The impact of ageing on the intestinal epithelial barrier and immune system

Angela L. Man, Nadezhda Gicheva, Claudio Nicoletti

Gut Health and Food Safety Institute Strategic Programme, Institute of Food Research, Norwich, United Kingdom

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1. Introduction

Ageing is an ill-defined process involving changes in various body systems which converts a mature, fit person into an increasingly infirm one. With the passage of time, individuals show a lower degree of adaptation with consequent increase in mortality, due to increased incidence of cancer and infectious disease [1,2], as well as a decline of mental health, wellbeing and cognitive abilities [3–5]. Advancements in science and medicine, and improved living standards have led to an increase of the ageing population in Western societies. For example, latest figures show that 10 million people in the United Kingdom are over 65 years old, estimating that there will be 5½ million more elderly people in 20 years’ time, with that number nearly doubling to around 19 million by 2050 [6]. This predicted increased life expectancy of the population has significant social and economic consequences. However, new research suggests that extending life span while reducing the prevalence of comorbidities is a realistic goal, and that developing strategies to delay ageing can produce significant economic

benefit, in excess of $7 trillion over a period of 50 years [7]. These data provide a compelling argument to increase our understanding of the ageing process, in order to improve the quality of life of an ever increasing segment of the population worldwide, and alleviate the economic burden associated to it.

One of the most important effects of the ageing process is a significant decline of the efficacy of both the adaptive and innate immune systems [8,9]; in particular, ageing has a profound influence on the intestinal immune system, and it would appear that age-associated alterations arise in the mucosal immune system of the GI tract earlier than in systemic immune compartments [10]. Although our knowledge of the age-related changes of the systemic immune system has improved over the past few years, our understanding of the mechanisms of immunosenescence of the intestine is still largely incomplete. The aim of this review is to summarise the age-associated changes affecting the different stages of the gut immune response, from the early events occurring at the mucosal interface mediated by various components of the epithelial barrier, to the generation of antigen-specific immune responses.

2. Introduction to the intestinal immune system

It is generally accepted that the GI tract represents the largest immunologic organ in terms of numbers of lymphocytes and at any given time, the gut-associated lymphoid tissue (GALT) can

Abbreviations: GI, gastrointestinal tract; GALT, gut-associated lymphoid tissue; PP, Peyer’s patch; M cell, microfold cell; FAE, follicle-associated epithelium; SED, subepithelial dome; LP, lamina propria; AMP, anti-microbial peptides; MLN, mesenteric lymph node; PRR, pathogen recognition receptor.

* Corresponding author. Address: Gut Health and Food Safety Programme, Institute of Food Research, Colney, Norwich NR4 7UA, United Kingdom.
E-mail address: claudio.nicoletti@ifr.ac.uk (C. Nicoletti).
accommodate nearly 70% of the total lymphocytes in the body [11]. Although this figure has been questioned by estimating the real number of lymphocytes in the gut to be closer to 5–20% of all lymphocytes [12], it is without doubt that the intestinal immune system copes daily with an antigen load that surpasses the one encountered by the systemic immune system during a lifetime. In the intestine, the immune system is present as isolated follicles scattered throughout the gut, or as aggregate follicles such as Peyer’s patches (PPs) in the small intestine [13] (Fig. 1). These structures form the inductive sites of the gut immune system, the locations where antigen-specific immune responses have their origin. PPs essentially harbour all the immune competent cells necessary for induction of an antigen-specific B and T cell response; antigen presenting cells (APCs) such as dendritic cells (DCs) and macrophages, B cells, and CD4+ T lymphocytes. The sub-epithelial dome (SED) area of PPs is the lymphoid microenvironment that is home to distinct subsets of DCs essential for T cell activation and regulation [14,15]. A unique adaptation of the specialised follicle-associated epithelium (FAE) of the PPs is the presence of antigen sampling microfold (M) cells which take up and transport antigens to the underlying immune machinery [16]. In contrast to the inductive sites, the effector sites consist of different anatomical compartments, such as the lamina propria (LP) populated with T cells, mostly CD8+ T cells, IgA-producing plasma cells, and mononuclear cells such as macrophages and DCs [17,18]. The intestinal immune system is separated from the external environment by a single cell layer that forms the intestinal epithelium whose importance in contributing to the intestinal immune homeostasis, elicitation of immune responses and shaping of the gut microbiota has become apparent over the past few years [19–21].

3. The intestinal epithelium in ageing

Although the main task of the epithelium overlying mucosal surfaces of the intestinal tract is the provision of an effective barrier to the vast majority of macromolecules and microorganisms present in the intestinal lumen, it has become evident in the past few years that the epithelial layer is much more than a mere physical barrier. Indeed, intestinal epithelial cells (IECs) engage in a dynamic cross-talk with the intestinal immune system [22,23] that helps to achieve the task of discriminating between invasive pathogenic organisms and harmless antigens, such as food, and the large number of microbes that make up the intestinal microbiota. In spite of its critical role, so far the effect of ageing on epithelial cell immune function has not been addressed in detail. The intestinal epithelium is a rapidly renewing tissue which sheds significant numbers of cells each day. It is organised into self-renewing ‘crypts’, with colonic stem cells at the crypt-base dividing asymmetrically to self-renew and form progenitor cells [24]. The progeny proliferate, migrate, differentiate and undergo shedding from the surface of the epithelium. Perpetual tissue renewal should...
minimise the accumulation of genomic abnormalities, but increasing evidence suggested that age-related molecular changes do accumulate in long-lived stem/progenitor cells, thus implying that tissue renewal and regeneration are compromised in the ageing intestinal epithelium [25,26]. Cytokine production is an important attribute and effector function of IECs that influence the activity of various cell types in the intestinal mucosa such as DCs and Treg cells [20,27]. Age-related changes in systemic cytokine production have led to the suggestion that the balance between inflammatory and anti-inflammatory cytokines is altered during ageing to favour excessive production of “geriatric” pro-inflammatory cytokines, such as IL-6, TNFα and IL-1β [1], and that this underpins the development of frailty and increased mortality in the elderly [28,29]. Indeed, IECs act as a significant source of “geriatric” cytokines in the GI tract and recently, animal studies showed that ageing may significantly affect the production of IEC-derived cytokines. Colonic biopsies from old baboons exhibit up-regulation of microRNA, miR-29a, and the inflammatory cytokines IFN-γ, IL-6, and IL-1β [30]. Increased levels of some of these cytokines may have a direct bearing on some of the features of the aged gut, such as increased permeability of the epithelial barrier (“leaky gut”) [31–33]; indeed, it has been shown that IL-1β caused an increase in intestinal epithelial tight junction (TJ) permeability, via activation of both canonical and non-canonical NF-κB pathways in IECs [34]. In turn, this led to reduced level of expression of the TJ proteins, zonula occludens (ZO)-1, occludin and junctional adhesion molecule (JAM)-A [30]. Interestingly, additional studies in Drosophila demonstrated that impairment of intestinal barrier function predicted age-onset mortality [35]. It is important to highlight that a defective intestinal barrier may have important consequences far beyond the gut; recently, it has been hypothesised that lack of barrier integrity might also be linked to systemic degenerative disorders affecting the central nervous system, such as Parkinson’s disease [36] and multiple sclerosis [37].

4. Interaction between the gut epithelium and the microbiota

Accommodating the vast microbiota is possibly the biggest challenge facing the intestinal epithelium. This is not a passive process, since it involves various host defence mechanisms which have evolved to regulate the composition of the microbiota, and to protect against infection and colonisation by pathogenic or opportunistic microbes. The primary mediators of this activity are various anti-microbial peptides (AMPs) that are secreted by IECs [38,39] and the mucus that coats the epithelium itself [40]. Members of the two major families of mammalian AMPs, defensins (cryptidins in the mouse), and cathelicidins are expressed in the GI tract [39,41]. Specialised epithelial cells in the small intestine known as Paneth cells, reside at the base of the crypts of Lieberkühn, and are the major source of AMPs [42]; whereas in the colon, AMP production appears to be a general property of colonic enterocytes [43]. In addition to targeting pathogens, AMPs also contribute to shaping the microbiota [44]. Production of AMPs by systemic peripheral blood mononuclear cells (PBMCs) does not appear to decline in healthy aged individuals [45], but it is not known how ageing impacts on AMP production in the gut. Early studies showed that in the human gut, the total number of Paneth cells increases to its maximum in early adult life, but their number and secretory function significantly decline with age [46]. This notion coupled with the observation of altered stem cell regeneration in intestinal crypts with ageing [25,26] may predict an impact on Paneth cell regeneration and possibly function. The mucus produced by goblet cells is an important but for a long-time ignored component of epithelial and GI tract antimicrobial defences; recent evidence showed that mucus is also critical for selecting and maintaining homeostatic interaction with the gut bacteria [40,47]. Knowledge of the impact of ageing on the GI tract mucus layer is incomplete and limited to reports of altered gastric mucus production [41,48]. The number of goblet cells does not decline in specialised FAE in aged mice [49] and the thickness of the gastric and duodenal mucus layers do not change with age in normal, healthy individuals [50]. This would suggest that the mechanical protection afforded by the mucus layer, at least in these two locations, is not affected by ageing. However, age-associated changes in the chemical composition and structure of the mucus may also have important effects on the intestinal microbiota. Bacterial adhesion to mucus changes with age; in particular, adhesion of bifidobacteria strains to mucus was found to be significantly reduced [51–53]. Since the binding of intestinal bacteria to mucus is mediated by molecules, such as mucin-binding proteins (MUBs) [54,55], it is plausible, for example to hypothesise that age-associated modification of mucus glycosylation might play an important role in the recently described alterations of the microbial community in the elderly [56]. Yet, it has been shown that mucus possesses important immunoregulatory properties [57] that affects the immunological environment of the gut. Thus, changes of the mucus chemistry, during ageing would have consequences relevant to both microbiota composition and inflammatory status of the gut.

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. Animal experiments and human intervention studies have shown the existence of a bidirectional relationship between the gut and brain, and aspects of behaviour and cognitive function [37,39,58–61]. 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.

5. Crossing the epithelial barrier

Antigen sampling across the FAE of the PPs via M cells is a critical step in enabling the immune system to monitor the luminal contents allowing for an appropriate course of action. M cells are specialised epithelial cells strategically located in the FAE overlying the PPs originally described by Owen and Jones [62], and are key in the transcytosis of microorganisms and macromolecules to the underlying immune system. Initially, conflicting results were reported whereby the intestinal uptake of microspheres across the epithelium was either increased or unaffected in the aged mice or rats [63,64]. More recently, it has been shown that ageing has a detrimental effect on the functional maturation of M cells. In aged mice, the number of glycoprotein 2 (GP2)+ M cells in PP was reduced significantly [49]. This reduction in M cell number led to a deficiency in their ability to transcytose particulate luminal antigen across the FAE, as demonstrated in ligated loop experiments where few or no microparticles passed through the FAE. Also, the number of Spi-B+ cells [65,66], are significantly reduced in the FAE of aged mice resulting in impairment of the downstream functional maturation of M cells [49]. Additionally, functional differentiation and maturation of M cells in the FAE is also dependent on a population of CCR6+CD11c+ expressing B cells that are recruited within the PP by the specific chemokine CCL20; age-associated reduction of CCL20 expression in the FAE of aged mice results in a decreased influx of CCR6+ B cells towards the FAE, and ultimately in a reduction of mature M cells in aged mice [49]. Currently, the impact of ageing on the production of other cytokines in the gut, such as macrophage migration inhibitory factor (MIF) that have a
significant role in the M cell function and transport [67] is not known. 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 [68]. Additional mechanisms for antigen sampling are present in the gut. Villous-associated M cells have been described [69] and LP CX3CR1+DCs/macrophages [70] are also thought to execute the same purpose by extending out dendrites between epithelial cell tight junctions to sample antigen. Currently, the impact of ageing on these two additional mechanisms for antigen-sampling is not known.

6. Immune response in the aged gut mucosa

6.1. Antigen-presentation

Antigen transported across the epithelial barrier to the inductive sites of the intestinal immune system is then dealt with by APCs. DCs are crucial for the presentation of antigen to immunocompetent B and T cells, and have a key role in the induction of peripheral tolerance [14]. T cell priming by DCs appears to be compromised in aged mice. Aged mice showed a suboptimal T cell priming to *Encephalitozoon cuniculi* that could be improved by using MLN–DCs from young animals to stimulate aged T cells; thus suggesting an age-related defective APC function [71]. Aged DCs were characterised by a significant decline of IL-12p70 and IL-15 production accompanied by a decreased expression of the CD80/CD86 co-stimulatory signals. In particular, production of IL-15 seems to be of significance to aged DC function; indeed, exogenous application of IL-15 to MLN DC derived from aged mice restores CD80/CD86 expression and the ability of the aged DC to prime the T cell response [71]. Others have reported that in absence of infection, aged-associated changes of the expression of CD86 is restricted to MLN and no difference was seen between young and aged DCs from LP and PPs [72]; whether DCs from LP and PP of aged mice also differed in their ability to prime T cells is currently unknown. In addition, in young mice, gut-derived DC differed from the systemic-DC in their ability to polarise Th1 responses; freshly isolated gut-derived DCs were found to prime T cells from the production of much lower levels of IFN–γ (T(1)) compared to splenic DCs, either directly via the production of TGF–β, or indirectly via the priming of TGF–β producing T cells [73]. The pattern appears to be different in ageing. Aged gut-DCs failed to induce TGF–β secretion and differentiation of CD4+CD25+ LAP+ T cells, while at the same time T cell production of IFN–γ was up-regulated [72]. The finding that gut-derived DCs from aged mice differentially regulate TGF–β and IFN–γ producing T cells is of particular interest. Firstly, optimal IgA-B cell differentiation [74] and effector functions of regulatory T cells induced after low dose antigen feeding is dependent on TGF–β [75]. Thus, a defect in the production of this cytokine may explain both reduced levels of IgA observed in some cases [76–78] and ability to establish oral tolerance to antigens observed by some authors in ageing [68]. Secondly, it would be interesting to assess the contribution of a more pronounced pro-inflammatory activity of gut-DCs to the development of the systemic low grade chronic inflammation, or inflamming [29,79] typical of the aged organism.

Furthermore age-associated changes of the number and function of DCs inhabiting the sub-epithelial dome (SED) area of PPs were reported to play a critical role in the lack of tolerance in aged mice [68]. This study, which also included the analysis of germinal centre follicular DCs (FDCs), clearly showed that CD11c+ DCs in the SED were decreased in mice >1 year of age. These data led the authors to suggest that, in addition to impaired T cell responses, lack of oral tolerance induction seen in aged mice may be associated with altered DC (and FDC) function. Although it is surprising that more detailed functional studies on the role of gut-DCs in ageing have not been completed at this time, these data taken together would indicate that ageing deficits in DC function play a key role in the decline of regulatory immune function at the gut mucosa.

6.2. IgA response

The most characteristic immunological activity at mucosal surfaces is the production of antibodies referred to as secretory immunoglobulin A (SlgA) that serve as a first line of defence against potentially invasive pathogenic microorganisms and participate in the host–microbe interaction. SlgA production, along with other adaptations of the intestinal mucosa, is critical for establishing and maintaining the intestinal microbiota community [80]. Also, in addition to immune exclusion of bacteria and viruses [81,82] and microbiota [83–85], SlgA also controls limited entry of antibody-coated bacteria via PP-associated M cells [86], and in so doing helps to modulate the local immune responses in an environment that limits the generation of pro-inflammatory response. PPs represent the most important site for IgA production [87]; PPs provide the environment necessary to favour IgA isotype switching with a large array of cells playing a role in determining the fate of B cells in the PPs, and their relocation (homing) to the lamina propria at distant mucosal sites. In particular, the cytokine TGF–β, which can be produced by a variety of PP-inhabiting cells, is of paramount importance [74]. Another important factor is represented by DC-derived retinoic acid (RA), which together with IL-6, promote IgA production [88] and simultaneously induces the expression of gut-homing molecules on IgA+ plasma cells to the gut LP [89]. Currently, the impact of ageing on many of the factors that are critical for an optimal IgA response is not known. For example, an age-related hypo-activation of the retinoid signalling pathways in PBMC has been reported [90], but the levels of RA in the aged gut is not known. A decline of RA in the gut mucosa could have wide ranging consequences on intestinal immunity, including disruption of the gut microflora via down-regulation of T(H)17 cells [91]. Overall, ageing appears to have a detrimental effect on IgA response [92]; however, so far, with regard to the magnitude of SlgA response in ageing, conflicting results have been reported. SlgA response to oral administration of CT declined in aged mice [76,78], rats [93] and non-human primates [94]. However, it has been suggested that reduced levels of IgA in the intestinal mucosa reflects impaired migration (homing) from inductor to effector sites of IgA-secreting cells rather than a reduction in their total numbers [78]. The observation of age-associated decline of the expression of MadCAM-1 in the LP and sub-mucosa venules, and a decrease in α4β7 integrin expression on systemic PMBCs [93] would lend support to this hypothesis. In contrast, others have reported unchanged or even increased levels of IgA in the serum and intestine [72,93,95–97]. An important aspect of humoral immunity in ageing that has to be taken into account is changes in the quality, rather than the magnitude of the antibody response. Animal studies have shown that the levels of systemic antigen-specific antibody response to a bacterial antigen vary according to the mouse strain, ranging from increased to significantly reduced [98]. However, when the protective ability of pneumococci-specific antibody was assessed in experiments of passive protection against lethal infection, “aged” antibodies failed to protect the recipients compared to “young” antibodies, independently of the mouse strain of origin [99], thus demonstrating a biologically relevant age-associated decline of the efficacy of the antibody response. Antigen-specific antibody response in aged mice was characterised by an increased heterogeneity of the V(H) gene repertoire [100] and a reduced affinity [99]. Recently, an extensive analysis of IgA repertoire in young and aged mice has been carried
out [101]. Young mice (4 weeks) have been shown to exhibit a lower frequency of IgA somatic hypermutation in comparison to aged mice (22 weeks). A high throughput sequencing analysis also showed that age affects complementarity determining region 3 (CDR3) sequence distributions, and the analysis of more than one million Vβ sequences showed that the IgA repertoire comprised both highly expanded and low frequency clones. Expanded clones were evenly distributed along the small intestine and represented the vast majority of the clones forming the repertoire in young mice. In aged mice, the repertoire diversity increased by the ongoing accumulation of low frequent clones and microbiota-, T cell-dependent, but PP-independent hypermutations. Whether SlgA from aged mice differs from their young counterpart in their efficacy to protect the intestinal mucosa remains to be determined.

7. Conclusions

Older people suffer from a decline in immune system function, which in the gut affects their ability to tolerate ingested nutrients or resident microbiota, response to infections and is often compounded by malnutrition and dehydation. All these factors contribute greatly to the increased morbidity and mortality rates within older populations. Compared with immunosenescence of systemic immunity, age-associated changes in the intestinal immune system (summary in Table 1) are less well understood in both humans and laboratory animals. In particular, within the intestinal immune system a major gap is represented by the lack of knowledge on events pertaining to intestinal innate immunity. Indeed, very basic aspects of how ageing affects the gut response to external stimuli including commensals, diet and pathogens are currently lacking; one of the most notable examples being the expression and function of molecules, such as pathogen recognition receptors (PRRs) on IECs and associated immune cells that play a critical role in sensing the environment. This is of particular significance given the fact that the intestinal epithelial barrier, mucosal innate immune system and microbiota represent a highly complex, multidimensional network that plays a critical role in controlling the immune homeostasis of the intestine, with potentially important consequences reaching far beyond the gut. Indeed, converging evidence suggest an association between the inflammation status and the presence of chronic disease in the elderly, and it has been suggested that the low grade chronic inflammation or “inflammaging” typical of old age can originate from the intestinal environment [102]. Also, understanding whether age-associated modifications of immune system are the cause or effect of the microbiota changes seen in the elderly is a goal of certain medical relevance, and may provide us with tool to impact on local inflammatory disorders and possibly systemic degenerative disorders.

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