cells (PBMC) is preserved (Castañeda-Delgado, 2013); however, currently the evidence of fluctuation of intestinal levels of AMPs in the elderly is not available.

In mice, it has been observed that the expression of important Paneth cell-derived AMPs, such as α-defenins and lysozyme declined with age; in contrast, other AMPs including regenerating islet-derived protein 3 beta (RegIIIB) and gamma (RegIIIG) as well as β-defensins 1,

angiogenin-4 and resistin-like molecule β (RELMβ) were significantly up-regulated in ageing (Tremblay et al. 2017). The exact mechanism underlying the age-related up-regulation of AMPs is not clear. It has been hypothesized that the accumulation of pathogenic insults throughout life might lead to a chronic activation status. Furthermore, it has been suggested that the problem might be linked to a defective immuno-regulation. A cell-intrinsic deficiency to terminate, but not initiate, inflammatory response to pro-inflammatory Toll-like receptor (TLR) stimulation that preceded manifestations of immunosenescence was described in both aged mice (Pattabiraman et al. 2017) and aged male, but not female *Drosophila* (Zerofsky et al. 2005).

A large number of experiments on intestinal AMPs in ageing have been performed in the fly *Drosophila*. This model harbours seven distinct families of inducible AMPs expressed systemically in the body fat or in the epithelial barrier. The expression of these AMPs is regulated by both TLR signalling and the immune deficiency (IMD) pathways, the latter pathway being specific for the expression of AMPs in the midgut section of the fly (Lemaitre and Hoffmann 2007). Other AMPs expressed in the *Drosophila* midgut are regulated by either the transcription factors *Drosophila* Forkhead box O (dFOXO) or Forkhead (FKH) (Buchon et al. 2009; Varma et al. 2014). In addition, in ageing flies, the production of AMPs appeared significantly up-regulated; the age-associated overexpression of the AMP drosomycin highly correlated with the loss of the intestinal barrier integrity (Rera et al. 2012). Interestingly, the genetically induced over-expression of specific AMPs in the midgut of *Drosohila*, including Drosocin and Cecdoprin A1 significantly extended the lifespan of the fly (Loch et al. 2017). The induced constitutive expression of these AMPs limited the intestinal stress response, regenerative and immune activity; these events were thought to be brought about by AMP-mediated reduction of bacterial challenges throughout life.

3.4- Immunoglobulin A (sIgA)

The presence of sIgA is the hallmark of mucosal surfaces. The main function of sIgA is to prevent the penetration of mucosal barriers *via* the immune exclusion of antigens and

pathogens (Stokes et al. 1975; Wijburg et al. 2006; Hapfelmeier et al. 2010). However, they also play a critical role at the host-microbe interface in the gut for establishing and maintaining the intestinal microbiota community (Macpherson and McCoy, 2013; Okai et al. 2016; Planer et al. 2016) and control entry of IgA-coated antigens *via* antigen-sampling M cells (Rey et al. 2004). Together, these activities prevent infections *via* mucosal surfaces and contribute to regulate the intestinal immune homeostasis by limiting the generation of pro-inflammatory response. The IgA production initiates within the PPs of the organized gut-associated lymphoid tissue (O-GALT) (Craig and Cebra, 1971). The PPs harbour the cells and signals required to create the appropriate immunological environment for antigen-specific B cells to undergo IgA isotype switching (MacPherson et al. 2000) and express the surface molecules required for the B cell homing to distant mucosal sites (Mora et al. 2006). These signals include the transforming growth factor-β (TGF-β), $CD103^+$ DC-secreted retinoic acid (RA) and interleukin 6 (IL- 6) (Agace et al. 2012). Currently, limited information is available on the effects of ageing on the intestinal factors that are critical for optimal IgA responses. For instance, RA signalling pathways was impaired in circulating PBMC (Feart, et al. 2005) but this analysis was not extended to the intestinal immune system.

So far, conflicting results have been reported on the magnitude of IgA-mediated responses in ageing. Levels of intestinal IgA to an oral antigen (i.e. cholera toxin, CT) declined with age in rodents and non-human primates (McDonald et al. 2011; Schmucker et al. 1988; Taylor et al. 1992). This could be attributed to age-associated defects in antigen presentation (Moretto et al. 2008; Vora et al. 2016) and T helper cell activity (Nicoletti, 1994; van der Geest et al. 2014, Carvalho et al. 2011; Daniels et al. 1988). However, the chronic low level of inflammation, or inflammageing typical of the ageing organism also contributed to dampen antibody responses by damaging the B cell development (Bulati et al. 2017). Indeed, the implementation of strategies to reduce inflammation, such as adoptive transfer of adipose mesenchymal stem cell from young donors restored a young-like CT-specific sIgA antibody response in aged mice (Tsuruhara et al. 2017). In contrast, others have observed that the IgA antibody response in ageing remained unchanged or even increased in both mice (Santiago et al. 2008; Haq and

Szewczuk, 1991; Arranz et al. 1992; Senda et al. 1988) and humans (de Bruijn et al. 1999). Accumulation of circulating IgA in ageing did not appear related to changes in the expression of the polymeric immunoglobulin receptor (poly-IgR) which was unaffected by ageing (Taylor et al. 1992; Daniels et al. 1998). In the past, investigation of the systemic antibody response showed that the magnitude of antibody production to a microbial component in ageing mice ranged from increased to reduced depending on the antigen utilized and the genetic make-up of the host (Nicoletti and Cerny, 1991). It is then possible that the same pattern applies to the magnitude of intestinal IgA responses. However, it is important to highlight that even antibodies from ageing mice of a "high responder" mouse strain failed to afford protection against lethal infection with *S. pneumoniae* when passively transferred into young mice (Nicoletti et al, 1993). In such a case, the antibody repertoire of aged mice displayed an increased heterogeneity of the variable heavy and light chains (V_H/V_L) gene repertoire and a much reduced antibody affinity to the bacterial epitope phosphorylcholine (Nicoletti et al. 1991). This would suggest that the process of affinity maturation/selection of antigen-specific clones is impaired in ageing. An additional study showed that in the mucosal immune system, ageing did not affect the process of hypermutation (Howards et al. 2006). However, in the same study, the analysis of the dynamics of the germinal centre selection process uncovered that in ageing mice a decrease in the extent of selection occurred in the germinal centres of mucosal tissue. Together, these data suggested that changes in the structure (quality) rather than the quantity of the antibody response is of significance in late life. More recently, a high throughput sequencing analysis (Lindner et al. 2012) showed that the repertoire diversity of intestinal sIgA increased in ageing mice, ultimately though, the protective capacity of the sIgA antibody response during ageing remains to be determined.

Finally, ageing also affected the expression of B cell homing-relevant molecules. Reduced levels of mucosal addressin cell adhesion molecule 1 (MadCAM-1) in the lamina propria (LP) and sub-mucosa venules α4b7 integrin on PMBCs (Thoreaux et al. 2000) were reported. The latter observation suggested that impaired homing of IgA-producing plasma cells rather than

a reduction of their overall number might underpin reduced sIgA responses in ageing (Schmucker et al. 2003).

4. Specialized epithelial function: monitoring the luminal contents *via* antigen sampling.

In addition to providing a formidable barrier to unwanted visitors and macromolecules the intestine has also evolved strategies to carry out the important task of allowing the intestinal immune system to constantly monitor the luminal contents. The activity of antigen-sampling is mainly carried out by membranous (M) cells (Nicoletti, 2000; Ohno, 2015). These cells are interspersed among conventional absorptive IECs and are strategically located within the follicle associated epithelium (FAE) of PPs, the inductive sites of the mucosal immune responses. In this way, luminal material is delivered directly to areas controlled by immune cells that distinguish between innocuous microbes or potentially harmful pathogens and undertake the appropriate course of action.

In the proximity of the PPs, the intestinal crypt presents two distinct axes of cell proliferation, migration and differentiation. While during the migration along the crypt villus axis emerging cells differentiate into IECs, goblet, enteroendocrine cells and Tuft cells, cells exiting the crypt zone on the FAE side of the crypt move onto the dome of the follicle, acquiring features of IECs and M cells (Barker, 2014) (Fig 1A). In the past, conflicting results on M cell activity in ageing were reported. M cell up-take and transport of microparticles were apparently unaffected or even increased in ageing (LeFevre et al. 1978; Simon et al. 1994). Instead, more recently, conclusive evidence showed that ageing does have a detrimental effect on M cell numbers, maturation and activity (Kobayashi et al. 2013), thus leading to a deficiency in their ability to transcytose particles across the FAE. The authors showed that the number of FAE cells expressing the M cell specific marker glycoprotein 2 $(GP2)^+$ M cells were reduced in ageing mice. Also, ageing significantly affected the functional maturation of M cells. First, the number of Spi-B^+ cells that play a critical role in M cell maturation are reduced within the FAE of aged mice. Second, the functional differentiation of M cells also depends on $CCR6^+$ $CD11c^+$

B cells that are recruited within the PPs by the chemokine CCL20. Age-associated decline of CCL20 levels in the FAE resulted in a decreased influx of $CCR6^+$ B cells; this in turn affected M cell maturation. Ultimately, M cell-mediated sampling in ageing appeared affected also by the reduction of the size of the FAE area overlying individual follicles (Kobayashi et al. 2013), but not of the total number of intestinal PPs (Kawanishi and Kiely, 1989). Within this context, it is important to highlight that the down-regulation of antigen sampling *via* M cells might contribute to impaired development of oral tolerance to protein in older mice (Kato et al. 2003). Other factors play a role on the development of fully operational M cells, such as the cytokine macrophage migration inhibitory factor (MIF) (Man et al. 2008) and receptor activator of NF-κB ligand (RANKL) (Knoop et al. 2009). Currently though, the effects of ageing on these soluble factors and the potential consequences on M cell biology/activity remain to be determined.

Furthermore, additional strategies for antigen sampling in the gut have been observed. First, a small number of functional villous-associated M cells have been described (Jang et al. 2004). Second, although its biological relevance has been recently questioned (Man et al. 2017; Regoli et al. 2017) it has been observed that LP-CX_3CR1^+ macrophages contributed to antigen sampling by sending cellular extensions between IECs (Rescigno et al. 2001). Third, colonic and small intestine goblet cells can deliver antigens to LP-$CD103^+$ DCs (McDole et al. 2012). Currently it is not known whether these routes for antigen-sampling are impaired in ageing.

5. Inflammatory balance in the gut

5.1- Pro-inflammatory cytokines and barrier integrity.

Intestinal levels of pro-inflammatory cytokines, in particular TNFα, IL-1β, IFNγ and IL-6 tend to increase with age. This observation has a direct bearing on barrier integrity; indeed, all these cytokines affect intestinal permeability by modulating the expression of TJ proteins. Experimental evidence stemming from investigation on the aetiology of inflammatory bowel disease revealed that IL-1β, TNF-α, or IFN-γ directly affected barrier integrity and intestinal permeability by TJ remodelling at the level of the perijunctional actomyosin cytoskeleton. This

event was linked to an increase of myosin light chain kinase (MLCK) gene and protein expression (Al-Sadi et al. 2012; Al-Sadi et al. 2013; Ma et al. 2005; Al-Sadi et al. 2016; Wang et al. 2005) (Fig. 2). Indeed, the inhibition of MLCK expression or kinase activity prevented the cytokine–driven TJ remodeling and the ensuing increase of the intestinal permeability. The cytokine IL-6 also increased the permeability to small molecules *via* the activation of JNK signalling cascade (Al-Sadi, 2014; Suzuki et al. 2009). The JNK-mediated activation of AP-1 resulted in AP-1 binding sequence targeting the claudin-2 promoter region, leading to a subsequent increase in claudin-2 gene transcription and protein production. In the small intestine of ageing humans, the up-regulation of claudin-2, a TJ protein that promotes the formation of pores that allows the paracellular movement of molecules with radii less than 4Å (Van Itallie, 2008) led to a significant decline of transepithelial electric resistance (TEER) (Man et al. 2015). Currently, there is no evidence linking the claudin-2 mediated increase in permeability to solutes to age-related disturbances of the GI-tract although the overexpression of claudin-2 has been reported in colitis (Heller et al. 2005). In the latter case, IL-13 and IL-17 appeared to be the governor of claudin-2 overexpression (Heller et al. 2005, Fujino et al. 2003). However, in healthy humans both *in vitro* and *ex-vivo* experiments demonstrated that the addition of IL-6 blocking antibody completely abolished the age-associated increase of claudin-2 mediated permeability. Thus, at least in the ageing human small intestine, claudin-2 up-regulation is triggered solely by IL-6 (Man et al. 2015).

5.2- *Contribution of IECs to gut inflammageing*

Most of the studies on cytokine levels in the ageing gut fell short of identifying the cellular source of the inflammatory cytokines. Then, the question remains as to what extent IECs contribute to the imbalanced inflammatory pattern in ageing.

The IECs are the bricks of the intestinal barrier but their role is not limited to separating microbes from the inside of the body. The constant and finely tuned dialogue between the microbiota, IECs and the underlying immune cells is central to the regulation of intestinal immune homeostasis and early responses to pathogens (Allaire et al. 2018). IECs express a

variety of pattern-recognition receptors (PRRs) including members of the TLR family and the nucleotidebindingoligomerization domain (NOD)-like receptors (NLRs) (Artis, 2008; Gribar et al. 2008). The differential and finely tuned expression of these receptors enable the IECs to discriminate between commensal and pathogenic bacteria (Neish et al, 2000; Kelly et al. 2004; Artis 2008).

At steady state, in the presence of commensal microbes, IECs produce anti-inflammatory molecules that include thymic stromal lymphopoietin (TSLP) (Rimoldi et al. 2005) and transforming growth factors (TGF-β) (Zeuthen et al. 2008). These cytokines shape the properties of local anti-inflammatory DCs and help the generation of T regulatory cells (Tregs) (Ilev et al. 2009) and in so doing, they prevent exaggerated inflammatory responses and regulate the gut microbiota (Bauché and Marie, 2017). In contrast, the presence of pathogenic bacteria triggers the production of pro-inflammatory responses such as CCL20, IL-8 and IL-6 (Mowat, 2003) required to clear the infection. In turn, immune cell-derived cytokines modulate a variety of functions and cytokine production by IECs (Man et al. 2008; Andrews et al. 2018; Regoli et al. 2018).

Up to date, the pattern of IEC-derived cytokine at steady state in ageing is not known, nor is the level of expression of most of the IEC-associated PRRs. Recently though, it has emerged that in aged humans the profile of cytokines production by IECs in response to microbial challenge varies according to the type of microbial challenge. Small intestine biopsies from healthy humans of different ages showed that IEC production of IL-8 in response to flagellin, a component of both commensal and pathogenic microbes, significantly declined in the elderly (67-77 years old) compared to younger individuals (Man et al. 2015). Importantly, neither the expression nor the distribution of the flagellin-specific TLR5 changed in IECs from ageing biopsies. This strongly suggested an age-associated alteration of the intracellular signalling pathways that follows the engagement of flagellin with TLR5. IL-8 was one of the first examples of IEC-derived immune mediators produced in the very early stages of infection in the gut (Eckmann et al. 1993; Eckman et al. 1995); thus, it is likely that reduced levels of this cytokine contribute to the increased susceptibility to pathogen infections in ageing. However,

it would appear that the ageing process does not affect all IEC-derived cytokines equally. Epithelial challenge of human biopsies with a different antigenic stimulus (i.e. probiotic mixture) triggered a similar production of TNFα by IECs from biopsies of individuals of different ages (Man et al. 2015).

Accumulating evidence also showed that components of the inflammasome are highly expressed in IECs. Experimental evidence obtained on freshly-isolated IECs and *in situ* staining demonstrated the expression of a variety of inflammasome components in IECs that included NAIPs (NAIP1, 2, 5, and 6 in mice; hNAIP in humans), NLRC4, NLRP1, NLRP6, AIM2, caspase-1, caspase-4(-11), ASC, and IL-18 (Sellin et al. 2015). IECs face an environment overloaded with microbe/pathogen-associated molecular patterns (M/PAMPs) and, most relevant to inflammasome expression, damage-associated molecular patterns (DAMPs) that dramatically increase in ageing (Kapetanovic et al. 2015). High levels of these molecules in ageing leads to a condition known as “garb-ageing” (Franceschi et al. 2017) and the ensuing activation of the inflammasome. Furthermore, the involvement of the inflammasome in the inflammageing of the GI-tract could be inferred by the overexpression of IL-1β, a hallmark of both the ageing colon (Tran and Greenwood-Van Meerveld, 2013) and inflammasome activation (Deng et al. 2019).

In contrast to the limited information available on the immunoregulatory properties of IECs in ageing, a large number of reports have shown that the microbiota composition changes with age (O’Toole and Jeffery, 2015). This notion was preceded by the hypothesis that the appearance or expansion of different bacterial species underpinned the imbalance between pro-and anti-inflammatory cytokines in gut and trigger inflammageing (Guigoz et al. 2008). Although an extensive review of the age-associated change of the gut microbiota, and its consequences on the host-microbe cross-talk, is beyond the scope of this review, it is important to address aspects of the evolution of the interaction between the epithelial barrier and microbiota in ageing and the potential effects on barrier integrity and local and systemic disturbances in late life.

6. Host-microbe interface in the gut: a strategic target to improve health in the elderly?

The composition of the gut microbiota in health and disease is, these days, a topic of exceptional interest for the scientific community. In ageing in particular, the important role of gut microbiota is highlighted by the notion that in centenarians the “longevity adaptation” is characterized by the enrichment in health-associated gut microbes (Biagi et al, 2016; Biagi et al. 2017; Kong et al, 2016; Kong et al 2019; Deng et al, 2019). However, how does the interaction between the gut microbiota and the host evolve across life? The ageing process is an important model for the study of the effects of the microbiota on the local and systemic changes occurring in the elderly. In the murine model, the adoption of strategies to minimize confounders and increase data quality for studies on the gut microbiota established that the intrinsic, environment-independent ageing is itself a driver of the gut microbiome composition (Miyoshi et al. 2018). The observation that mice from the same colony and subjected to the same environmental conditions (i.e. housing, diet, temperature, humidity, etc.) showed an age-dependent drift of their microbiota profile (Langille et al. 2014; Conley et al. 2016) also lends support to this conclusion. Then, it would appear that the ageing process triggers major changes that profoundly alter the intestinal environment. These include altered cytokine balance and immunological profile, different expression of PPRs, impaired nutrient absorption and hormonal status. In turn, these changes create the habitat favourable for the expansion and establishment of different microbial species. However, this seems to be in contrast with the observation that, in some instances the change of the microbiota composition appeared to precede the age-related alterations of the intestinal epithelial barrier. In mice, the passive transfer of microbiota from aged mice triggered local and systemic inflammageing in young recipients (Fransen et al. 2017) and in the fly *Drosophil*a distinct shift in the profile of midgut microbiota in ageing affected intestinal function and drove mortality (Clark et al. 2015). The latter study carried out a detailed analysis of the kinetics of microbiota changes in *Smurf* flies, so called for the presence of non-absorbable blue dye within the body tissues and outside the GI-tract due to loss of barrier integrity (Rera et al. 2012). The authors reported that microbiota

dysbiosis preceded and predicted the age-dependent onset of intestinal barrier failure. The loss of barrier integrity was then followed by a second and more significant change of the microbiota composition (Clark et al. 2015). Then, the question on how the interaction between the components of the intestinal epithelial barrier and the microbiota change/evolve during ageing remains to be answered. Yet, how do these changes occurring at the host-microbe interface (Fig. 3) affect resistance to diseases in the ageing organism? The answers to these questions lay in the dynamics of host-microbe cross-talk based on the continuous exchange of signals required to establish and maintain intestinal homeostasis.

The intricate reciprocal control and regulation of the epithelial barrier function and microbiota composition is epitomized by the outcome of the cross-talk between IEC-associated NLRP6 inflammasome and microbial metabolites. A variety of microbiota-derived metabolites including taurine, histamine, spermine modulate the activity of the NLRP6 inflammasome, which in turn regulates the host-microbe interface *via* the production of IL-18 and the downstream expression of AMPs (Elinav et al. 2011; Levy et al. 2015). Ultimately, the microbiota and IEC-inflammasome loop plays an important role in determining the local microbiota profile. This was further confirmed by the observation that inflammasome-deficient mice “physiologically” harboured an aberrant microbiota community (Elinav et al. 2013). Furthermore, the impact of microbiota on IECs also extended to metabolic and barrier functions. Microbe-derived short-chain fatty acids (SCFAs) are important sources of energy for IECs and modulate oxygen absorption and the secretion of factors, such as hypoxia-inducible factor (HIF) that improves barrier function (Kelly et al. 2015). The microbial metabolite indole also has an impact on barrier function through the nuclear receptor subfamily 1 group I member2 (NR1I2) (Venkatesh et al. 2014). Thus, the disruption at any level of the intricate IEC-microbe interaction in ageing could lead to the development of an intestinal environment supportive of an expansion of certain microbial species and inflammatory imbalance.

Ultimately, understanding the dynamics of the evolution of host-microbe interaction at the mucosal interface in the gut across life and its potential role in the age-associated loss of

barrier integrity is key to provide information to design novel intervention strategies to improve the life span and ameliorate the quality of life of the elderly. Experiments in *C. elegans* and *Drosophila* have shown that the intervention on the intestinal barrier held the potential to promote longevity (Libina et al. 2003; Rera et al. 2013). Since most of the genetic factors involved in defining the lifespan are conserved in all vertebrates (Kenyon, 2010) it is then plausible to foresee that interventions aiming to restoring the integrity of the gut barrier could be beneficial to elderly humans as well. Yet, dysfunctions of the intestinal epithelial barrier have been associated to devastating age-related neurodegenerative diseases (Köhler et al. 2016; Clairembault et al. 2015) and behavioural disorders (Maes et al. 2013, Pearson-Leary et al. 2019). Then, the possibility to halt or delay the progression of these diseases in ageing and improve the overall quality of life including the psychological well-being of the elderly by targeting the gut barrier is certainly very appealing. In the past, optimistic expectations were placed on probiotic-, prebiotic- and symbiotic-based therapeutic approaches to restore barrier integrity and combat intestinal and systemic inflammation. Although full of promises, until now this approach has not been extremely successful (Bron et al. 2017). Recently though, the success of microbiota transplant (MT) as a highly effective cure for *C. difficile* infection (Leffler and Lamont, 2015) and the promises of MT on the treatment of certain intestinal and systemic pathologies (Bouri and Hart, 2018; Vrieze et al. 2012; Borody and Khoruts, 2011) have laid the foundation to hypothesize a microbiota-based strategy to intervene on barrier integrity. The recent advances in the development of more economical, safer and more patient-friendly way for MT administration (Petrof et al, 2013; Zhang, 2013) are important steps towards the definition of this strategy. The identification of a “defined flora” including a selected panel of intestinal microbes that can deliver the same health-promoting effects as MT could also contribute to increase the safety and the feasibility of this approach. The microbiota-based therapy could be integrated by the adoption of dietary interventions aiming to avoid food components with detrimental effects on barrier integrity while including dietary components that restore it (Nicoletti, 2015; Arújo et al. 2017; Gleeson, 2017).

7. Concluding remarks

We are living in an ageing world. However, the extra years of life are often accompanied by an increase of a wide array of ailments and the number of people suffering from age-associated disturbances is increasing rapidly. The result of this is a poor quality of life for millions of people worldwide and astronomical socio-economic costs (Lee and Mason, 2017). Recent advances in medical sciences have brought to the surface the critical role of the intestinal epithelial barrier in health and disease. However, although significant progress has been made in understating the basic biology of the various components of the intestinal epithelial barrier, many of the mechanisms underlying its functional decline in ageing remain unknown. In particular, the age-associated alteration of the signalling network operating at the mucosal interface and interlinking the gut microbiota, the intestinal epithelium and the underlying immune system still harbours many unsolved mysteries. We believe that given the potential role of intestinal barrier dysfunctions on the onset of important disorders in ageing, future investigation in this area is highly needed. Expanding our knowledge in this area is a goal of very high medical and socio-economic relevance and it will help us to progress from “adding extra years to life” to “adding quality to the extra years of life” for this ever-increasing demographic segment of modern societies.

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References

Agace, W.W., Persson, E.K., 2012. How vitamin A metabolizing dendritic cells are generated in the gut mucosa. Trends Immunol. 33, 42-48.

Al-Sadi, R., Guo, S., Dokladny, K., Smith, M.A., Ye, D., Kaza, A., Watterson, D.M., Ma, T.Y., 2012. Mechanism of interleukin-1β induced-increase in mouse intestinal permeability *in vivo*. J. Interferon Cytokine Res. 32, 474-484.

Al-Sadi, R., Guo, S., Ye, D., Dokladny, K., Alhmoud, T., Ereifej, L., Said, H.M., Ma, T.Y., 2013. Mechanism of IL-1β modulation of intestinal epithelial barrier involves p38 kinase and activating transcription factor-2 activation. J. Immunol. 190, 6596-6606.

Al-Sadi, R., Guo, S., Ye, D., Rawat, M., Ma, T.Y., 2016. TNF-α Modulation of Intestinal Tight Junction Permeability Is Mediated by NIK/IKK-α Axis Activation of the Canonical NF-κB Pathway. Am. J. Pathol. 186, 1151-1165.

Al-Sadi, R., Ye, D., Boivin, M., Guo, S., Hashimi, M., Ereifej, L., Ma, T.Y., 2014. Interleukin-6 modulation of intestinal epithelial tight junction permeability is mediated by JNK pathway activation of claudin-2 gene. PLoS One 9, e85345.

Allaire, J.M., Crowley, S.M., Law, H.T., Chang, S.Y., Ko, H.J., Vallance, B.A., 2018. The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. Trends Immunol. 39, 677-696.

Andrews, C., McLean, M.H., Durum, S.K., 2018. Cytokine Tuning of Intestinal Epithelial function. Front. Immunol. 9, 1270.

Araújo, J.R., Tomas, J., Brenner, C., Sansonetti, P.J., 2017. Impact of high-fat diet on the intestinal microbiota and small intestinal physiology before and after the onset of obesity. Biochimie. 141, 97-106.

Arranz, E., O'Mahony, S., Barton, J.R., Ferguson, A., 1992. Immunosenescence and mucosal immunity: significant effects of old age on secretory IgA concentrations and intraepithelial lymphocyte counts. Gut 33, 882-886.

Artis, D., 2008. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. Nat. Rev. Immunol. 8, 411-420.

Barker, N., 2014. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat. Rev. Mol. Cell. Biol. 15, 19-33.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., Clevers, H., 2007. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003-1007.

Bauché, D., Marie, J.C., 2017. Transforming growth factor β: a master regulator of the gut microbiota and immune cell interactions. Clin. Transl. Immunology 6, e136.

Behrens, A., van Deursen, J.M., Rudolph, K.L., Schumacher, B., 2014. Impact of genomic damage and ageing on stem cell function. Nat Cell Biol. 16, 201-207.

Bennett, E. P., Mandel, U., Clausen, H., Gerken, T. A., Fritz, T. A., Tabak, L. A., 2012. Control of mucin-type O-glycosylation: a classification of the polypeptide GalNAc-transferase gene family. Glycobiology 22, 736-756.

Bergstrom, K. S. B., Xia, L. J., 2013. Mucin-type O-glycans and their roles in intestinal homeostasis. Glycobiology 23, 1026-1037.

Bevins, C.L., Salzman, N.H., 2011. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat. Rev. Microbiol. 9, 356-368.

Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, Consolandi C, Quercia S, Scurti M, Monti D, Capri M, Brigidi P, Candela M. 2016. Gut Microbiota and Extreme Longevity. Curr Biol. 26, 1480-1485

Biagi E, Rampelli S, Turroni S, Quercia S, Candela M, Brigidi P. 2017. The gut microbiota of centenarians: Signatures of longevity in the gut microbiota profile. Mech Ageing Dev.165, 180-184

Biteau, B., Hochmuth, C.E., Jasper, H., 2008. JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. Cell Stem Cell. 3, 442-455.

Blokzijl, F., de Ligt, J., Jager, M., Sasselli, V., Roerink, S., Sasaki, N., Huch, M., Boymans, S., Kuijk, E., Prins, P., Nijman, I.J., Martincorena, I., Mokry, M., Wiegerinck, C.L., Middendorp, S., Sato, T., Schwank, G., Nieuwenhuis, E.E., Verstegen, M.M., van der Laan, L.J., de Jonge, J., IJzermans, J.N., Vries, R.G., van de Wetering, M., Stratton, M.R., Clevers, H., Cuppen, E., van Boxtel, R., 2016. Tissue-specific mutation accumulation in human adult stem cells during life. Nature 538, 260-264.

Borody, T.J., Khoruts, A., 2011. Fecal microbiota transplantation and emerging applications. Nat. Rev. Gastroenterol. Hepatol. 9, 88-96.

Bouri, S., Hart, A., 2018. Fecal microbial transplantation: an update. Curr. Opin. Clin. Nutr. Metab. Care 21, 405-410.

Brockhausen, I., Schachter, H., Stanley, P., 2009. O-GalNAc Glycans, in: Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C.R., Hart, G. W., Etzler, M. E. (Eds.), Essentials of Glycobiology, 2nd edition. Cold Spring Harbor, New York, pp. 115-127.

Bron, P.A., Kleerebezem, M., Brummer, R.J., Cani, P.D., Mercenier, A., MacDonald, T.T., Garcia-Ródenas, C.L., Wells, J.M., 2017. Can probiotics modulate human disease by impacting intestinal barrier function? Br. J. Nutr. 117, 93-107.

Buchon, N., Broderick, N.A., Poidevin, M., Pradervand, S., Lemaitre, B., 2009. Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. Cell Host Microbe 5, 200-211.

Buckley, A., Turner, J.R., Cell Biology of Tight Junction Barrier Regulation and Mucosal Disease. Cold Spring Harb. Perspect. Biol. 10, pii a029314

Bulati, M., Caruso, C., Colonna-Romano, G., 2017. From lymphopoiesis to plasma cells differentiation, the age-related modifications of B cell compartment are influenced by "inflamm-aging". Ageing Res. Rev. 36, 125-136.

Camara-Lemarroy, C.R., Metz, L., Meddings, J.B., Sharkey, K.A., Wee Yong, V., 2018. The intestinal barrier in multiple sclerosis: implications for pathophysiology and therapeutics. Brain. 141, 1900-1916.

Carvalho, C.R., Weiner, H.L., Faria, A.M., 2011. Aging correlates with reduction in regulatory-type cytokines and T cells in the gut mucosa. Immunobiology 216, 1085-1093.

Castañeda-Delgado, J.E., Miranda-Castro, N.Y., González-Amaro, R., González-Curiel, I., Montoya-Rosales, A., Rivas-Calderon, B., Rivas-Santiago, B., 2013. Production of antimicrobial peptides is preserved in aging. Clin. Immunol. 148, 198-205.

Cereijido, M., Valdés, J., Shoshani, L., Contreras, R.G., 1998. Role of tight junctions in establishing and maintaining cell polarity. Annu. Rev. Physiol. 60, 161-177.

Choi, J., Rakhilin, N., Gadamsetty, P., Joe, D.J., Tabrizian, T., Lipkin, S.M., Huffman, D.M., Shen, X., Nishimura, N., 2018. Intestinal crypts recover rapidly from focal damage with coordinated motion of stem cells that is impaired by aging. Sci. Rep. 8, 10989.

Choi, N.H., Kim, J.G., Yang, D.J., Kim, Y.S., Yoo, M.A. Age-related changes in Drosophila midgut are associated with PVF2, a PDGF/VEGF-like growth factor. Aging Cell. 7, 318-334.

Choi, W., Yeruva, S., Turner, J.R., 2017. Contributions of intestinal epithelial barriers to health and disease. Exp. Cell. Res. 358, 71-77.

Clairembault, T., Leclair-Visonneau, L., Coron, E., Bourreille, A., Le Dily, S., Vavasseur, F., Heymann, M.F., Neunlist, M., Derkinderen, P., 2015. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. Acta Neuropathol. Commun. 3, 12.

Clark, R.I., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana, A., Rera, M., Pellegrini, M., Ja, W.W., Walker, D.W., 2015. Distinct Shifts in Microbiota Composition during Drosophila Aging Impair Intestinal Function and Drive Mortality. Cell Rep. 12, 1656-1667.

Conley, M.N., Wong, C.P., Duyck, K.M., Hord, N., Ho, E., Sharpton, T.J., 2016. Aging and serum MCP-1 are associated with gut microbiome composition in a murine model. PeerJ. 4, e1854.

Corazza, G.R., Ginaldi, L., Quaglione, G., Ponzielli, F., Vecchio, L., Biagi, F., Quaglino, D., 1998. Proliferating cell nuclear antigen expression is increased in small bowel epithelium in the elderly. Mech. Ageing Dev. 104, 1-9.

Craig, S.W., Cebra, J.J., 1971. Peyer’s patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. J. Exp. Med. 134, 188-200.

Daniels, C.K., Schmucker, D.L., Bazin, H., Jones, A.L., 1988. Immunoglobulin A receptor of rat small intestinal enterocytes is unaffected by aging. Gastroenterology 94, 1432-1440.

de Bruijn, I.A., Remarque, E.J., Jol-van der Zijde, C.M., van Tol, M.J., Westendorp, R.G., Knook, D.L., 1999. Quality and quantity of the humoral immune response in healthy elderly and young subjects after annually repeated influenza vaccination. J. Infect. Dis.179, 31-36.

Deng F, Li Y, Zhao J. 2019. The gut microbiome of healthy long-living people. Aging. 15; 11, 289-290

Deng, J., Yu, X.Q., Wang, P.H., 2019. Inflammasome activation and Th17 responses. Mol. Immunol. 107, 142-164.

Diao, D., Wang, H., Li, T., Shi, Z., Jin, X., Sperka, T., Zhu, X., Zhang, M., Yang, F., Cong, Y., Shen, L., Zhan, Q., Yan, J., Song, Z., Ju, Z., 2018. Telomeric epigenetic response mediated by Gadd45a regulates stem cell aging and lifespan. EMBO Rep. 19, pii e45494.

Eckmann, L., Kagnoff, M.F., Fierer, J., 1993. Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. Infect. Immun. 61, 4569-4574.

Eckmann, L., Kagnoff, M.F., Fierer, J., 1995. Intestinal epithelial cells as watchdogs for the natural immune system. Trends Microbiol. 3, 118-120.

Elderman, M., Sovran, B., Hugenholtz, F., Graversen, K., Huijskes, M., Houtsma, E., Belzer, C., Boekschoten, M., de Vos, P., Dekker, J., Wells, J., Faas, M., 2017. The effect of age on the intestinal mucus thickness, microbiota composition and immunity in relation to sex in mice. PLoS One 12, e0184274.

Elinav, E., Strowig, T., Kau, A.L., Henao-Mejia, J., Thaiss, C.A., Booth, C.J., Peaper, D.R., Bertin, J., Eisenbarth, S.C., Gordon, J.I., Flavell, R.A., 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 145, 745-757.

Elinav, E., Thaiss, C.A., Flavell, R.A., 2013. Analysis of microbiota alterations in inflammasome-deficient mice. Methods Mol. Biol. 1040, 185-194.

Fasano, A., Not, T., Wang, W., Uzzau, S., Berti, I., Tommasini, A., Goldblum, S.E., 2000. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet 355, 1518-1519.

Feart, C., Pallet, V., Boucheron, C., Higueret, D., Alfos, S., Letenneur, L., Dartigues, J.F., Higueret, P., 2005. Aging affects the retinoic acid and the triiodothyronine nuclear

receptor mRNA expression in human peripheral blood mononuclear cells. Eur. J. Endocrinol. 152, 449-458.

France, M.M., Turner, J.R., 2017. The mucosal barrier at a glance. J. Cell. Sci. 130, 307-314.

Franceschi, C., Garagnani, P., Vitale, G., Capri, M., Salvioli, S., 2017. Inflammaging and 'Garb-aging'. Trends Endocrinol. Metab. 28, 199-212.

Fransen, F., van Beek, A.A., Borghuis, T., Aidy, S.E., Hugenholtz, F., van der Gaast-de Jongh, C., Savelkoul, H.F.J., De Jonge, M.I., Boekschoten, M.V., Smidt, H., Faas, M.M., de Vos, P., 2017. Aged Gut Microbiota Contributes to Systemic Inflammaging after Transfer to Germ-Free Mice. Front. Immunol. 8, 1385.

Fujino, S., Andoh, A., Bamba, S., Ogawa, A., Hata, K., Araki, Y., Bamba, T., Fujiyama, Y., 2003. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 52, 65-70.

Furuse, M., 2010. Molecular basis of the core structure of tight junctions. Cold Spring Harb. Perspect. Biol. 2, a002907.

Furuse, M., Tsukita, S., 2006. Claudins in occluding junctions of humans and flies. Trends Cell. Biol. 16, 181-188.

Garrod, D., Chidgey, M., 2008. Desmosome structure, composition and function. Biochim. Biophys. Acta. 1778, 572-587.

Gervais, L., Bardin, A.J., 2017. Tissue homeostasis and aging: new insight from the fly intestine. Curr. Opin. Cell. Biol. 48, 97-105.

Gleeson, J.P., 2017. Diet, food components and the intestinal barrier. Nut. Bull. 42, 123-131.

Greicius, G., Virshup, D.M., 2019. Stromal control of intestinal development and the stem cell niche. Differentiation S0301-4681, 30178-30186.

Gribar, S.C., Richardson, W.M., Sodhi, C.P., Hackam, D.J., 2008. No longer an innocent bystander: epithelial toll-like receptor signaling in the development of mucosal inflammation. Mol Med. 14, 645-659.

Grivennikov, S.I., Wang, K., Mucida, D., Stewart, C.A., Schnabl, B., Jauch, D., Taniguchi, K., Yu, G.Y., Osterreicher, C.H., Hung, K.E., Datz, C., Feng, Y., Fearon, E.R., Oukka, M., Tessarollo, L., Coppola, V., Yarovinsky, F., Cheroutre, H., Eckmann, L., Trinchieri, G., Karin, M., 2012. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 491, 254-258.

Guigoz, Y., Doré, J., Schiffrin, E.J., 2008. The inflammatory status of old age can be nurtured from the intestinal environment. Curr. Opin. Clin. Nutr. Metab. Care 11, 13-20.

Guo, Z., Lucchetta, E., Rafel, N., Ohlstein, B., 2016. Maintenance of the adult Drosophila intestine: all roads lead to homeostasis. Curr. Opin. Genet. Dev. 40, 81-86.

Gusils, C., Morata, V., González, S., 2004. Determination of bacterial adhesion to intestinal mucus. Methods Mol. Biol. 268, 411-415.

Hao, L.Y., Armanios, M., Strong, M.A., Karim, B., Feldser, D.M., Huso, D., Greider, C.W., 2005. Short telomeres, even in the presence of telomerase, limit tissue renewal capacity. Cell 123, 1121-1131.

Hapfelmeier, S., Lawson, M.A., Slack, E., Kirundi, J.K., Stoel, M., Heikenwalder, M., Cahenzli, J., Velykoredko, Y., Balmer, M.L., Endt, K., Geuking, M.B., Curtiss, R. 3rd, McCoy, K.D., Macpherson, A.J., 2010. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. Science 328, 1705–1709.

Haq, J.A., Szewczuk, M.R., 1991. Differential effect of aging on B-cell immune responses to cholera toxin in the inductive and effector sites of the mucosal immune system. Infect. Immun. 59, 3094-3100.

He, C., Yu, T., Shi, Y., Ma, C., Yang, W., Fang, L., Sun, M., Wu, W., Xiao, F., Guo, F., Chen, M., Yang, H., Qian, J., Cong, Y., Liu, Z., 2017. MicroRNA 301A Promotes Intestinal Inflammation and Colitis-Associated Cancer Development by Inhibiting BTG1. Gastroenterology 152, 1434-1448.

He, F., Ouwehand, A.C., Isolauri, E., Hashimoto, H., Benno, Y., Salminen, S., 2001a. Comparison of mucosal adhesion and species identification of bifidobacterial isolated from healthy and allergic infants. FEMS Immunol. Med. Microbiol. 30, 43-47.

He, F., Ouwehand, A.C., Isolauri, E., Hosoda, M., Benno, Y., Salminen, S., 2001b. Differences in composition and mucosal adhesion of bifidobacteria isolated from healthy adults and healthy seniors, Curr. Microbiol. 43, 351-354.

Heller, F., Florian, P., Bojarski, C., Richter, J., Christ, M., Hillenbrand, B., Mankertz, J., Gitter, A.H., Bürgel, N., Fromm, M., Zeitz, M., Fuss, I., Strober, W., Schulzke, J.D., 2005. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 129, 550-564.

Heller, T.D., Holt, P.R., Richardson, A., 1990. Food restriction retards age-related histological changes in rat small intestine. Gastroenterology 98, 387-391.

Hernandez, S., Chavez Munguia, B., Gonzalez-Mariscal, L., 2007. ZO-2 silencing in epithelial cells perturbs the gate and fence function of tight junctions and leads to an atypical monolayer architecture. Exp Cell Res. 313, 1533-1547.

Hochmuth, C.E., Biteau, B., Bohmann, D., Jasper, H., 2011. Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in Drosophila. Cell Stem Cell. 8, 188-199.

Hollander, D., Tarnawski, H., 1985. Aging-associated increase in intestinal absorption of macromolecules. Gerontology 31, 133-137.

Holt, P.R., Pascal, R.R., Kotler, D.P., 1984. Effect of aging upon small intestinal structure in the Fischer rat. J. Gerontol. 39, 642-647.

Howard, W.A., Gibson, K.L., Dunn-Walters, D.K., 2006. Antibody quality in old age. Rejuvenation Res. 9, 117-125.

Iliev, I.D., Mileti, E., Matteoli, G., Chieppa, M., Rescigno, M., 2009. Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning. Mucosal Immunol. 2, 340-350.

Izumi, Y., Furuse, M., 2014. Molecular organization and function of invertebrate occluding junctions. Semin. Cell. Dev. Biol. 36, 186-193.

Jang, M.H., Kweon, M.N., Iwatani, K., Yamamoto, M., Terahara, K., Sasakawa, C., Suzuki, T., Nochi, T., Yokota, Y., Rennert, P.D., Hiroi, T., Tamagawa, H., Iijima, H., Kunisawa, J., Yuki, Y., Kiyono, H., 2004. Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proc. Natl. Acad. Sci. USA 101, 6110-6115.

Jeffery, V., Goldson, A.J., Dainty, J.R., Chieppa, M., Sobolewski, A., 2017. IL-6 Signaling Regulates Small Intestinal Crypt Homeostasis. J. Immunol. 199, 304-311.

Johansson, M.E., 2012. Fast renewal of the distal colonic mucus layers by the surface goblet cells as measured by in vivo labeling of mucin glycoproteins. PLoS One 7, e41009.

Johansson, M.E., Larsson, J.M., Hansson, G.C., 2011. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proc. Natl. Acad. Sci. U S A 108, 4659-4665.

Johansson, M.E., Phillipson, M., Petersson, J., Velcich, A., Holm, L., Hansson, G.C., 2008. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc. Natl. Acad. Sci. U S A 105, 15064-15069.

Johansson, M.E.V., Hansson, G.C., 2011. Keeping Bacteria at a Distance. Science 334, 182-183.

Kapetanovic, R., Bokil, N.J., Sweet, M.J., 2015. Innate immune perturbations, accumulating DAMPs and inflammasome dysregulation: A ticking time bomb in ageing. Ageing Res. Rev. 24, 40-53.

Kato, H., Fujihashi, K., Kato, R., Dohi, T., Fujihashi, K., Hagiwara, Y., Kataoka, K., Kobayashi, R., McGhee, J.R., 2003. Lack of oral tolerance in aging is due to sequential loss of Peyer's patch cell interactions. Int. Immunol. 15, 145-158.

Kawanishi, H., Kiely, J., 1989. Immune-related alterations in aged gut-associated lymphoid tissues in mice. Dig. Dis. Sci. 34, 175-184.

Kelly, C.J., Zheng, L., Campbell, E.L., Saeedi, B., Scholz, C.C., Bayless, A.J., Wilson, K.E., Glover, L.E., Kominsky, D.J., Magnuson, A., Weir, T.L., Ehrentraut, S.F., Pickel, C., Kuhn, K.A., Lanis, J.M., Nguyen, V., Taylor, C.T., Colgan, S.P., 2015. Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. Cell Host Microbe 17, 662-671.

Kelly, D., Campbell, J.I., King, T.P., Grant, G., Jansson, E.A., Coutts, A.G., Pettersson, S., Conway, S., 2004. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat. Immunol. 5, 104-112.

Kenyon, C.J., 2010. The genetics of ageing. Nature 464, 504-512.

Kenyon, J., Gerson, S.L., 2007. The role of DNA damage repair in aging of adult stem cell. Nucleic Acids Res. 35, 7557-7565.

Knoop, K.A., Kumar, N., Butler, B.R., Sakthivel, S.K., Taylor, R.T., Nochi, T., Akiba, H., Yagita, H., Kiyono, H., Williams, I.R., 2009. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. J. Immunol. 183, 5738-5747.

Kobayashi, A., Donaldson, D.S., Erridge, C., Kanaya, T., Williams, I.R., Ohno, H., Mahajan, A., Mabbott, N.A., 2013. The functional maturation of M cells is dramatically reduced in the Peyer's patches of aged mice. Mucosal Immunol. 6, 1027-1037.

Köhler, C.A., Maes, M., Slyepchenko, A., Berk, M., Solmi, M., Lanctôt, K.L., Carvalho, A.F., 2016. The Gut-Brain Axis, Including the Microbiome, Leaky Gut and Bacterial Translocation: Mechanisms and Pathophysiological Role in Alzheimer's Disease. Curr. Pharm. Des. 22, 6152-6166.

Kong F, Hua Y, Zeng B, Ning R, Li Y, Zhao J. 2016. Gut microbiota signatures of longevity. Curr Biol. 26, R832-R833

Kong F, Deng F, Li Y, Zhao J. 2019. Identification of gut microbiome signatures associated with longevity provides a promising modulation target for healthy aging. Gut Microbes.10, 210-215

Krištić, J., Vučković, F., Menni, C., Klarić, L., Keser, T., Beceheli, I., Pučić-Baković, M., Novokmet, M., Mangino, M., Thaqi, K., Rudan, P., Novokmet, N., Sarac, J., Missoni, S., Kolčić, I., Polašek, O., Rudan, I., Campbell, H., Hayward, C., Aulchenko, Y., Valdes, A., Wilson, J.F., Gornik, O., Primorac, D., Zoldoš, V., Spector, T., Lauc, G., 2014. Glycans are a novel biomarker of chronological and biological ages. J. Gerontol. A Biol. Sci. Med. Sci. 69, 779-789.

Langille, M.G., Meehan, C.J., Koenig, J.E., Dhanani, A.S., Rose, R.A., Howlett, S.E., Beiko, R.G., 2014. Microbial shifts in the aging mouse gut. Microbiome 2, 50.

Lee, R., Mason, A., 2017. Cost of Aging. Finance Dev. 54, 7-9.

LeFevre, M.E., Vanderhoff, J.W., Laissue, J.A., Joel D.D., 1978. Accumulation of 2-micron latex particles in mouse Peyer's patches during chronic latex feeding. Experientia 34, 120-122.

Leffler, D.A., Lamont, J.T., 2015. Clostridium difficile infection. N. Engl. J. Med. 372, 1539-1548.

Lemaitre, B., Hoffmann, J., 2007. The host defense of Drosophila melanogaster. Annu. Rev. Immunol. 25, 697-743.

Levy, M., Thaiss, C.A., Zeevi, D., Dohnalová, L., Zilberman-Schapira, G., Mahdi, J.A., David, E., Savidor, A., Korem, T., Herzig, Y., Pevsner-Fischer, M., Shapiro, H., Christ, A., Harmelin, A., Halpern, Z., Latz, E., Flavell, R.A., Amit, I., Segal, E., Elinav, E., 2015. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. Cell 163, 1428-1443.

Libina, N., Berman, J.R., Kenyon, C., 2003. Tissue-specific activities of C. elegans DAF-1 in the regulation of lifespan. Cell 115, 489-502.

Lindner, C., Wahl, B., Fohse, L., Suerbaum, S., Macpherson, A.J., Prinz, I., Pabst, O., 2012. Age, microbiota, and T cells shape diverse individual IgA repertoires in the intestine. J. Exp. Med. 209, 365-377.

Loch, G., Zinke, I., Mori, T., Carrera, P., Schroer, J., Takeyama, H., Hoch, M., 2017. Antimicrobial peptides extend lifespan in Drosophila. PLoS One 12, e0176689.

Ma, T.Y., Boivin, M.A., Ye, D., Pedram, A., Said, H.M., 2005. Mechanism of TNF-{alpha} modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. Am. J. Physiol. Gastrointest. Liver Physiol. 288, G422-G430.

Ma, T.Y., Hollander, D., Dadufalza, V., Krugliak, P., Effect of aging and caloric restriction on intestinal permeability. Exp. Gerontol. 27, 321-333.

MacDermott, R.P., Donaldson, R.M., Jr., Trier, J.S., 1974 Glycoprotein synthesis and secretion by mucosal biopsies of rabbit colon and human rectum. J. Clin. Invest. 54, 545-554.

Macpherson, A.J., McCoy, K.D., 2013. Stratification and compartmentalisation of immunoglobulin responses to commensal intestinal microbes, Semin. Immunol. 25, 358-363.

Macpherson, A.J., Gatto, D., Sainsbury, E., Harriman, G.R., Hengartner, H., Zinkernagel, R.M., 2000. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 288, 2222-2226.

Maes, M., Kubera, M., Leunis, J.C., Berk, M., Geffard, M., Bosmans, E., 2013. In depression, bacterial translocation may drive inflammatory responses, oxidative and nitrosative stress (O&NS), and autoimmune responses directed against O&NS-damaged neoepitopes. Acta Psychiatr. Scand. 127, 344-354.

Man, A.L., Bertelli, E., Rentini, S., Regoli, M., Briars, G., Marini, M., Watson, A.J., Nicoletti C., 2015. Age-associated modifications of intestinal permeability and innate immunity in human small intestine. Clin. Sci. 129, 515-527.

Man, A.L., Gicheva, N., Regoli, M., Rowley, G., De Cunto, G., Wellner, N., Bassity, E., Gulisano, M., Bertelli, E., Nicoletti, C., 2017. CX3CR1+ Cell-Mediated Salmonella Exclusion Protects the Intestinal Mucosa during the Initial Stage of Infection. J. Immunol. 198, 335-343.

Man, A.L., Lodi, F., Bertelli, E., Regoli, M., Pin, C., Mulholland, F., Satoskar, A.R., Taussig, M.J., Nicoletti, C., 2008. Macrophage migration inhibitory factor plays a role in the regulation of microfold (M) cell-mediated transport in the gut. J Immunol. 181, 5673-5680.

Maróti, G., Kereszt, A., Kondorosi, E., Mergaert, P., 2011. Natural roles of antimicrobial peptides in microbes, plants and animals. Res. Microbiol. 162, 363-374.

Martin, K., Potten, C.S., Roberts, S.A., Kirkwood, T.B., 1998. Altered stem cell regeneration in irradiated intestinal crypts of senescent mice. J. Cell. Sci. 111, 2297-2303.

Matter, K., Balda, M.S., 2003. Signalling to and from tight junctions. Nat Rev Mol. Cell. Biol. 4, 225-236.

McDole, J.R., Wheeler, L.W., McDonald, K.G., Wang, B., Konjufca, V., Knoop, K.A., Newberry, R.D., Miller, M.J., 2012. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 483, 345-349.

McDonald, K.G., Leach, M.R., Huang, C., Wang, C., Newberry, R.D., Aging impacts isolated lymphoid follicle development and function. Immun. Ageing 8, 1.

Miura, Y., Endo, T., 2016. Glycomics and glycoproteomics focused on aging and age-related diseases-Glycans as a potential biomarker for physiological alterations. Biochim. Biophys. 1860, 1608-1614.

Miyoshi, J., Leone, V., Nobutani, K., Musch, M.W., Martinez-Guryn, K., Wang, Y., Miyoshi, S., Bobe, A.M., Eren, A.M., Chang, E.B., 2018. Minimizing confounders and increasing data quality in murine models for studies of the gut microbiome. PeerJ. 6, e5166.

Moorefield, E.C., Andres, S.F., Blue, R.E., Van Landeghem, L., Mah, A.T., Santoro, M.A., Ding, S., 2017. Aging effects on intestinal homeostasis associated with expansion and dysfunction of intestinal epithelial stem cells. Aging (Albany NY) 9, 1898-1915.

Mora, J.R., Iwata, M., Eksteen, B., Song, S.Y., Junt, T., Senman, B., Otipoby, K.L., Yokota, H., Takeuchi, P., Ricciardi-Castagnoli, K., Rajewsky, D.H., Adams, U.H., von

Andrian, U.H., 2006. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science 314, 1157-1160.

Moretto, M.M., Lawlor, E.M., Khan, I.A., 2008. Aging mice exhibit a functional defect in mucosal dendritic cell response against an intracellular pathogen, J. Immunol. 181, 7977-7984.

Mowat, A.M., 2003. Anatomical basis of tolerance and immunity to intestinal antigens. Nat. Rev. Immunol. 4, 331-341.

Na, H.J., Park, J.S., Pyo, J.H., Lee, S.H., Jeon, H.J., Kim, Y.S., Yoo, M.A., 2013. Mechanism of metformin: inhibition of DNA damage and proliferative activity in Drosophila midgut stem cell. Mech. Ageing Dev. 134, 381-390.

Nalapareddy, K., Nattamai, K.J., Kumar, R.S., Karns, R., Wikenheiser-Brokamp, K.A., Sampson, L.L., Mahe, M.M., Sundaram, N., Yacyshyn, M.B., Yacyshyn, B., Helmrath, M.A., Zheng, Y., Geiger, H., 2017. Canonical Wnt Signaling Ameliorates Aging of Intestinal Stem Cells. Cell Rep. 18, 2608-2612.

Neish, A.S., Gewirtz, A.T., Zeng, H., Young, A.N., Hobert, M.E., Karmali, V., Rao, A.S., Madara, J.L., 2000. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science 289, 1560-1563.

Newgard, C.B., Sharpless, N.E., 2013. Coming of age: molecular drivers of aging and therapeutic opportunities. J. Clin. Invest. 123, 946-950.

Newton, J.L., Jordan, N., Pearson, J., Williams, G.V., Allen, A., James, O.F., 2000. The adherent gastric antral and duodenal mucus gel layer thins with advancing age in subjects infected with Helicobacter pylori, Gerontology 46, 153-157.

Nicoletti, C., 1994. Antibody response in aged C57BL/6 mice: T helper cells are responsible for the decline of the primary antibody response to a bacterial antigen in aging. Immunobiology 190, 127-137.

Nicoletti, C., 2000. Unsolved mysteries of intestinal M cells. Gut 47, 735-739.

Nicoletti, C., Borghesi, C., Yang, X.H., Schulze, D.H., Cerny, J., 1991. Repertoire diversity of antibody response to bacterial antigens in aged mice. II. Phosphorylcholine-antibody in young and aged mice differ in both VH/VL gene repertoire and in specificity. J. Immunol. 147, 2750–2755.

Nicoletti, C., Cerny, J., 1991. The repertoire diversity and magnitude of antibody responses to bacterial antigens in aged mice: I. Age-associated changes in antibody responses differ according to the mouse strain. Cell. Immunol. 133, 72–83.

Nicoletti, C., Yang, X., Cerny, J., 1993. Repertoire diversity of antibody response to bacterial antigens in aged mice. III. Phosphorylcholine antibody from young and aged mice differ in structure and protective activity against infection with *Streptococcus pneumoniae*. J. Immunol. 150, 543-549.

Nicoletti C., 2015. Age-associated changes of the intestinal epithelial barrier: local and systemic implications. Expert Rev Gastroenterol. Hepatol. 9, 1467-1469

Pearson-Leary J, Zhao C, Bittinger K, Eacret D, Luz S, Vigderman AS, Dayanim G, Bhatnagar S. 2019. The gut microbiome regulates the increases in depressive-type behaviors and in inflammatory processes in the ventral hippocampus of stress vulnerable rats. Mol Psychiatry. Doi: 10.1038/s41380-019-0380-x

O'Toole, P.W., Jeffery, I.B., 2015. Gut microbiota and aging. Science 350, 1214-1215.

Ohlstein, B., Spradling, A., 2006. The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature 439, 470-474.

Ohno, H., 2016. Intestinal M cells. J. Biochem. 159, 151-160.

Okai, S., Usui, F., Yokota, S., Hori-I, Y., Hasegawa, M., Nakamura, T., Kurosawa, M., Okada, S., Yamamoto, K., Nishiyama, E., Mori, H., Yamada, T., Kurokawa, K., Matsumoto, S., Nanno, M., Naito, T., Watanabe, Y., Kato, T., Miyauchi, E., Ohno, H.,

Shinkura, R., 2016. High-affinity monoclonal IgA regulates gut microbiota and prevents colitis in mice. Nat. Microbiol. 1, 16103.

Okuyama, H., Urao, M., Lee, D., Abe, A., Drongowski, R.A., Harmon, C.M., Coran, A.G., 1998. Changes, with age, in the phospholipid content of the intestinal mucus layer of the newborn rabbit. J. Pediatr. Surg. 33, 358.

Ouwehand, A.C., Isolauri, E., Kirjavainen, P.V., Salminen, S.J., 1999. Adhesion of four Bifidobacterium strains to human intestinal mucus from subjects in different age groups, FEMS Microbiol. Lett. 172, 61-64.

Park, J.S., Jeon, H.J., Pyo, J.H., Kim, Y.S., Yoo, M.A., 2018. Deficiency in DNA damage response of enterocytes accelerates intestinal stem cell aging in Drosophila. Aging (Albany NY) 10, 322-338.

Park, J.S., Kim, Y.S., Yoo, M.A., 2009. The role of p38b MAPK in age-related modulation of intestinal stem cell proliferation and differentiation in Drosophila. Aging. 1, 637-651.

Park, J.S., Lee, S.H., Na, H.J., Pyo, J.H., Kim, Y.S., Yoo, M.A., 2012. Age- and oxidative stress-induced DNA damage in Drosophila intestinal stem cells as marked by Gamma-H2AX. Exp. Gerontol. 47, 401-405.

Park, J.S., Na, H.J., Pyo, J.H., Jeon, H.J., Kim, Y.S., Yoo, M.A., 2015. Requirement of ATR for maintenance of intestinal stem cells in aging Drosophila. Aging. 7, 307-318.

Park, J.S., Pyo, J.H., Na, H.J., Jeon, H.J., Kim, Y.S., Arking, R., Yoo, M.A., 2014. Increased centrosome amplification in aged stem cells of the Drosophila midgut. Biochem. Biophys. Res. Commun. 450, 961-965.

Parker, A., Maclaren, O.J., Fletcher, A.G., Muraro, D., Kreuzaler, P.A., Byrne, H.M., Maini, P.K., Watson, A.J., Pin, C., 2017. Cell proliferation within small intestinal crypts is the principal driving force for cell migration on villi. FASEB J. 31, 636-649.

Pattabiraman, G., Palasiewicz, K., Galvin, J.P., Ucker, D.S., 2017. Aging-associated dysregulation of homeostatic immune response termination (and not initiation). Aging Cell 16, 585-593.

Pelaseyed, T., Bergström, J.H., Gustafsson, J.K., Ermund, A., Birchenough, G.M., Schütte, A., van der Post, S., Svensson, F., Rodríguez-Piñeiro, A.M., Nyström, E.E., Wising, C., Johansson, M.E., Hansson, G.C., 2014. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. Immunol Rev. 260, 8-20.

Peters, B.M., Shirtliff, M.E., Jabra-Rizk, M.A., 2010. Antimicrobial peptides: primeval molecules or future drugs? PLoS Pathog. 6, e1001067.

Petrof, E.O., Gloor, G.B., Vanner, S.J., Weese, S.J., Carter, D., Daigneault, M.C., Brown, E.M., Schroeter, K., Allen-Vercoe, E., 2013. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. Microbiome 1, 3-8.

Planer, J.D., Peng, Y., Kau, A.L., Blanton, L.V., Ndao, I.M., Tarr, P.I., Warner, B.B., Gordon, J.I., 2016. Development of the gut microbiota and mucosal IgA responses in twins and gnotobiotic mice. Nature 534, 263-266.

Potten, C.S., Martin, K., Kirkwood, T.B., 2001. Ageing of murine small intestinal stem cells. Novartis Found. Symp. 235, 66-79.

Qi, Y., Goel, R., Kim, S., Richards, E.M., Carter, C.S., Pepine, C.J., Raizada, M.K., Buford, T.W., 2017. Intestinal Permeability Biomarker Zonulin is Elevated in Healthy Aging. J. Am. Med. Dir. Assoc. 18, 810.e1-810.e4.

Regan, J.C., Khericha, M., Dobson, A.J., Bolukbasi, E., Rattanavirotkul, N., Partridge, L., 2016. Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. Elife 5, e10956.

Regoli, M., Bertelli, E., Gulisano, M., Nicoletti, C., 2017. The Multifaceted Personality of Intestinal CX_3CR1^+ Macrophages. Trends Immunol. 38, 879-887.

Regoli, M., Man, A., Gicheva, N., Dumont, A., Ivory, K., Pacini, A., Morucci, G., Branca, J.J.V., Lucattelli, M., Santosuosso, U., Narbad, A., Gulisano, M., Bertelli, E., Nicoletti, C., 2018. Morphological and Functional Characterization of IL-12Rβ2 Chain on Intestinal Epithelial Cells: Implications for Local and Systemic Immunoregulation. Front. Immunol. 9, 1177.

Rera, M., Azizi, M.J., Walker, D.W., 2013. Organ-specific mediation of lifespan extension: more than a gut feeling? Ageing Res. Rev. 12, 436-444.

Rera, M., Clark, R.I., Walker, D.W., 2012. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. Proc. Natl. Acad. Sci. U S A. 109, 21528-21533.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., Ricciardi-Castagnoli, P., 2001- Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. 2, 361-367.

Rey, J., Garin, N., Spertini, F., Corthesy, B., 2004. Targeting of secretory IgA to Peyer's patch dendritic and T cells after transport by intestinal M cells. J. Immunol. 3026-3033.

Rimoldi, M., Chieppa, M., Salucci, V., Avogadri, F., Sonzogni, A., Sampietro, G.M., Nespoli, A., Viale, G., Allavena, P., Rescigno, M., 2005. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. Nat. Immunol. 6, 507-514.

Rudolph, K.L., Chang, S., Lee, H.W., Blasco, M., Gottlieb, G.J., Greider, C., DePinho, R.A., 1999. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96, 701-712.

Santiago, A.F., Fernandes, R.M., Santos, B.P., Assis, F.A., Oliveira, R.P., Carvalho, C.R., Faria, A.M., 2008. Role of mesenteric lymph nodes and aging in secretory IgA production in mice. Cell. Immunol. 253, 5-10.

Sato, T., Clevers, H., 2013. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 340, 1190-1194.

Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., Clevers, H., 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459, 262-265.

Schmidt, A., Heid, H.W., Schäfer, S., Nuber, U.A., Zimbelmann, R., Franke, W.W., 1994. Desmosomes and cytoskeletal architecture in epithelial differentiation: cell type-specific plaque components and intermediate filament anchorage. Eur. J. Cell. Biol. 65, 229-45.

Schmucker, D.L., Daniels, C.K., Wang, R.K., Smith, K., 1988. Mucosal immune response to cholera toxin in ageing rats. I. Antibody and antibody-containing cell response, Immunology 64, 691-695.

Schmucker, D.L., Owen, R.L., Outenreath, R., Thoreux, K., 2003. Basis for the age-related decline in intestinal mucosal immunity. Clin. Dev. Immunol. 10, 167-172.

Schwiertz, A., Spiegel, J., Dillmann, U., Grundmann, D., Bürmann, J., Faßbender, K., Schäfer, K.H., Unger, M.M., 2018. Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease. Parkinsonism Relat. Disord. 50, 104-107.

Seishima, R., Barker, N., 2019. A contemporary snapshot of intestinal stem cells and their regulation. Differentiation. pii: S0301-4681(18) 30147-30156.

Sellin, M.E., Maslowski, K.M., Maloy, K.J., Hardt, W.D., 2015. Inflammasomes of the intestinal epithelium. Trends Immunol. 36, 442-450.

Senda, S., Cheng, E., Kawanishi, H., 1988. Aging-associated changes in murine intestinal immunoglobulin A and M secretions. Scand. J. Immunol. 27, 157-164.

Shan, M., Gentile, M., Yeiser, J.R., Walland, A.C., Bornstein, V.U., Chen, K., He, B., Cassis, L., Bigas, A., Cols, M., Comerma, L., Huang, B., Blander, J.M., Xiong, H., Mayer, L., Berin, C., Augenlicht, L.H., Velcich, A., Cerutti, A., 2013. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals, Science 342, 447-453.

Simon, L., Shine, G., Dayan, A.D., 1994. Effect of animal age on the uptake of large particulates across the epithelium of the rat small intestine. Int. J. Exp. Pathol. 75, 369-373.

Skoczek, D.A., Walczysko, P., Horn, N., Parris, A., Clare, S., Williams, M.R., Sobolewski, A., 2014. Luminal microbes promote monocyte-stem cell interactions across a healthy colonic epithelium. J. Immunol. 193, 439-451.

Song, J., Wolf, S.E., Wu, X.W., Finnerty, C.C., Gauglitz, G.G., Herndon, D.N., Jeschke, M.G., 2009. Starvation-induced proximal gut mucosal atrophy diminished with aging. J. Parenter. Enteral. Nutr. 33, 411-416.

Sovran, B., Hugenholtz, F., Elderman, M., Van Beek, A.A., Graversen, K., Huijskes, M., Boekschoten, M.V., Savelkoul, H.F.J., De Vos, P., Dekker, J., Wells, J.M., 2019. Age-associated Impairment of the Mucus Barrier Function is Associated with Profound Changes in Microbiota and Immunity. Sci. Rep. 9, 1437.

Spindler, V., Meir, M., Vigh, B., Flemming, S., Hütz, K., Germer, C.T., Waschke, J., Schlegel, N., 2015. Loss of Desmoglein 2 Contributes to the Pathogenesis of Crohn's Disease. Inflamm. Bowel Dis. 21, 2349-2359.

Steenstrup, T., Kark, J.D., Verhulst, S., Thinggaard, M., Hjelmborg, J.V.B., Dalgård, C., Kyvik, K.O., Christiansen, L., Mangino, M., Spector, T.D., Petersen, I., Kimura, M.,

Benetos, A., Labat, C., Sinnreich, R., Hwang, S.J., Levy, D., Hunt, S.C., Fitzpatrick, A.L., Chen, W., Berenson, G.S., Barbieri, M., Paolisso, G., Gadalla, S.M., Savage, S.A., Christensen, K., Yashin, A.I., Arbeev, K.G., Aviv, A., 2017. Telomeres and the natural lifespan limit in humans. Aging. 9, 1130-1142.

Stevens, B.R., Goel, R., Seungbum, K., Richards, E.M., Holbert, R.C., Pepine, C.J., Raizada, M.K., 2018. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. Gut 67, 1555-1557.

Stokes, C.R., Soothill, J.F., Turner, M.W., 1975. Immune exclusion is a function of IgA. Nature 255, 745-746.

Suenaert, P., Bulteel, V., Lemmens, L., Noman, M., Geypens, B., Van Assche, G., Geboes, K., Ceuppens, J.L., Rutgeerts, P., 2002 Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. Am. J. Gastroenterol. 97, 2000-2004.

Suzuki, T., Yoshinaga, N., Tanabe, S., 2009. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J. Biol. Chem. 286, 31263-31271.

Tailford, L.E., Crost, E.H., Kavanaugh, D., Juge, N., 2015. Mucin glycan foraging in the human gut microbiome. Front. Genet. 6, 81.

Tan, D.W., Barker, N., 2014. Intestinal stem cells and their defining niche. Curr. Top. Dev. Biol. 107, 77-107.

Tariq, H., Bella, J., Jowitt, T.A., Holmes, D.F., Rouhi, M., Nie, Z., Baldock, C., Garrod, D., Tabernero, L., 2015. Cadherin flexibility provides a key difference between desmosomes and adherens junctions. Proc. Natl. Acad. Sci. U S A. 112, 5395-5400.

Taylor, L.D., Daniels, C.K., Schmucker, D.L., 1992. Ageing compromises gastrointestinal mucosal immune response in the rhesus monkey. Immunology 75, 614-618.

Tepass, U., Tanentzapf, G., Ward, R., Fehon, R., 2001. Epithelial cell polarity and cell junctions in Drosophila. Annu. Rev. Genet. 35, 747-784.

Thoreux, K., Owen, R.L., Schmucker, D.L., 2000. Intestinal lymphocyte number, migration and antibody secretion in young and old rats. Immunology 101, 161-167.

Tran, L., Greenwood-Van Meerveld, B., 2013. Age-associated remodeling of the intestinal epithelial barrier. J. Gerontol. A Biol. Sci. Med. Sci. 68, 1045-1056.

Tremblay, S., Côté, N.M.L., Grenier, G., Duclos-Lasnier, G., Fortier, L.C., Ilangumaran, S., Menendez, A., 2017. Ileal antimicrobial peptide expression is dysregulated in old age. Immun. Ageing 14, 19.

Tsukita, S., Yamazaki, Y., Katsuno, T., Tamura, A., Tsukita, S., 2008. Tight junction-based epithelial microenvironment and cell proliferation. Oncogene 27, 6930-6938.

Tsuruhara, A., Aso, K., Tokuhara, D., Ohori, J., Kawabata, M., Kurono, Y., McGhee, J.R., Fujihashi, K., 2017. Rejuvenation of mucosal immunosenescence by adipose tissue-derived mesenchymal stem cells. Int. Immunol. 29, 5-10.

Turner, J.R., 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9, 799-809.

Ungewiß, H., Vielmuth, F., Suzuki, S.T., Maiser, A., Harz, H., Leonhardt, H., Kugelmann, D., Schlegel, N., Waschke, J., 2017. Desmoglein 2 regulates the intestinal epithelial barrier via p38 mitogen-activated protein kinase. Sci. Rep. 7, 6329.

van Beek, A.A., Sovran, B., Hugenholtz, F., Meijer, B., Hoogerland, J.A., Mihailova, V., van der Ploeg, C., Belzer, C., Boekschoten, M.V., Hoeijmakers, J.H., Vermeij, W.P., de Vos, P., Wells, J.M., Leenen, P.J., Nicoletti, C., Hendriks, R.W., Savelkoul, H.F., 2016.

Supplementation with Lactobacillus plantarum WCFS1 Prevents Decline of Mucus Barrier in Colon of accelerated Aging Ercc1-/Δ7 Mice. Front. Immunol. 7, 408.

van der Geest, K.S., Abdulahad, W.H., Tete, S.M., Lorencetti, P.G., Horst, G., Bos, N.A., Kroesen, B.J., Brouwer, E., Boots, A.M., 2014. Aging disturbs the balance between effector and regulatory CD4+ T cells. Exp. Gerontol. 60, 190-196.

Van der Sluis, M., De Koning, B.A., De Bruijn, A.C., Velcich, A., Meijerink, J.P., Van Goudoever, J.B., Büller, H.A., Dekker, J., Van Seuningen, I., Renes, I.B., Einerhand, A.W., 2006. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology 131, 117-129.

Van Itallie, C.M., Anderson, J.M., 2006. Claudins and epithelial paracellular transport. Annu Rev Physiol. 68, 403-429.

Van Itallie, C.M., Holmes, J., Bridges, A., Gookin, J.L., Coccaro, M.R., Proctor, W., Colegio, O.R., Anderson, J.M., 2008. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. J. Cell. Sci. 121, 298-305.

Varela, E., Muñoz-Lorente, M.A., Tejera, A.M., Ortega, S., Blasco, M.A., 2016. Generation of mice with longer and better preserved telomeres in the absence of genetic manipulations. Nat. Commun. 7, 11739.

Varma, D., Bülow, M.H., Pesch, Y.Y., Loch, G., Hoch, M., 2014. Forkhead, a new cross regulator of metabolism and innate immunity downstream of TOR in Drosophila. J. Insect Physiol. 69, 80-88.

Venkatesh, M., Mukherjee, S., Wang, H., Li, H., Sun, K., Benechet, A.P., Qiu, Z., Maher, L., Redinbo, M.R., Phillips, R.S., Fleet, J.C., Kortagere, S., Mukherjee, P., Fasano, A., Le Ven, J., Nicholson, J.K., Dumas, M.E., Khanna, K.M., Mani, S., 2014. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity 41, 296-310.

Vora, R., Bernardo, D., Durant, L., Reddi, D., Hart, A.L., Fell, J.M., Al-Hassi, H.O., Knight S.C., 2016. Age-related alterations in blood and colonic dendritic cell properties. Oncotarget 7, 11913-11922.

Vrieze, A., Van Nood, E., Holleman, F., Salojärvi, J., Kootte, R.S., Bartelsman, J.F., Dallinga-Thie, G.M., Ackermans, M.T., Serlie, M.J., Oozeer, R., Derrien, M., Druesne, A., Van Hylckama Vlieg, J.E., Bloks, V.W., Groen, A.K., Heilig, H.G., Zoetendal, E.G., Stroes, E.S., de Vos, W.M., Hoekstra, J.B., Nieuwdorp, M., 2012. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 143, 913-916.e7.

Wang, F., Graham, W.V., Wang, Y., Witkowski, E.D., Schwarz, B.T., Turner, J.R., 2005. Interferon-gamma and tumor necrosis factor-alpha synergize to induce intestinal epithelial barrier dysfunction by up-regulating myosin light chain kinase expression. Am. J. Pathol.166, 409-419.

Wijburg, O.L., Uren, T.K., Simpfendorfer, K., Johansen, F.E., Brandtzaeg, P., Strugnell, R.A., 2006. Innate secretory antibodies protect against natural Salmonella typhimurium infection. J. Exp. Med. 203, 21-26.

Willott, E., Balda, M.S., Fanning, A.S., Jameson, B., Van Itallie, C., Anderson, J.M., 1993. The tight junction protein ZO-1 is homologous to the Drosophila discs-large tumor suppressor protein of septate junctions. Proc. Natl. Acad. Sci. U S A. 90, 7834-7838.

Wilson, Q.N., Wells, M., Davis, A.T., Sherrill, C., Tsilimigras, M.C.B., Jones, R.B., Fodor, A.A., Kavanagh, K., 2018. Greater Microbial Translocation and Vulnerability to Metabolic Disease in Healthy Aged Female Monkeys. Sci. Rep. 8, 11373.

Xiao, Z.Q., Moragoda, L., Jaszewski, R., Hatfield, J.A., Fligiel, S.E., Majumdar, A.P., 2001. Aging is associated with increased proliferation and decreased apoptosis in the colonic mucosa. Mech. Ageing Dev. 122, 1849-1864.

Yeaman, C., Grindstaff, K.K., Nelson, W.J., 2004. Mechanism of recruiting Sec6/8 (exocyst) complex to the apical junctional complex during polarization of epithelial cells. J. Cell. Sci. 117, 559-570.

Zerofsky, M., Harel, E., Silverman, N., Tatar, M., 2005. Aging of the innate immune response in Drosophila melanogaster. Aging Cell 4, 103.

Zeuthen, L.H., Fink, L.N., Frokiaer, H., 2008. Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta. Immunology 123, 197-208.

Zhang, S., 2013. Faeces-filled pill stops gut infection. Nature 2013.

Zihni, C., Munro, P.M., Elbediwy, A., Keep, N.H., Terry, S.J., Harris, J., Balda, M.S., Matter, K., 2014. Dbl3 drives Cdc42 signaling at the apical margin to regulate junction position and apical differentiation. J. Cell. Biol. 204, 111-127.

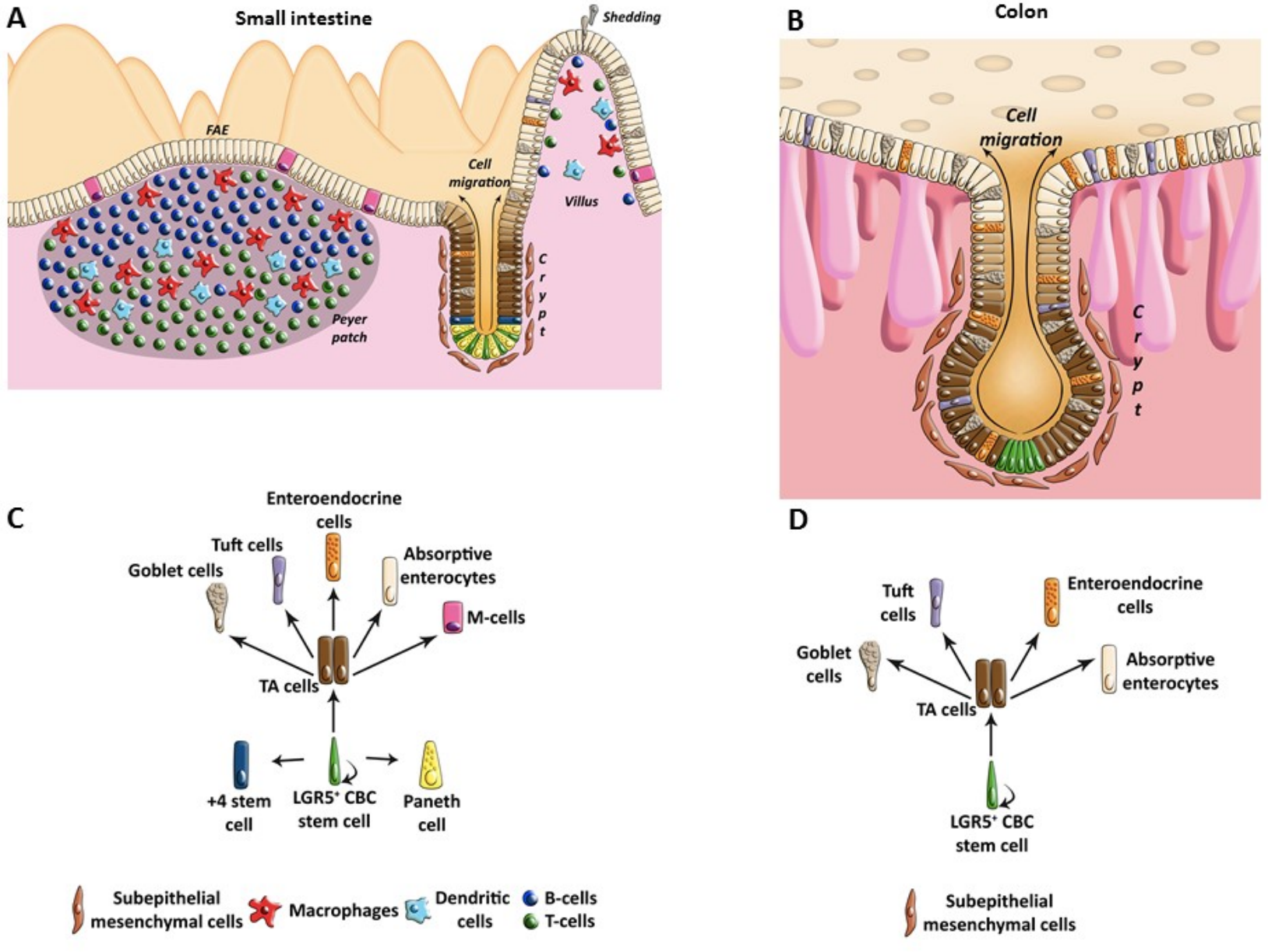
Legends

Figure 1. **Renewal of the intestinal epithelium: the intestinal crypt**. The architecture of the intestinal crypt that houses dedicated populations of stem and progenitor cells that self-renew to maintain epithelial function throughout life differs between the small and large intestine. The small intestine (A) is the main site for nutrient absorption and the presence of villi and microvilli ensures a large surface area for such a critical task. In the colon (C), the flat surface is specialized in absorption of water leading to the formation of faeces. In the small intestine each villus is encircled by at least six crypts of Lieberkühn, while in the colon the mucosal layer is arranged into multiple crypts connected to the flat luminal surface *via* circular openings. The continuous renewal of the epithelium relies on intestinal epithelial stem cells $Lgr5^{+}$ (Leu-rich repeat-containing G protein-coupled receptor 5-expression) located in specialized regions of the intestinal crypt. In the small intestine (B) $Lgr5^{+}$ cells, or crypt base columnar cells (CBC) are interspersed among Paneth cells at the bottom of the crypt and divide to give origin to proliferating transit-amplifying (TA) cells that ultimately differentiate into the absorptive (enterocytes) or secretory (enteroendocrine, Tuft and Goblet cells) lineages. The +4 stem cells that occupy the fourth position counting from the bottom represent a reservoir of stem cells that can replace injured CBC. In the proximity of the Peyer's patches (PPs) the cells emerging from the crypt and moving onto the follicle-associated epithelium (FAE) of the PPs also include the membranous (M) cells specialized in antigen sampling. In the colonic crypt (C), the $Lgr5^{+}$ cells occupy the bottom of the crypt that generate TA cells that, similarly to the small intestine give origin to the various epithelial cells (D), although the proportion of each cell type varied according to the area of the gut. The epithelial turnover is completed in 3-5 days in the small intestine and 5-7 days in the colon. The intestinal crypt niche is completed by mesenchymal and immune cells that provide important signals for cell proliferation and differentiation. In ageing, several changes occurred to the intestinal crypt, affecting cell proliferation, migration and epithelium formation.

Figure 2. **Intestinal barrier integrity in ageing**. An electron micrograph of the intestinal epithelium (A) and detail of the intercellular junctional complex (inset). The intestinal epithelium is formed by a single layer of enterocytes (E) provided with well-organized microvilli (mV) that prevent the adhesion of microbes to the apical domain of the epithelial cells. Intercellular junctional complexes (arrow heads) seal the paracellular space. At the base of the mV the plasma membrane of adjacent enterocytes (inset) is in intimate contact at the tight junction (TJ) level. Here, claudins, zonula occludens (ZO-1), occluding and F-actin filament interact to form a highly integrated complex. Just below the TJ complex the proteins E‐cadherin, α‐catenin 1, β‐catenin, catenin δ1 form the adherens junction (AJ) a structure important for the assembly and function of TJs. Both TJs and AJs are supported by a perijunctional ring of actin and myosin. Beneath the AJ, the desmosome (DE) participates in the sealing of the paracellular space by strengthening the adhesion bonds between adjacent IECs. Similarly to the TJs and AJ, the DE encompasses numerous interacting proteins that include desmoglein, desmocollin, desmoplakin and keratin filaments. In ageing, increased levels of the inflammatory cytokines IL-1β, TNF-α, or IFN-γ can directly affect barrier integrity and intestinal permeability by TJ remodelling at the level of the perijunctional actomyosin cytoskeleton by triggering myosin light chain kinase (MLCK) activity. Age-associated increase of intestinal levels of IL-6 impaired barrier function *via* the JNK-mediated activation of AP-1 that target the claudin-2 promoter region. The up-regulation of claudin-2 affects barrier function by *de novo* formation of pores that allows the paracellular movement of molecules with radii less than 4Å.

Figure 3. **Gut-microbe interaction in ageing: impact on local and systemic homeostasis**. An array of modifications occur at the host-microbe interface in the gut during ageing that involve several key components of the intestinal epithelial barrier. These age-associated alterations are likely to contribute to local and systemic

inflammageing. In young individuals the tight junction (TJs) complexes seal the intestinal epithelial cells (IECs) to provide a barrier to microbes and macromolecules. Also, at steady state intestinal epithelial cells secrete anti-inflammatory cytokines, such as thymic stromal lymphopoietin (TSLP) and transforming growth factors (TGF-β) that help maintain intestinal (mucosal) and systemic immune homeostasis, thus creating the optimal environment for a proper gut-brain axis communication. The advance of age is paralleled by changes in the composition of the microbiome with a decline of microbial diversity. This event is accompanied by the thinning of the mucus layer in certain areas of the gut exposing the intestinal epithelium to an increase barrage of microbial stimuli. In both humans and laboratory animals, the integrity of the epithelial barrier appears compromised with the advancing of age by the reduced/modified expression of key tight junction (TJs) proteins leading to a leaky gut. The continuous influx of microbes and their products, in turn triggers a sustained chronic production of pro-inflammatory cytokines that further contribute to disrupt barrier integrity and increase intestinal permeability. In addition, the activation of IEC-associated components of the inflammasome might contribute to exacerbate local inflammation while at the same time directly participating to the age-associated change of the microbiota composition. Alteration of intestinal and systemic homeostasis has a significant impact on the finely tuned gut-brain axis communication. Ultimately, this could lead to the decline of functions of the central nervous system (CNS) with severe consequences on cognition and behavior. All these events are strongly interlinked; however, currently their exact sequence is not clear and the question of “what is causing what” remains unanswered. Disentangling this intricate scenario is a challenging goal of very high medical relevance in order to devise strategies to improve the physical, neurological and psychological well-being of the elderly.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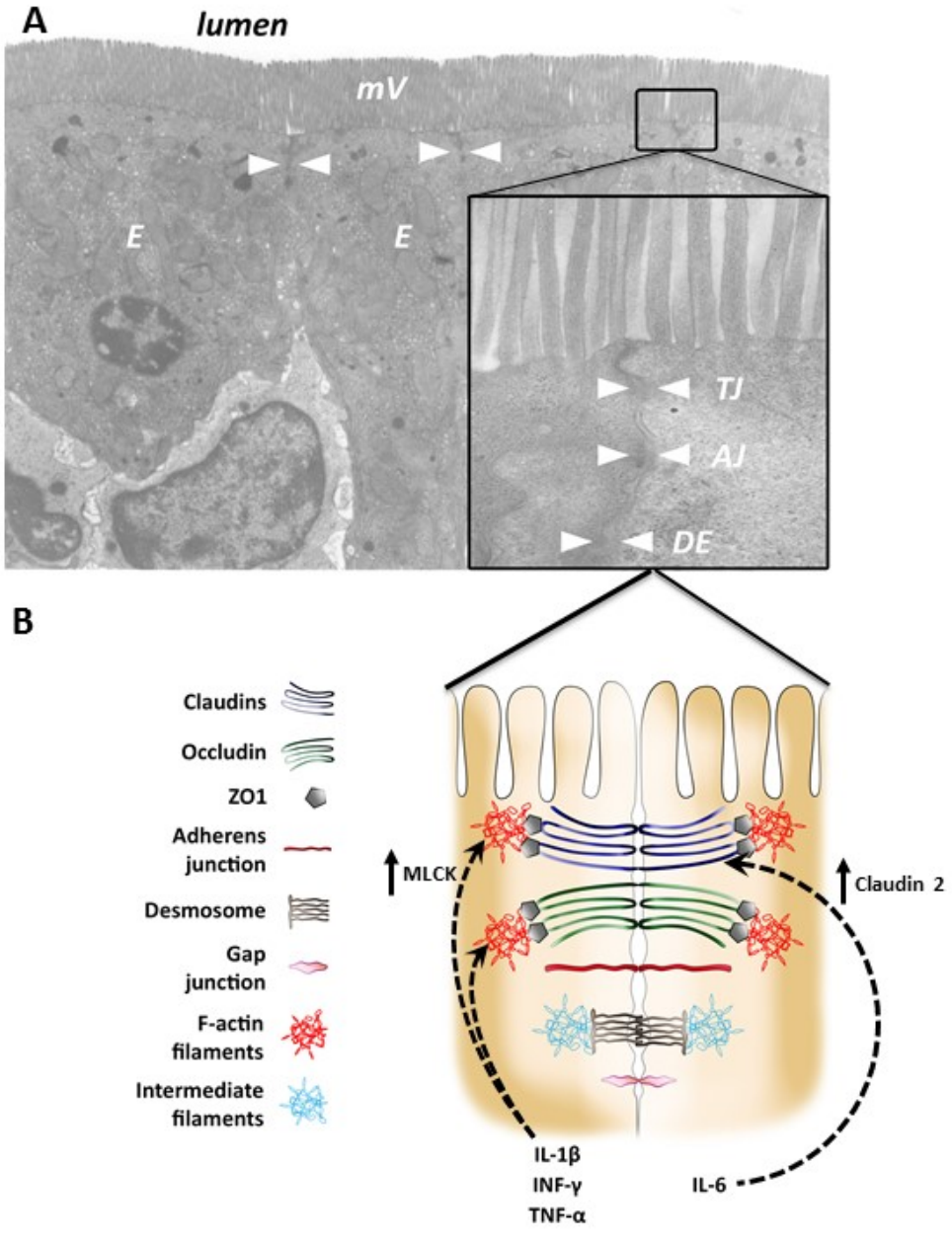
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INF-γ
TNF-α
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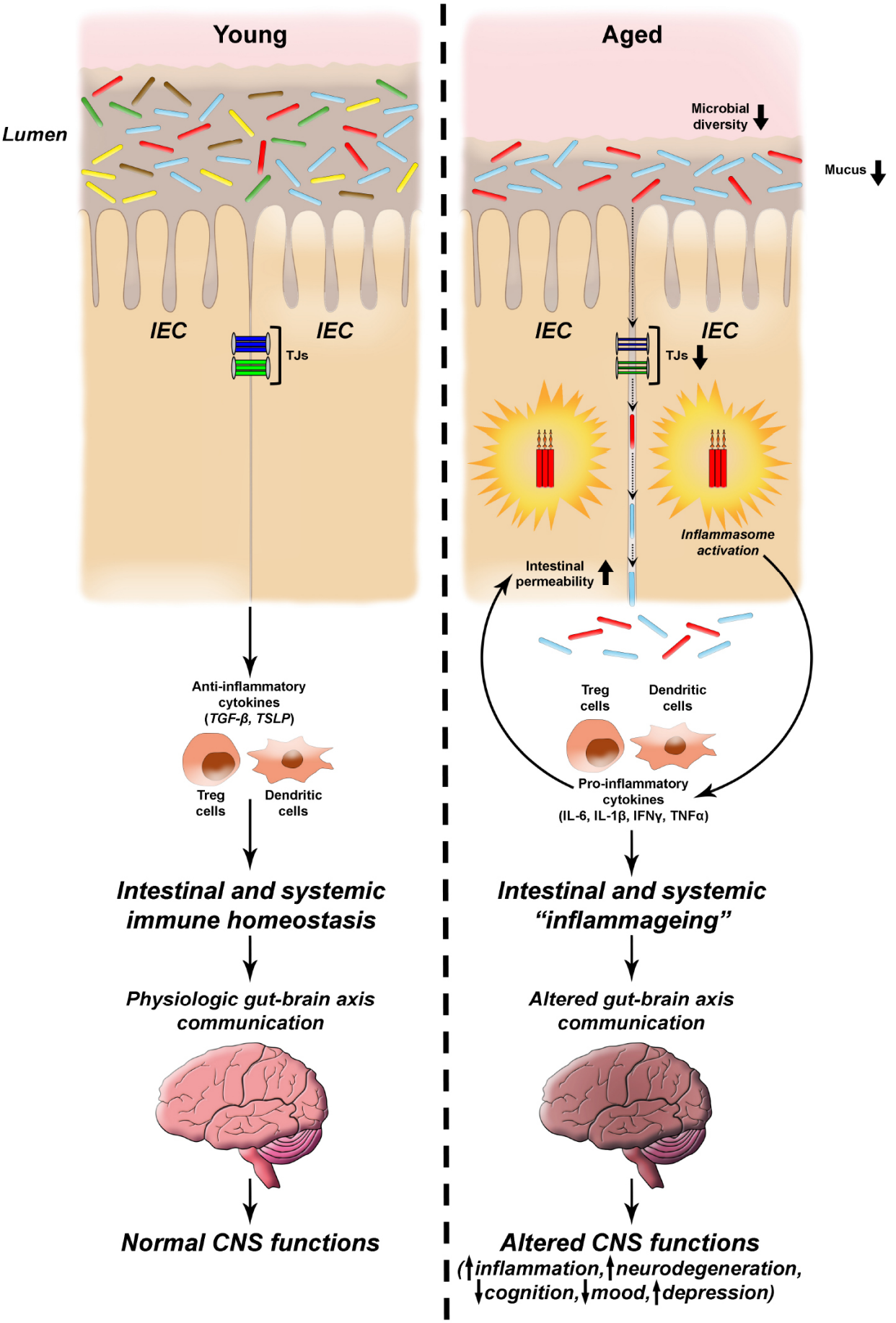
Young
Aged
Lumen
Microbial diversity
Mucus
IEC
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TJs
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IEC
TJs
Inflammasome activation
Intestinal permeability
Anti-inflammatory cytokines (TGF-β, TSLP)
Treg cells
Dendritic cells
Treg cells
Dendritic cells
Pro-inflammatory cytokines (IL-6, IL-1β, IFNγ, TNFα)
Intestinal and systemic immune homeostasis
Intestinal and systemic "inflammageing"
Physiologic gut-brain axis communication
Altered gut-brain axis communication
Normal CNS functions
Altered CNS functions
(↑inflammation, ↑neurodegeneration, ↓cognition, ↓mood, ↑depression)