The "Inner Tube of Life": How Does the Gastrointestinal Tract Age?

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Abstract

The gastrointestinal (GI) tract is the primary and largest area of contact with environmental factors and antigens, and it contains the largest number of immune cells in the body. The intestinal barrier is integral to GI-defense in preventing or limiting exposure of the host and its immune system to luminal antigens. One of the main consequences of this is that such a vast mucosal surface of the intestine requires constant and effective patrolling by a large number of lymphocytes forming the intestinal immune system. Recent advance in the field suggested that alterations of the intestinal epithelial barrier, including its associated immune system, are linked to both local and systemic disorders of various natures. However, like any other system in the body, the various components of the intestinal epithelial barrier, including the immune system deteriorate with the advancing of age; therefore, the identification of the events underlying the ageing process in the gut might have important consequences on health and well-being far beyond the GI-tract. In spite of its critical role in

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maintaining health, up to date very little attention has been given to how ageing affects this critical structure; as a result, our knowledge on the effects of ageing on the physical and immunological properties of the intestinal epithelial barrier is still poor. This chapter describes the impact of ageing on the highly integrated components of the intestinal epithelial barrier; also, the possibility to devise novel strategies to achieve a healthier ageing by targeting the GI-tract is discussed.

Keywords
Ageing · Intestine, gut epithelium · Mucosal immunity · Microbiota · Gut-brain axis

Introduction: The Intestinal Epithelial Barrier and Its Components

The world population is ageing. Due to advancements in science and medicine and improved living standards, the last 25 years has seen a disproportionate increase in the number of people aged 65, which in the UK alone currently total more than ten million. The fastest population increase is seen in the number of those 85 years and over, which by 2050 is projected to double and reach eight million (Smith 2015; Cracknell 2010). These extra years however are not necessarily spent in good health nor free from disability with life expectancy increasing at a faster rate than healthy and disability-free life expectancy. In order to benefit from adding "years to life," it is important that this includes the concept of adding "quality to life," which requires a more complete understanding of the ageing process. Older people suffer from a decline in immune function (immunosenescence) and are at increased risk of infection and disease that target or affect the gastrointestinal (GI)-tract. The GI-tract, also termed "the inner tube of life" (2005) for its critical role in establishing and maintaining health, is protected from injury and invading microbes that are consumed in contaminated food or drinking water by a multilayered barrier: the epithelial barrier. In the past few years, increasing evidence suggested that alterations of the intestinal epithelial barrier, including its associated immune system are linked to both local and systemic disorders of various natures. Indeed, maintaining barrier integrity appeared to be essential for health, and defects in intestinal barrier function can lead to persistent immune activation and contribute to the pathogenesis of intestinal diseases including coeliac disease, colorectal cancer, inflammatory bowel disease (IBD), and metabolic disorders such as obesity and diabetes (Turner 2009; Marchiando et al. 2010). Remarkably, alterations of the intestinal epithelial barrier have been associated also to the central nervous system (CNS)-related disorders myalgic encephalomyelitis, multiple sclerosis (MS), Parkinson's disease, and autism (Aroniadis and Brandt 2013) among others. However, in spite of the numerous and important debilitating health problems that might stem from a malfunctioning gut barrier, up to date little attention has been given to how ageing affects this critical structure, and as a result, our knowledge on the effects of ageing is still limited. Most importantly, the lack of knowledge is particularly profound in humans, and only recently, new data have shed some light on the ageing human gut (Man et al. 2015).

The GI-tract is the primary and largest area (~300 m^2) of contact with environmental factors and antigens, and it contains the largest number of immune cells in the body. The intestinal barrier is integral to GI-defense in preventing or limiting exposure of the host and its immune system to luminal antigens. It is made up of several integrated and interactive components that are physical (the epithelium and mucus), biochemical (enzymes, antimicrobial proteins (AMP), immunological (IgA and epithelia-associated immune cells), and microbial (the microbiota) in nature (Turner 2009; Marchiando et al. 2010). The microbiota is vital for human development and health and in addition to playing a role in pathogen resistance, influences host metabolism providing essential nutrients and vitamins as part of a complex mutualistic relationship that is a product of co-evolution. Signals originating from the microbiota can directly or indirectly affect intestinal barrier integrity and function and ultimately, the balance between health and disease states by influencing mucus production (Jakobsson et al. 2015), epithelial permeability (Maffeis et al. 2016) and turnover (Hausmann 2010), AMP production (Vaishnava et al. 2008), immune cell maturation (Chung et al. 2012), and IgA production (Hooper et al. 2012). Many of these effects are seen during human development and microbial colonization of the neonate (Arrieta et al. 2014) or as a result of dysbioisis.

The immune component of the intestinal epithelial barrier accommodates large numbers of lymphocytes, and it represents the largest branch of the immune system. Indeed the gut-associated lymphoid tissue (GALT) contains nearly 70% of the total lymphocytes in the body (Pabst et al. 2008). Although the number of immune cells inhabiting the intestine have been set by some authors closer to 15–20% of all lymphocytes (Ganusov and De Boer 2007), it is a fact that due to the constant antigenic bombardment the intestinal immune system has to handle an antigen load much larger than that one encountered by the systemic immune system throughout the life. Aggregate lymphoid follicles such as the Peyer's patches (PPs) or isolated lymphoid follicle dispersed along the gut form the inductive sites of the gut immune system, the anatomical locations where the immune responses start **(**Fig. 1**)**. An important feature of both isolated lymphoid follicle and PPs is the presence of highly specialized epithelium, termed the follicle-associated epithelium (FAE). This is characterized by the presence of the antigen-sampling membranous (M) cells (Nicoletti 2000) whose unique role is to capture and transport but not process both particulate and soluble antigens to the underlying immune system. The latter is present within the lymphoid tissue of the PP with all the competent cells necessary for the induction of an antigen-specific response; these include B and CD4+ T cells, antigen-presenting cells (APCs), and macrophage. Distinct subsets of antigen-presenting DCs that are instrumental for T cell activation and regulation (Coombes and Powrie 2008) are located immediately underneath the FAE, within the sub-epithelial dome (SED) area of PPs. The main effector site of the mucosal immune response is the lamina propria (LP), which is populated with different subsets of immune cells mostly $CD8^+$T cells, IgA-producing plasma cells, and mononuclear cells such as macrophages and DCs (Brandtzaeg et al. 2008). In addition, it has become apparent over the past few years that the intestinal epithelium is key to the intestinal homeostasis, elicitation of appropriate immune response, and the

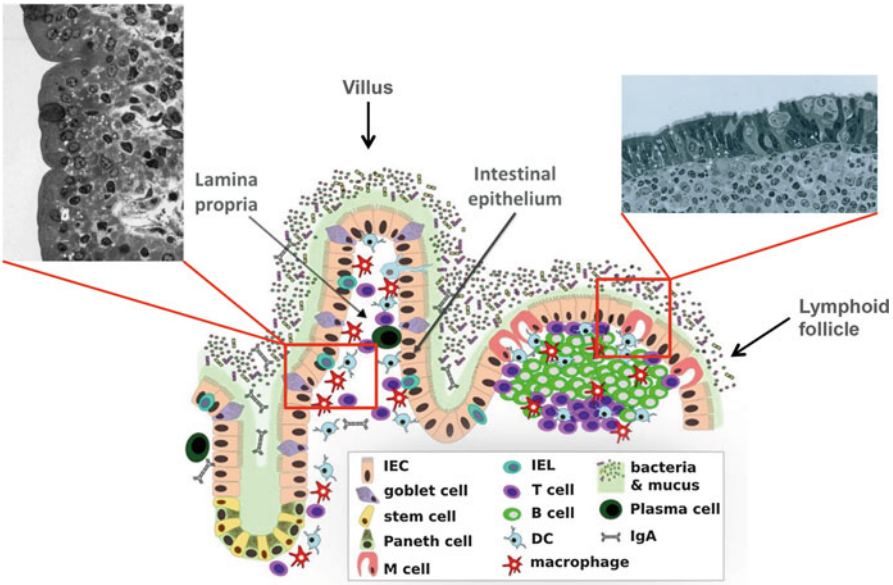

Fig. 1 Schematic illustration of the structure and various components of the intestinal immune system. The microbes (microbiota), intestinal epithelium, and the underlying immune system closely work in a "three-way cross talk" to establish and maintaining intestinal immune homeostasis. In addition to providing a barrier against potentially harmful pathogens and macromolecules, the intestinal epithelium constantly relay signals to the underlying immune system. The presence of pattern recognition receptors (PPRs) on intestinal epithelial cells and antigen transport across follicle-associated epithelium (FAE) by M cells allow the gut immune system to constantly survey the intestinal luminal contents and to generate rapid and effective innate and antigen-specific (adaptive) immune responses to pathogenic organisms. After the initial interaction within lymphoid follicles of the Peyer's patches, the inductive sites of mucosal immunity, antigen-specific B and T cells recirculate to the lamina propria of distant mucosal effector sites. The surface of the epithelium is covered by a mucus layer produced mainly by goblet cells that contains large amount of S-IgA the main role of which is to prevent microbes from crossing the epithelial barrier. Ageing has a detrimental effect on many components of the intestinal immune system that ultimately leads to a reduced ability to mount effective immune responses and to control the local, and possibly systemic, inflammatory status to establish and maintaining intestinal immune homeostasis

composition of the intestinal microbiota (Saenz et al. 2008). The intestinal immune system is separated from the external environment by a single cell layer that forms the intestinal epithelium which contributes to the intestinal immune homeostasis, elicitation of immune responses, and shaping of the gut microbiota.

A hallmark feature of ageing is immunosenescence and the functional decline of the adaptive and innate immune system and compromised immunity to microbial pathogens (Franceschi et al. 2000). This is attributed to an imbalance between inflammatory and anti-inflammatory networks, resulting in low-grade chronic inflammation termed "inflammaging"; it is very likely that events taking place in the GI-tract play a central role in this process.

Ageing and the Intestinal Epithelium

During many years, it has been generally accepted that the main task of the intestinal epithelium overlying mucosal surfaces was to provide a barrier to macromolecules and microorganisms present in the intestinal lumen. More recently, however, it has become evident that the epithelial layer of the gut is not only a formidable physical barrier but also it is fully part of a dynamic three-way cross talk that includes the epithelium itself, the immune system, and the intestinal microbiota (Saenz et al. 2008). The finely tuned interaction between these components is instrumental to discriminate between invasive pathogenic organisms and harmless antigens, including dietary components and microbes that make up the intestinal microbiota. Unfortunately, up to date, the real extent of the age-associated modifications of the intestinal epithelium and their consequences on the immunoregulatory functions of the IECs remains largely unknown. Ethical reasons and technical difficulties intrinsic to the nature of the IECs have hindered major advances in this field. More recently, the development of in vitro intestinal organoids have provided a promising tool to address fundamental questions on IEC biology (Sato and Clevers 2013). However, also this model has its limitations, the most notable being the lack of the large variety of immune-derived signals that are not present in culture but that represent an important component of the real-life intestinal environment.

The intestinal epithelium is a rapidly renewing tissue characterized by a rapid and constant turn over. Epithelial cells are generated within self-renewing "crypts," with stem cells located at the base that crypt that form progenitor cells (Yen and Wright 2006). Stem cell-derived cells proliferate, migrate, and differentiate along the crypt-villus axis and eventually undergo shedding from the surface of the epithelium. This continuous tissue renewal minimize the possibility of accumulating genomic abnormalities; however, an increasing body of evidence suggested that in ageing, the accumulation of molecular changes in long-lived stem/progenitor cells have a significant impact on tissue renewal and regeneration of the intestinal epithelium (Kirkwood 2004). The IECs are an important source of cytokines with the ability to influence the function of a variety of important regulatory cells of the LP, including DCs (Rimoldi et al. 2005) and Treg (Spadoni et al. 2012). The balance between anti- and pro-inflammatory cytokine at systemic level is altered in ageing with a significant increase of the production of pro-inflammatory cytokines, such as IL-6, TNFα, and IL1β that are likely to contribute a chronic low-grade inflammatory status termed "inflammageing" that underpins the development of frailty and increased mortality in the elderly (Morley and Baumgartner 2004). The intestinal epithelium is an important source of "geriatric" cytokines and studies in primates have suggested that the production of IEC-derived cytokine might be affected in ageing. Levels of IFN-y, IL-6, TNFα were significantly upregulated in aged baboons compared to young subjects (Tran and Greenwood-Van Meerveld 2013) with direct impact on important function of the intestinal epithelial barrier such as permeability to macromolecules that was increased in these animals. Also, aged baboons had higher levels of IL-1β in the lower GI-tract (colon) that can also contribute to altered barrier function typical of the aged individual (leaky gut) (Katz et al. 1987). Indeed,

IL-1β via the activation of both canonical and noncanonical NF-κB pathways in IECs triggered intestinal epithelial tight junction (TJ) permeability (Al-Sadi et al. 2010). Thus, it is likely that increased levels of certain pro-inflammatory cytokines might down-regulate the expression, and possibly redistribution of the tight junction (TJ) proteins, zonula occludens (ZO)-1, occludin, and junctional adhesion molecule (JAM)-A (Tran and Greenwood-Van Meerveld 2013). The pattern was found to be different in biopsies from the terminal ileum of humans. Recently, the first study has been reported that shed some light on how ageing affects several aspects of the intestinal epithelial barrier, including levels of pro-inflammatory cytokine, intestinal permeability, and innate immunity to bacterial components in humans (Man et al. 2015). By using biopsy tissues from the terminal ileum of healthy individuals ranging from 7 to 77 years of age, it was observed that expression of the cytokine IL-6, but not other pro-inflammatory cytokines including IFNγ, TNF-α, and IL-1β, significantly increased in the elderly. When trying to identify the major source of the IL-6 production, it became apparent that gut-derived $CD11c^{+}$ DCs constitutively produced significantly higher levels of IL-6 compared to their counterparts from younger individuals. Most importantly, this event had an impact from a functional point of view. Increased intestinal levels of IL-6 directly affected intestinal permeability by upregulating the expression but not the distribution of the TJ protein claudin-2. The latter promotes the formation of pores that allow the paracellular movement of cations and small molecules with radii less than 4 Å (Van Itallie et al. 2008) and therefore leads to a reduced transepithelial electric resistance (TEER) (Man et al. 2015), a measure of intestinal permeability. The discordant observations between humans and nonhuman primates would overall suggest intrinsic differences between distinct areas of the intestine, such as the colon and ileum. The latter hypothesis is supported by the observation that in humans, the regulatory features of DCs varied according to their geographical location in the gut (Mann et al. 2015). Thus, it is possible that while terminal ileum DCs are characterized by increased production of IL-6 in ageing, DCs located in the large intestine will display a broader array of age-related modifications that might lead to more diverse alterations of the local inflammatory status and intestinal permeability. Overall these novel observations might be instrumental in understanding the role of a poorly functioning intestinal epithelial barrier in age-associated disorders both locally and systemically. This notion might have important consequences far beyond the gut. Recently, alterations of barrier integrity and a consequent increase of the intestinal permeability has been directly linked to systemic disorders, including disturbances of the central nervous system, such as Parkinson's disease (Forsyth et al. 2011) and multiple sclerosis (Vrieze et al. 2013).

Host-Microbiota Interaction in Ageing

Accommodating the microbiota is the most daunting challenge for the intestinal epithelium. This is not a passive process; instead, it is achieved by a variety of processes that have evolved to fight infections and protect against the colonization by pathogenic or opportunistic microbes and include various host defence mechanisms. Antimicrobial peptides (AMPs) that are secreted mainly by enterocytes and

Paneth cells (Gallo and Hooper 2012) and the abundant production of mucus by goblet cells (Johansson et al. 2008) that coats the epithelium itself are among the most important factors that contribute to shape the composition of the microbiota. Specific AMPs in addition to targeting pathogens also contribute to shaping the microbiota (Vaishnava et al. 2011). These peptides are produced by specialized epithelial cells in the small intestine known as Paneth cells that are located at the base of the crypts of Lieberkuhn (Elphick and Mahida 2005; Bevins and Salzman 2011). Instead, in the colon, AMP production appears to be a property of conventional colonic enterocytes (Gallo and Hooper 2012). It has been shown that the production of AMPs by systemic peripheral blood mononuclear cells (PBMCs) did not decline in healthy aged individuals (Castaneda-Delgado et al. 2013); however, up to date, the impact of ageing on either qualitative or quantitative changes of AMP production in the gut is still unknown. It has been reported in the human gut that the total number of Paneth cells increased with age (Moorefield et al. 2017), and it is likely that this event might impact on the ability to secrete AMPs and, as a consequence, to select the appropriate host microbiota (Moorefield et al. 2017).

A major unanswered question is how does the interaction between the host and its intestinal microbiota evolve during ageing, and what are the consequences of age-related changes in this interaction for maintaining health and resisting disease? Because of its physical and immunological properties, the mucus produced by goblet cells is likely to be a key player in regulating the epithelium-microbiota interaction. The mucus, whose biological relevance has been neglected for long time, provides an important protection at the host-microbe interface; on top of this, recent evidence also suggested that the mucus plays a central role in selecting and maintaining homeostatic interaction with the gut bacteria (Juge 2012; Johansson et al. 2011). Similarly to IECs, up to date, our understanding of the impact of ageing on the mucus layer is rather limited. The number of goblet cells does not decline in the colon of aged mice (Kobayashi et al. 2013), and in healthy humans, it has been observed that the thickness of the gastric and duodenal mucus layers do not change with age (Valenkevich and Zhukova 1976). These preliminary observations in mice and humans support the hypothesis that the ageing process does not affect the mechanical protection afforded by the mucus layer, at least in these two locations. However, it is important to keep in mind that also changes in the chemical composition and structure of the mucus during ageing might affect the contribution of the mucus to shaping the intestinal microbiota. Indeed, molecules, such as mucin-binding proteins (MUBs) (Etzold et al. 2013), mediate the binding of intestinal bacteria to mucus, and these mucus-bacteria interactions are critical to allow the colonization of certain microbial species. In line with this hypothesis is the observation that bacterial adhesion to mucus changes with age; in particular, adhesion of bifidobacteria strains to mucus was found to be significantly reduced (Newton et al. 2000; He et al. 2001; Ouwehand et al. 1999). Although the mechanism underpinning this event remains to be determined, taken together these considerations make it plausible, for example, to hypothesize that alterations of mucus glycosylation that might occur with age might play an important role in the recently described alterations of the microbial community in the elderly (Claesson et al. 2011).

Age-associated quantitative and qualitative changes of the production of mucus in the gut might be biologically relevant also for the intestinal immune homeostasis. Mucus possesses properties that affect the immunological environment of the gut by shaping the regulatory features of DCs (Shan et al. 2013). Thus, changes of the mucus chemistry, during ageing, would have consequences relevant to both microbiota composition and possibly on the inflammatory status of the gut.

Antigen Sampling

Membranous, originally microfold (M), cells (Owen and Jones 1973) of the FAE of the Peyer's patch are the most important route for antigen sampling. These cells transport luminal antigens across the FAE and deliver them to the underlying immune system. Antigens are then "checked out" by the immune machinery that carries out a constant activity of immune surveillance of the luminal contents. Functional maturation of M cells appears to be significantly affected by the ageing process. In mice, ageing was accompanied by a significant reduction in the number of glycoprotein 2 $(GP2)^+$ M cells (Kobayashi et al. 2013) that led to a deficiency in the transport of particulate antigen across the epithelium. In addition, the age-associated decline of the number of Spi-B^+ cells, (Kanaya et al. 2012) also impacted on M cell activity by contributing to impaired downstream functional maturation of M cells (Kobayashi et al. 2013). Additional age-associated changes of the cell populations inhabiting the PPs had an impact on M cells' number and function. Reduced production of the chemokine CCL20 in ageing results in a decline of the number of $CCR6^+CD11c^+$ B cell recruited within the PP lymphoid tissue. This B cell subset plays an important role in the functional maturation of FAE-M cells, and the reduced influx of these B cell subsets towards the FAE ultimately resulted in a reduction of the number of mature M cells in aged mice (Kobayashi et al. 2013). M cell function is also under the regulation of cytokines. Following bacterial challenge, $CD11c^+$ cells inhabiting the SED area start producing the cytokine macrophage migration inhibitory factor (MIF) that rapidly led to an increase in number and function of operational M cells (Man et al. 2008). However, at present, the impact of ageing on the production of (MIF) in the gut has not been investigated. The relevance of age-associated decline of antigen transport across the FAE of the PPs is also highlighted by its contribution to impaired development of oral tolerance to protein observed in ageing mice (Kato et al. 2003a). Also, a smaller number M cells located outside the FAE of PP, termed villous-associated M cells, have been observed (Jang et al. 2004). However, also in this case, up to date, the effect of ageing on the number and maturation process of this distinct M cells subset has not been investigated.

The importance of antigen-sampling in the monitoring of the contents of the intestinal lumen and the beginning of mucosal immune response is further stressed by the presence of an additional, M cell-independent mechanism to transport luminal antigens across the intestinal epithelium. Indeed, a LP-CX_3CR1^+DC/macrophage-based mechanism has been described (Rescigno et al. 2001). Although the real biological role of these cells have been recently questioned (Man et al. 2017; Regoli

et al. 2017), these cells are thought to sample luminal antigens by extending out dendrites between IECs. At present, though the impact of ageing on DC/macrophage-mediated sampling is not known.

Age-Associated Changes of Intestinal Immunity

Innate Immunity

A critical component of immunity to pathogens is provided by the rapid response carried out by the innate immune system. Recently, it has been shown that the initial innate immune response to pathogenic stimuli declined in the elderly (Man et al. 2015). The production of IL-8, a critical cytokine in the early stage of anti-bacterial immunity, significantly declined across life (children>adults>elderly) in response to a pathogenic stimulus, such as flagellin. Interestingly, the expression of flagellin-specific TLR5 on IECs remained unchanged in ageing. The latter observation suggested that the progressive age-dependent decline in production of IL-8 is due to alteration of intracellular signalling pathways following the engagement of flagellin with TLR5. Ultimately, it is likely that reduced levels of IL-8 may play an important role in the increased susceptibility of the elderly to infections. However, the same study (Man et al. 2015) also shows that innate immune response to bacterial product may or may not decline, possibly depending on the nature of the antigenic stimulus. Indeed, while epithelial production of IL-8 in response to flagellin progressively declined across life and it is significantly compromised in ageing, the production of TNFα in response to a more complex microbial challenge, such as live VSL#3 probiotic mixture, did not show significant variation between the age groups. The latter finding is of potential interest. First, although studies conducted in mice and cell lines have shown that probiotics promoted gut health by inducing the production of the pro-inflammatory cytokine TNFα, rather than its suppression (Pagnini et al. 2010), their effect on the human gut was still unknown. Second, very little attention has been given so far to how the intestinal response to probiotics varies between individuals of different age. These results demonstrated that probiotics, or at least certain probiotic strains, induced the production in the human gut of TNFα, which plays an important role in preventing/ameliorating ileitis in mice (Pagnini et al. 2010), and that age does not influence the production of a cytokine required for the beneficial effects of probiotics. The identification of the triggering events affecting aspects of the epithelial barrier integrity and intestinal epithelial innate immune response to certain pathogenic stimuli is a goal of certain medical relevance and may provide important information to improve resistance to infections in the elderly.

Adaptive Immunity

Antigen transported by M cells across the epithelial barrier to the inductive sites is the first critical step in the induction of mucosal and systemic immune responses.

After reaching the lymphoid tissue, antigen is dealt with by DCs, professional APCs that are crucial for the presentation of antigen to immunocompetent B and T cells, and in the induction of peripheral tolerance (Coombes and Powrie 2008). DC priming of T cells in ageing appeared to be compromised. Suboptimal T cell priming to *Encephalitozoon cuniculi* in aged mice was improved/restored by using MLN-DCs from young animals to stimulate aged T cells, thus suggesting an age-related defective APC function of DCs (Moretto et al. 2008). Yet, modification of the cytokine repertoire produced by aged DCs was reported. Aged DCs produced significantly less amount of the cytokines IL-12 and IL-15 and showed an impaired expression of the CD80/CD86 co-stimulatory molecules. Importantly, defective production of IL-15 and reduced levels CD80/CD86 by DCs seemed to be closely linked. The exogenous application of IL-15 to MLN DC from aged mice restored the expression of CD80/CD86 and in so doing significantly improved the ability of the aged DCs to activate T cell (Moretto et al. 2008).

Others have suggested that age-associated modifications of DCs functions varied according to the compartment of the intestinal immune system. Changes of the expression of CD86 was reported in MLN-derived DCs whereas no difference was observed between both LP- and PP-derived DCs (Santiago et al. 2011). However, changes in the expression of CD86 does not necessarily reflect changes in function, and currently, it is not clear whether DCs from LP and PPs of aged mice also differ in their ability to prime T cells. Furthermore, in young mice, freshly isolated DCs from the PPs display distinct regulatory features compared to DCs isolated from the systemic (spleen) immune system. Gut-derived DC were found to prime T cells to produce significantly lower amount of IFN-γ (a canonical T_H1 cytokine) compared to splenic DCs. This differential activity was mediated either directly via the production of TGF-β or indirectly via the priming of TGF-β producing T cells (Iwasaki and Kelsall 1999). By contrast, PP-derived DCs from ageing mice failed to induce TGF-β secretion and differentiation of $CD4^+CD25^+LAP^+$ T cells; this was paralleled by an increased production of IFN-γ by T cells (Santiago et al. 2011). The differential regulation of TGF-β and IFN-γ producing T cells could have far-reaching consequences for the immune regulation during ageing. First, TGF-β plays an important role in the optimal IgA-B cell differentiation (Cazac and Roes 2000) and effector functions of regulatory T cells (Treg) induced after low-dose antigen feeding (Friedman and Weiner 1994). Thus, a defect in the production of this cytokine could explain age-associated reduced levels of IgA (Fulton and Cuff 2004; Thoreux et al. 2000) and difficulties to establish oral tolerance (Kato et al. 2003b). Secondly, increased production of IFN-y by T cells implies a more pronounced pro-inflammatory activity of gut-DCs in ageing; this is likely to have a direct bearing on the development of the systemic low-grade chronic inflammation or "inflammaging" typical of the aged organism.

The lack of immunological tolerance in ageing has been attributed to reduced number and function of DCs inhabiting the SED area (Kato et al. 2003b). A significant decline of $CD11c^+$DCs in the SED were in fact observed in mice >1 year of age. Importantly, this was paralleled by a similar pattern for germinal center follicular DCs (FDC). These data taken together led the authors to suggest that

the problematic induction of oral tolerance in ageing might be associated to modifications of the DC/FDC function. It is surprising that many more mechanistic studies of DC function have not been undertaken so far, although data collected so far point to a significant role of this cell type in the age-associated decline of immune effectiveness.

The abundant production of secretory immunoglobulin A (S-IgA) at mucosal surfaces is one of the most remarkable adaptation of the mucosal immune system. The main role of S-IgA produced by LP-plasma cells represents, together with the mucus layer and AMPs, the first line of defense against pathogens. In addition to this, S-IgA also play important roles in host-microbe interaction that include establishing and maintaining the intestinal microbiota community (Macpherson and McCoy 2013). S-IgA critically mediate the immune exclusion of bacteria and viruses (Asahi et al. 2002; Wijburg et al. 2006) and microbiota (Macpherson and Uhr 2004; Sait et al. 2007) and control limited entry of antibody-coated bacteria across M cells (Rey et al. 2004) and in so doing help to modulate the local immune responses in an environment that tends to limit pro-inflammatory response. The first step in the generation of antigen-specific S-IgA response is the encounter of antigen with the immune system of the PPs (Craig and Cebra 1971). A large array of cells and cytokines plays a role in determining the fate of B cells within the PPs and provide the necessary environment for the IgA isotype switching and subsequent relocation (homing) of IgA producing B cells to the LP at distant mucosal sites. Among others, the cytokine TGF-β (Cazac and Roes 2000) and the combination of retinoic acid (RA) and IL-6 are of paramount importance to promote both IgA production (Mora et al. 2006) and the expression of gut-homing molecules on IgA+ plasma cells to the gut LP (Agace and Persson 2012). Currently, the impact of ageing on many of the factors that are critical for an optimal IgA response remains to be determined. The activation of the RA signalling pathways in PBMC declines in ageing (Feart et al. 2015); however, whether this also applies to the GI-tract is not known. Given the critical role of the RA in the gut mucosa, it is likely that a marked decline could have significant consequences on a variety of aspects that pertain to intestinal immune homeostasis, including disruption of the gut microbiota via downregulation of T_H17 cells (Cha et al. 2010). Overall, it would appear that like other components of the mucosal immune system the S-IgA response is negatively affected by the ageing process (McDonald et al. 2011). However, the mechanisms underlying the decline of the production of S-IgA are not clear. Intestinal levels of S-IgA in response to oral administration of cholera toxin significantly declined in aged mice (McDonald et al. 2011), rats (Schmucker et al. 1988), and nonhuman primates (Taylor et al. 1992). It remains to be determined, however, whether this reflects a reduction of the total number of IgA-producing B cells or an impaired migration (homing) from inductor to effector sites of IgA-secreting cells as suggested. Indeed, the decline of the expression of certain homing-critical molecules have been observed in ageing (Ogino et al. 2004).

The investigations of the magnitude of S-IgA response, at times, have brought about surprising results. Indeed, in various experimental systems, it has been observed that IgA antigen-specific response displays unchanged or even increased

levels of IgA in the serum and intestine (Arranz et al. 1992; Haq and Szewczuk 1991; Senda et al. 1988). The reason for this variability is not known. One possibility is that the magnitude of the IgA response in ageing varies according to the nature of the antigen used. A parallel can be drawn by the analysis of cytokine production by intestinal biopsies from individuals of different age following challenge with different antigens. While the production of IL-8 in response to challenge with flagellin significantly declined in tissue from elderly individuals, levels of TNF-α in response to different bacterial antigens did not change in tissues from young, adult, or aged individuals (Man et al. 2015).

When examining the antibody response in ageing, it is of paramount importance to take into account changes in the quality of the antibody molecules rather than simply the magnitude of the response. At systemic level, the amount of antigen-specific antibody response to a pneumococcal vaccine depends on the genetic makeup of the mouse ranging from increased to significantly reduced (Nicoletti and Cerny 1991). However, anti-pneumococci antibody response in aged mice was characterized by an increased heterogeneity of the V_H gene repertoire (Nicoletti et al. 1991) and reduced antigen affinity (Nicoletti et al. 1993). Thus, in addition of being structurally different, they were less effective in combating pneumococcal infection and failed to protect the recipients compared to "young" antibodies, independently of the mouse strain of origin (Nicoletti et al. 1993).

Currently, whether S-IgA from aged mice differs from their young counterpart in their efficacy to protect the intestinal mucosa remains to be determined. The modality of the IgA production in ageing has been recently assessed via a high throughput sequencing analysis (Lindner et al. 2012). Overall, it was reported that young mice displayed a lower frequency of IgA somatic mutation and age-associated changes of the complementarity-determining region 3 (CDR3) sequence distributions. Furthermore, the analysis of more than one million V_H sequences also showed that the IgA repertoire comprised both highly expanded and low frequency clones, and differences were observed between young and aged mice. In young mice, the vast majority of the clones forming the repertoire comprised expanded clones that were evenly distributed along the small intestine. By contrast, in ageing mice, the repertoire diversity was the result of the ongoing accumulation of low frequency clones and microbiota-, T cell-dependent but PP-independent hypermutations.

Future Directions: Targeting the Gut for a Healthier Ageing?

Experiments in *C. elegans* and *Drosophila* have shown that the intestine represents potentially an important target for intervention in order to promote longevity (Libina et al. 2003; Rera et al. 2013). This notion, given the fact that most of the genetic factors involved in defining the life span are conserved in all vertebrates (Kenyon 2010), makes it plausible to hypothesize that interventions that aim to restore both physical and immunological properties of the gut barrier could be beneficial to ageing humans as well. The gut represents a complex system, the development

and function of which is determined by a finely tuned interaction between the host and the microbiota. It is then possible that the profound changes in the intestinal microbiota observed in ageing (Claesson et al. 2011) will significantly contribute to compromise the intestinal barrier. Currently, how the interaction between the host and its intestinal microbiota evolves during ageing and what are the consequences of age-related changes in this interaction for maintaining health/resisting disease remains to be determined. Nonetheless, the possibility of restoring a fully operational epithelial barrier via manipulation of the intestinal microbiota is certainly very appealing. What are the options currently available? Some studies have already suggested a potentially important role of probiotics, prebiotics and their combination (symbiotics) to reverse dysbiosis and overall improving parameters of intestinal barrier integrity and local and systemic inflammation. However, the success of this approach has been highly variable mainly due to the application of individual probiotic strains that can elicit strain-dependent effects on the host. To this end, the implementation of carefully designed studies with the aim of dissecting in details the mechanisms underlying the effects of probiotic and prebiotics on the microbiota, the gut epithelium, and immune system, is of paramount importance. Recently, the success of fecal microbial transplant (FMT) in treating *C. difficile* infection and ameliorating certain gut pathologies (Rossen et al. 2015) has certainly broadened the options available to intervene more drastically on the composition/profile of the microbiota. Importantly, the interest raised by FMT has recently led to significant improvements in the way faecal material or "stool substitute" (Petrof et al. 2013) could be effectively delivered in the near future by "bacterial pills" in an easier, safer, and more acceptable way as recently suggested (Zhang 2013). These approaches could be integrated by dietary strategies that include avoidance of high amounts sugar and fat and poorly absorbed short-chain carbohydrates (FODMAPs) that directly affect intestinal permeability and microbiota composition (Ahmad and Akbar 2015).

Understanding the role of the intestinal epithelial barrier and immune system in shaping the intestinal microbiota may prove beneficial for an array of debilitating disorders in the elderly. Indeed, animal experiments and human intervention studies have shown the existence of a bidirectional relationship between the gut and brain (the gut-brain axis) and aspects of behavior and cognitive function (Bercik et al. 2011; Bravo et al. 2011; Nishino et al. 2013) **(**Fig. 2**)**. This notion opens the way to pursuing the fascinating hypothesis that identification of molecular and cellular events regulating host-microbe cross talk at the mucosal interface in the gut might help to design strategies to improve psychological well-being late in life. For example, novel approaches including manipulation of the intestinal microbiota via either FMT or probiotics supplementation could be devised to combating geriatric anxiety and depression (Maes et al. 2013; Stanton and Kohn 2012) and possibly improving mood and appetite, the latter being one of the leading cause of malnutrition in the elderly (Volkert 2013).

A positive outcome on behavior/mood and cognitive ability of the elderly following intervention to restore the integrity of the intestinal epithelial barrier is a goal of very high medical and socioeconomic relevance achievable in the near future.

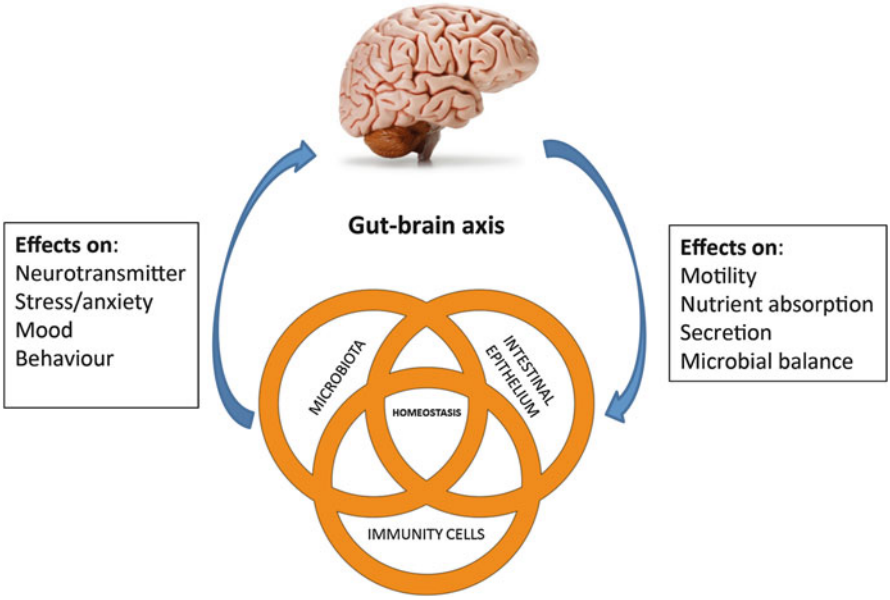

Fig. 2 There is increasing evidence of the ability of events taking place at the mucosal interface in the gut to influence bidirectional neurohumoral communication between the GI-tract and brain (the gut-brain axis). Studies in animals suggest an association between GI-tract microbiota and levels of depression and anxiety that may have potential in developing therapeutic interventions in humans. The gut-brain axis consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with intestinal functions. Insights into the gut-brain cross talk have revealed a complex communication system that not only ensures the proper maintenance of gastrointestinal homeostasis but also likely to have multiple effects on affect, motivation, and higher cognitive functions. This notion is of particular significance in the ageing society of today. The possibility of acting on the components of the "three-way cross talk" in the gut: the microbiota, the gut epithelium, and immune system open the way to pursuing the fascinating hypothesis. The identification of molecular and cellular events regulating host-microbe interaction in the gut might help to design strategies to improve age-related disturbances such as geriatric depression, anxiety, and malnutrition

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