Trends in Immunology

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Review

T cell control of the intestinal barrier and gut microbiota during ageing

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The epithelial, microbial, and immune components of the intestinal barrier coexist in harmony to prevent undesirable inflammatory outcomes and ensure homeostasis in the host. In this review, we outline molecular mechanisms by which T cells regulate intestinal homeostasis and how the ageing-associated dysfunction of T cells could disturb host–microbiota symbiosis and the physical integrity of the intestinal barrier, ultimately driving inflammageing and poor health outcomes. Finally, we propose microbiota- and T cell-based therapeutic interventions aimed at strengthening the intestinal barrier to promote healthier longevity. Namely, we discuss the transplantation of youthful microbiota, the use of designed probiotics, and the adoptive transfer of competent or engineered T cells.

The fall of the intestinal protective barriers with ageing

The intestinal tract is constantly exposed to a dynamic milieu replete with potential noxious agents such as microbes, toxins, xenobiotics, and dietary compounds. Beyond nutrition, the intestinal mucosa upholds the challenging task of hindering the uncontrolled flux of harmful agents across the epithelium. The intestine consists of a physical barrier formed by a monolayer of intestinal epithelial cells (IECs) that arise from intestinal stem cells (ISCs) located at the bottom of the crypts. IECs are intimately attached by sophisticated intercellular junctional complexes, such as tight junctions, that seal the intercellular space to restrict the free passage of molecules. In addition, specialised IECs secrete layers of mucus and **antimicrobial peptides (AMPs)** (see Glossary), acting as a first line of defence [1,2]. The intestine harbours a diverse and intricate ecosystem of microorganisms, known as the gut microbiota. This biological layer not only protects against pathogen colonisation but also supports host immunity and metabolism through the synthesis of bioactive metabolites [3,4]. To deal with the potential threat of the microbiota and other luminal antigens, the intestinal barrier is equipped with a variety of immune cells that comprise the **gut-associated lymphoid tissue (GALT)**. This network of macrophages, dendritic cells (DCs), innate lymphoid cells, and T and B cells is coordinated to survey the intestine, detect intruders, and elaborate specific immune responses to prevent undesired inflammatory reactions [5].

These three layers of protection—the epithelial barrier, the commensal microbiota, and mucosal immune cells—provided by the intestine are instrumental in preserving homeostasis in the organism throughout life. A century ago, the Nobel laureate Élie Metchnikoff, one of the founders of modern immunology, formulated the pioneering theory that ageing was driven by the rupture of the intestinal barrier and the subsequent translocation of bacteria to the periphery, which could trigger hyperinflammation [6]. By studying the leakage of a nonabsorbable dye in *Drosophila*, scientists identified that intestinal barrier disruption predicted metabolic and inflammatory alterations, as well as the imminent death of flies, in a more accurate manner than chronological age [7]. During the last decade, these revolutionary concepts linking the collapse of the intestinal barrier to the sustained low-grade inflammation that appears with ageing—or **inflammageing**—disease, and mortality

Highlights

The intestinal epithelium, resident immune cells, and the commensal microbiota coexist in harmony to prevent unwanted inflammatory responses in the host.

T cells have emerged as key players in the establishment of beneficial host–microbiota relationships and the preservation of intestinal barrier integrity.

Age-related shifts in the T cell compartment can lead to gut dysbiosis and intestinal barrier disruption, driving inflammageing and tissue senescence in aged organisms.

Understanding the interplay between T cells, the intestine, and its microbiota could allow the design of new therapeutic strategies to foster healthier ageing.

Significance

The integrity of the intestine is compromised during ageing, which contributes to the chronic inflammation that appears with age and the development of age-related conditions. T cells play a vital role in the maintenance of the intestinal barrier and its commensal microbiota. Understanding how T cell dysfunction during ageing compromises intestinal homeostasis could offer novel therapeutic opportunities to mitigate ageing-related pathologies.

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have drawn the attention of the scientific community in the field of ageing biology [8]. Therefore, exploring the physical, microbial, and immune components of the intestinal barrier could shed light on the molecular mechanisms driving age-related intestinal barrier dysfunction.

The physical integrity of the intestinal epithelium is disturbed during ageing. The intestine of naturally aged mice shows diminished regenerative capacity [9], suggesting exhaustion and depletion of ISCs, which compromise their ability to sustain a high-rate turnover in this epithelium [10–13] (Figure 1). This is accompanied by age-dependent dismantling of intercellular junctional complexes and shrinkage of mucus layers in the intestinal epithelium, notably increasing the permeability of the intestine across species [7,14–27]. Moreover, with ageing, the commensal microbiota undergoes significant shifts in the intestines, collectively termed **gut dysbiosis**, which is characterised by a reduction in microbial diversity and diminished abundance of health-promoting taxa that are replaced by pathobionts (Box 1) [16,18,20,21,28–31,43,47,48].

Transplantation of microbiota from aged organisms into young recipients has illustrated that age-related gut dysbiosis enhances intestinal permeability due to the downregulation of tight junctions

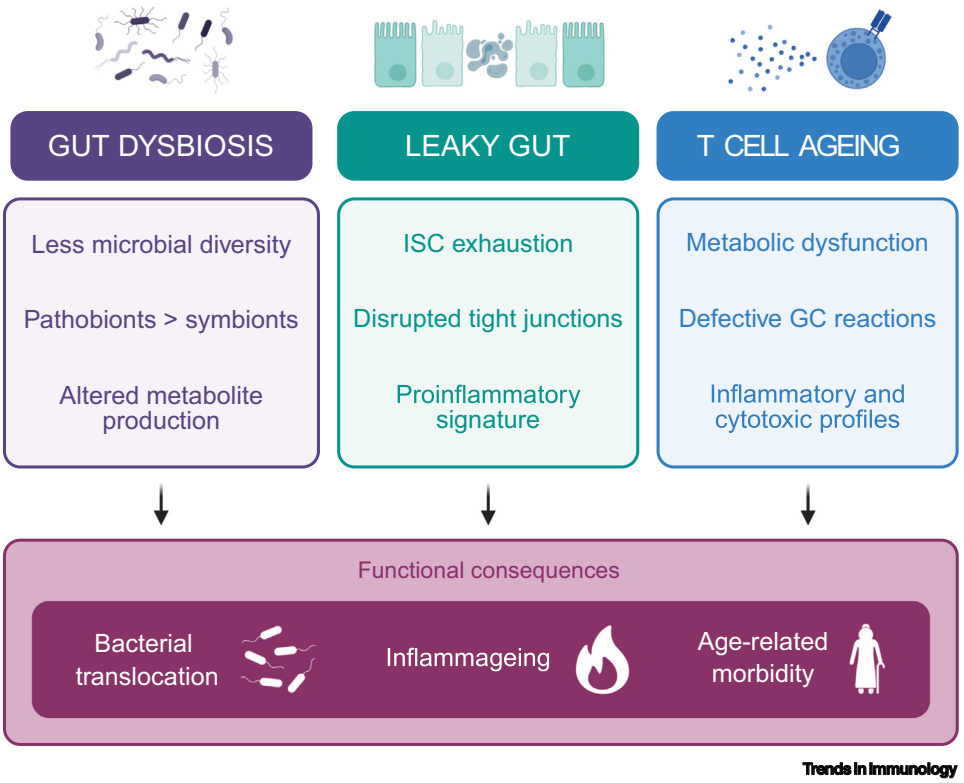

Figure 1. The fall of the microbial, epithelial, and immune barriers of the intestine during ageing. The three protection layers provided by the intestine experience notable age-related changes that threaten host homeostasis. The intestinal epithelium loses its regenerative capacity and becomes more permeable due to exhaustion of intestinal stem cells (ISCs) and dismantling of cell-to-cell junctions. This is favoured by shifts in the intestinal microbiota, mainly characterised by lack of microbial diversity, expansion of disease-associated taxa, and altered production of metabolites. Moreover, dysfunctional T cells, in part due to intrinsic metabolic alterations, acquire highly inflammatory and cytotoxic profiles, abrogate specific germinal centre (GC) reactions, and disrupt intestinal physiology. Altogether, these alterations foster translocation of luminal bacteria to the periphery and fuel inflammageing, ultimately driving ageing-related conditions and mortality in the host. This figure was created using BioRender (https://biorender.com/).

Glossary

Antimicrobial peptide (AMP): small proteins secreted into the intestinal lumen to neutralise and eliminate potentially harmful microbes through different mechanisms, such as cell lysis, RNase and ion-binding activities, or by inducing the expression of proinflammatory genes to recruit immune cells.
CD4 cytotoxic T lymphocyte (CD4 CTL): a subset of CD4 T cells characterised by enhanced expression of CCL5, which exhibits enhanced cytotoxic properties conventionally attributed to CD8 T cells. Although first described in the context of chronic viral infections and autoimmune disorders, CD4 CTLs have also been found in association with ageing.
Cell senescence: a state of permanent cell proliferation arrest in response to stressors, such as DNA damage, oncogene activation, mitochondrial dysfunction, or proinflammatory mediators. Among other features, senescent cells acquire a hypersecretory profile, named the senescence-associated secretory phenotype, to enhance immune surveillance. Although cell senescence plays a vital role in embryogenesis, wound healing, and tumour suppression, the persistent accumulation of senescent cells contributes to tissue decline, chronic inflammation, and age-related diseases.
Germinal centre (GC): transient and specialised microstructures within lymphoid follicles of secondary lymphoid organs, in which B cells are activated and then mature and differentiate into antibody-secreting PCs to elaborate specific immune responses against external and internal threats.
Gut-associated lymphoid tissue (GALT): a specialised network of immune cells located throughout the gastrointestinal tract in different compartments, such as lymphoid follicles, PPs, the LP, and the epithelium, that provides immune surveillance by protecting against pathogens while ensuring tolerance to the commensal microbiota and dietary antigens.
Gut dysbiosis: abnormal variations in the composition and metabolic activities of the intestinal microbiota, in which microbial diversity diminishes and disease-associated microbes outnumber health-promoting ones. Beyond gastrointestinal complications, it is associated with noncommunicable

Box 1. The gut microbiota of aged and long-lived individuals

The ageing process is accompanied by a progressive reconfiguration of the intestinal bacteria owing to host-intrinsic alterations in the digestive system (i.e., disturbed peristalsis, reduced synthesis of digestive enzymes, or mucosal immunosenescence), as well as social and environmental variations (e.g., diet, polypharmacy, lifelong infections, and comorbidities) [28].

Age-associated bacterial dysbiosis is featured by reduced microbial diversity and increased inter-individual variability. Common compositional changes comprise the loss of dominant commensal taxa, such as *Prevotella*, *Faecalibacterium*, *Eubacterium*, Lachnospiraceae, *Coprococcus*, and *Bifidobacterium*, which are replaced by a second group of commensal bacteria (e.g., *Akkermansia*, Christensenellaceae, *Butyricimonas*, *Odoribacter*, and *Butyricicoccus*) and putative pathobionts, including *Eggerthella*, *Desulfovibrio*, *Fusobacterium*, *Streptococcus*, and members of the Enterobacteriaceae family [28–32]. Interestingly, having low microbial uniqueness and retaining a Bacteroides-dominant enterotype is sufficient to predict decreased survival in the elderly in the upcoming years [33]. These compositional shifts are often translated into altered metabolic outputs. One of the hallmarks of an aged gut microbiota is the decreased synthesis of beneficial metabolites, such as SCFAs and secondary bile acids. Moreover, the higher abundance of opportunistic pathogens results in the overproduction of detrimental molecules that compromise host homeostasis, for instance, hyperinflammatory lipopolysaccharides, D-galactose, reactive oxygen species, and DNA-damaging toxins [28,34,35].

Studies indicate that extremely aged individuals harbour a gut microbiota that diverges from the typical ageing profile. Centenarians display stress-adapted microbiota in the intestine, with enrichment in health-associated bacteria, including *Akkermansia*, *Alistipes*, *Parabacteroides*, Christensenellaceae, *Methanobrevibacter*, and certain *Bifidobacterium* spp., while accommodating potentially harmful bacteria, such as Enterobacteriaceae [33,36–40]. Evaluation of the gut microbiome in the world's oldest living person revealed an anti-inflammatory enterotype, with high abundance of *Bifidobacterium* and Christensenellaceae, and low levels of Proteobacteria and *Clostridium*, which could participate in the healthy longevity of this subject [41]. In addition, metagenomic and metabolomic analyses have uncovered that elevated abundance of bile-acid modifying Odoribacteraceae could exert beneficial effects in long-lived individuals [42]. On top of this, the centenarian microbial signature preserves metabolic routes for xenobiotic degradation, redox homeostasis, and synthesis of SCFAs [39,40]. These biochemical pathways correlate with improved health outcomes in centenarians, which could help compensate for the declining metabolism during ageing.

Mouse models have been particularly useful for dissecting cause–effect relationships between microbial shifts and host ageing. The gut microbiota follows a similar trajectory in mice and humans over time. Both species display age-related decrease in microbial diversity and a shift towards less stable and proinflammatory communities, which is accompanied by reduction in SCFA-producing and bile-acid-modifying symbionts [16,21,43–46]. Although conserved compositional and metabolic microbial signatures have been observed in aged individuals and mouse models of ageing, taxonomic differences and highly inter-individual variations in the human microbiota could hinder direct extrapolation from controlled mouse microbiota studies.

and direct damage to the mucosa [16,21,28,47]. Interestingly, most AMPs appear upregulated in the intestines of aged animals, probably as a compensatory mechanism to overcome gut dysbiosis and epithelium damage [7,49]. Because of leaky gut, luminal bacteria and their byproducts translocate to peripheral tissues, which contribute to inflammageing [16,21,50]. Hence, gut dysbiosis and intestinal barrier dysfunction have been associated with conditions linked to ageing, including **sarcopenia**, locomotor disability, and dysregulation of glucose metabolism [22,50]. Interrogation of the gut–brain axis has unveiled the role of microbiota in neurodegenerative disorders, for instance, Parkinson's disease [51]. Furthermore, metabolites derived from intestinal bacteria can trigger atherosclerosis [52], endothelial **cell senescence** [53], cognitive dysfunction [54,55], and neuroinflammation [56], supporting the idea that loss of gut homeostasis can precipitate the onset of age-related pathologies.

Single-cell RNA sequencing (scRNA-seq) analyses of several tissues in aged mice have uncovered that, among all cell types detected, immune cells—in particular, T cells—manifest the greatest alterations in their gene expression dynamics during ageing [57]. These observations were confirmed in peripheral immune cells throughout the human lifespan, emphasising that T cells are transcriptionally the most affected in the aged immune landscape [58]. This is probably the result of three main factors. First, the thymus starts to involute very early in life, thereby limiting the output of new T cells throughout life. Secondly, while young individuals show a balance between lymphoid and myeloid progenitors in the bone marrow, ageing shifts haematopoiesis

metabolic, cardiovascular, and neuropsychiatric disorders, as well as with the ageing process.

Inflammageing: low-grade and sustained inflammation that appears with age under sterile conditions due to gut dysbiosis, cellular senescence, and dysfunction of the immune system. It has been associated with the development of age-related conditions, including cardiometabolic and neurodegenerative diseases, cancer, and frailty.

Phage therapy: the therapeutic use of naïve or engineered bacteriophages to specifically target and eliminate bacteria as an alternative to combat antibiotic resistance. They are currently under development as antitumour weapons or as delivery systems for therapeutic and diagnostic compounds.

Postbiotic: preparation of nonliving microorganisms and/or their components, including microbially derived metabolites, cell wall derivatives, and other bioactive compounds that can modulate the immune system, support intestinal barrier function, and influence host metabolism. Postbiotics are considered more stable and safer than live probiotics in certain applications.

Probiotic: live microorganisms, such as bacteria and yeasts, that maintain or restore host–microbiota symbiosis, providing health benefits, including the suppression of pathogen colonisation and the enhancement of the immune response. They can be naturally present in fermented foods, such as yogurt (naïve probiotics), or genetically modified to produce bioactive compounds (engineered probiotics).

Sarcopenia: the progressive loss of skeletal muscle mass and strength associated with ageing, physical inactivity, inadequate nutritional intake, or chronic disease, which leads to impaired physical performance and an increased risk of disability, falls, and mortality.

T follicular helper (T_{FH}) and T follicular regulatory (T_{FR}) cells: T_{FH} cells are a specialised subset of CD4 T cells mainly located in the immune follicles of secondary lymphoid organs that support GC reactions. In brief, naïve CD4 T cells identify antigen epitopes in antigen-presenting cells and acquire a T_{FH} cell phenotype by expressing the GC homing receptor CXCR5 and the transcription factor Bcl6. Once they migrate to the GCs, T_{FH} cells encounter and activate B cells to achieve affinity maturation and finally differentiate into

towards myeloid over lymphoid progenitors—myeloid skewing—which reduces the pool of T cell precursors. Finally, T cells are under high replicative pressure to compensate for the reduced production of new cells and as a result of clonal expansion after lifelong antigen exposure. Consequently, T cells progressively acquire terminally differentiated profiles at the expense of losing the naïve/resting pools [59]. Analysis of hundreds of wild type mice undertaking different nutritional interventions revealed that the proportion of effector/memory-like T cells that accumulate with age correlates with poor health outcomes and shortened lifespan [60]. Compositional analyses of these memory-like T cells in scRNA-seq studies have shown expansion of CD4 and CD8 T cells with highly cytotoxic, exhausted, and regulatory profiles during ageing [61,62]. This age-related dysfunction in T cells abrogates **germinal centre (GC)** reactions, limiting the capacity to mount effective humoral responses upon new antigen encounters and vaccination [63,64]. On top of compromising host defence, memory-like T cells engage in dysregulated inflammatory reactions that fuel inflammageing [50,62,65,66], which plays a crucial role in tissue deterioration and the onset of diseases related to ageing [67,68]. Importantly, these alterations in the T cell compartment are mirrored in the intestinal mucosa of naturally aged mice [10,11,43,50], suggesting a failure in gut immunity with age.

Age-associated shifts in T cells can pose a threat to the delicate harmony between the epithelium, the resident microbiota, and immune cells in the intestinal barrier, which could trigger disease and mortality in advanced ages. In this review, we discuss mechanisms by which T cells fail to preserve host–microbiota symbiosis and intestinal barrier integrity during ageing. We further debate strategies to manipulate the gut microbiota and/or T cells to maintain a healthy state of the intestinal barrier and foster healthier longevity in the ageing population.

Age-related remodelling of T cells in the intestine

T cells are located in every compartment of the GALT. Gut-associated lymphoid follicles, such as Peyer's patches (PPs) or colonic patches in the small and large intestines, respectively, are mainly composed of B cells and CD4 T follicular cells [69]. By contrast, the intestinal lamina propria (LP) is primarily enriched in T cells expressing the T cell receptor αβ (TCRαβ$^+$) showing effector profiles, such as type 17 CD4 T helper (T_H17) or regulatory CD4 T (T_{reg}) cells and, to a lesser extent, CD8 αβ T cells [5]. This connective tissue is covered by the intestinal epithelium, which shows a high proportion of intraepithelial lymphocytes (IELs). IELs are classified into natural IELs, which are thymus-derived CD8αα$^{\pm}$ αβ or TCRγδ$^+$ (γδ) T cells, and induced IELs, which arise from conventional αβ T cells [70].

The well-adjusted activity of intestinal T cells safeguards a healthy and balanced state of the gut microbiota, mainly through IgA responses, and maintains the integrity of the epithelial barrier by regulating ISC behaviour and cell-to-cell junctions (Box 2). Therefore, ageing-associated alterations in the composition and function of the intestinal T cell compartment can critically compromise gut homeostasis, leading to poor health outcomes in the host.

Age-related changes in intestinal T cell subsets and function

In the intestine, T cells acquire extremely differentiated phenotypes with age (Figure 2). Contrary to the T cell lymphopenia observed in peripheral blood and secondary lymphoid organs, CD4 and CD8 T cells progressively accumulate in gut-associated lymphoid follicles, IELs, and the intestinal LP of naturally aged mice [50,88–91]. In PPs, while the frequency of naïve cells decreases, the proportion of memory-like subsets showing exhausted, cytotoxic, and regulatory profiles increases in both the CD4 and CD8 T cell compartments of aged mice [50]. This ageing-related contraction of naïve CD4 and CD8 T cells is also observed in gut-associated lymphoid follicles in humans [92]. Functionally, antigen-specific B cell reactions are notably repressed in

antibody-secreting PCs. This process is negatively controlled by T_{FR} cells, a subset of regulatory CD4 T cells that prevents excessive or autoimmune reactions in the GCs.

Tissue-resident T (T_{RM}) cells: T_{RM} cells are a subset of long-lived memory T cells that permanently reside in nonlymphoid tissues, such as the skin, intestine, liver, or lung, to mount quicker and more localised immune reactions at barrier sites. Compared with effector/memory T cells, T_{RM} cells display unique phenotypic markers, including CD69 and CD103, that favour their retention in tissues without recirculating between blood, lymphoid organs, and peripheral tissues.

Box 2. The role of T cells in intestinal homeostasis

Beyond mucosal immunity, T cells are key regulators of intestinal function, helping maintain host–microbiota mutualism while ensuring tissue integrity.

In GCs of gut-associated lymphoid follicles, **T follicular helper (T_{FH}) cells** dictate the differentiation and maturation of B cells into PCs to secrete high-affinity IgA, the most predominant antibody in the intestinal mucosa, to restrain enteric pathogens and control commensal microbes [69]. Some works report that some T_H17 cell [71], T_{reg} cell [72], and γδ T cell subsets [73] acquire T_{FH} cell profiles to support IgA responses. GC reactions are negatively regulated by a specialised subset of T_{reg} cells, **T follicular regulatory (T_{FR}) cells**, which prevents uncontrolled antibody reactions [74]. IgA responses can also be elicited in a T cell-independent manner through factors secreted by DCs that foster the generation of IgA-producing PCs outside the lymphoid follicles [5,69]. Although the precise role of T cells in IgA-mediated bacterial targeting is still unclear, analysis of taxa-specific IgA coating by antibody-based cell sorting combined with *16S* rRNA gene sequencing is clarifying this process [75,76]. T cell-dependent IgAs display high affinity and specificity towards defined members of the gut microbiota, excluding disease-associated bacteria while ensuring microbiota diversity [69]. Contrarily, T cell-independent IgAs are polyreactive and opsonises a broader set of bacteria without affecting microbiota composition. Apart from IgA responses, T cells help generate mutualistic host–microbiome interactions through alternative mechanisms. For instance, γδ T cells synthesise AMPs such as regenerating islet-derived protein 3 γ (RegIIIγ) in response to the gut microbiota [77]. Moreover, interleukin (IL)-17 and IL-23 derived from γδ T cells and T_H17 cells confine disease-associated members of the gut microbiota, limiting their systemic leakage in mice [78,79].

On top of this, T cells actively shape gut barrier integrity. Chiefs among them are T_{reg} cells, which restrain inflammatory reactions and promote tissue healing [80,81]. Upon damage, IECs release signals such as IL-33, driving T_{reg} cell differentiation [82]. The reparative potential of these cells lies in the secretion of mediators such as amphiregulin and keratinocyte growth factor (KGF) to coordinate IEC proliferation. Moreover, T_{reg} cell-derived IL-10 favours a balanced activity of ISCs by inducing self-renewal over differentiation [83]. ISCs are further subjected to T cell immune surveillance via the MHC class I to avoid aberrant or exceeding cycling [84]. γδ T cells are also a relevant source of regenerating factors including KGF, insulin growth factor-1 or transforming growth factor-β to accelerate tissue repair after injury [70]. Along with T_H17 cells [85], γδ T cells contribute to intestinal integrity through secretion of IL-17 and IL-22 [86]. Furthermore, a balanced synthesis of anti- and proinflammatory mediators by T cells modulates the architecture of intercellular junctions, thus regulating epithelial integrity [1,87].

the intestine of aged mice [43,93], which is associated with the accumulation of dysfunctional T_{FH} cells and, to a greater extent, suppressive T_{FR} cells [64]. In addition to the absence of positive signals derived from aged T_{FH} cells, GC B cells are intrinsically subjected to cellular senescence as a result of sustained stimulation from intestinal bacteria over time [93]. Despite faulty GC reactions, two independent scRNA-seq analyses have underscored a notorious expansion of IgA-producing plasma cells (PCs) in the intestinal LP of aged mice [10,11]. While the total frequency of IgA-coated bacteria in faeces remains comparable, taxon-specific IgA responses to intestinal bacteria are diminished in mice and humans during ageing [43,94], which could partially explain the age-related expansion of potentially pathogenic bacteria in the intestine. These data suggest that faulty GC B cell responses could favour T cell-independent IgA responses that could disturb host–microbiota symbiosis during ageing.

Outside gut-associated lymphoid follicles, scRNA-seq and conventional flow cytometry analyses of the intestinal LP reveal a marked drop in naïve CD4 T cells, alongside an accumulation of memory-like CD4 T cells expressing C–C motif chemokine ligand 5 (CCL5) in aged mice [10,11,50]. CCL5 identifies a subset of CD4 T cells displaying cytotoxic features, termed **CD4 cytotoxic T lymphocytes (CD4 CTLs)**, which are characterised by the expression of Eomesodermin (EOMES) and notably accumulate in tissues with age [61,95,96]. Strikingly, a unique CD4 T cell subset expressing granzyme K (GzmK)—a cytolytic molecule that distinguishes age-associated T cells in mouse and human [62]—in an EOMES-dependent manner has also been described in colitis in both species [97]. Likewise, two subsets of $CD8\alpha\alpha^+$ CD4 T cells that display a similar cytotoxic transcriptomic fingerprint, including expression of *Ccl5* and cytolytic molecules, increase within the small intestine IEL fraction of mice during ageing [91], suggesting a pathological role for different CD4 CTLs in aged intestines. Although **tissue-resident memory T (T_{RM}) cells**

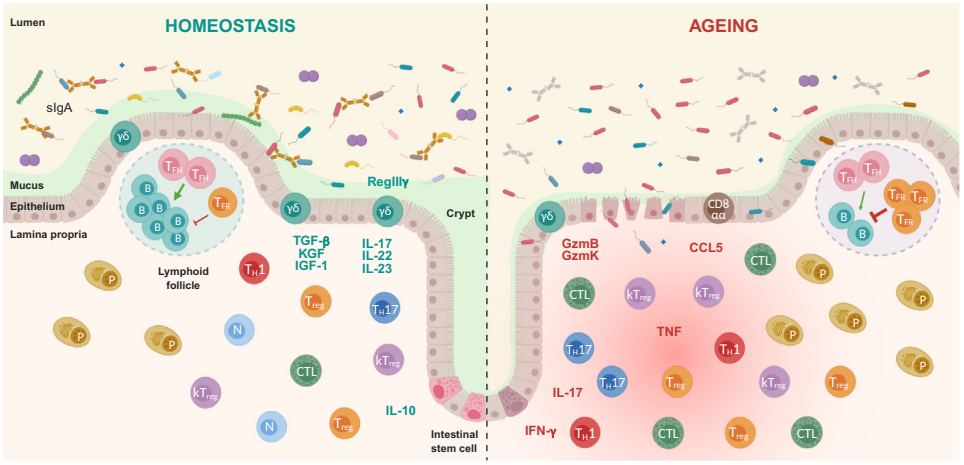

Figure 2. Age-related remodelling of T cells in the intestine during ageing. T cells shift from naïve towards extremely differentiated profiles in the intestine with age. In gut-associated lymphoid follicles, dysfunctional T follicular helper (T_{FH}) and accumulation of T follicular regulatory (T_{FR}) cells lead to defective germinal centre reactions and dysregulated T cell-independent IgA responses, which is associated with microbial dysbiosis. The frequency of intraepithelial γδ T cells, which support relevant functions for immune surveillance and wound healing, decreases during ageing. Together with γδ T cells and CD8αα$^+$ CD4 T cells within the epithelium, CD4 and CD8 αβ T cells acquire highly cytotoxic features in the intestinal LP that could indiscriminately damage the tissue with age. Furthermore, the intestine is dominated by T helper (T_H) cell type 1 and 17 proinflammatory cytokines during ageing, which could disassemble intercellular junctions in the epithelium and precipitate intestinal stem cell (ISC) exhaustion, favouring disruption of the intestinal barrier. A higher abundance of CD4 and CD8 T regulatory (T_{reg}) cells, in particular a subset of KLRG1$^+$ CD4 T_{reg} (kT_{reg}) cells, is also found in aged intestines. Rather than restraining inflammation, promoting tissue repair, and ISC self-renewal, aged T_{reg} cells further contribute to proinflammatory events. This figure was created using BioRender (https://biorender.com/). B: B cell; CCL5: C–C motif chemokine ligand 5; CTL: cytotoxic T lymphocyte; Gzm: granzyme; IFN-γ: interferon gamma; IGF-1: insulin growth factor 1; IL: interleukin; KGF: keratinocyte growth factor; N: naïve; P: plasma cell; RegIIIγ: regenerating islet-derived protein 3 gamma; sIgA: secretory IgA; TGF-β: transforming growth factor beta; TNF: tumour necrosis factor.

maintain site-specific phenotypes without developing immunosenescent traits in the intestines of elderly individuals [98], transcriptomic analysis of CD4 T_{RM} cells in the human small intestine shows ageing-related enrichment of gene pathways involved in the inflammatory response (i.e., *IFNG*, *TNF, IL23R*, and *NFKBID*) and repression of crucial genes for intestinal homeostasis, such as *IL22* [99].

Regulatory T cell (T_{reg}) cells do not appear in scRNA-seq analyses of mouse intestines [10,11]. The apparent lack of T_{reg} cells in these datasets is unlikely to reflect a true biological absence but rather some technical and/or analytical issues due to their relatively low frequency within the CD4 T cell compartment, which could have limited their detection within broader populations. Nonetheless, T_{reg} cells can be easily detected in the intestinal LP of naturally aged mice by using conventional flow cytometry techniques. The frequency of total T_{reg} cells is remarkably increased in the colonic LP of aged mice, particularly T_{reg} cells with activated/effector phenotypes and a subset of T_{reg} cells expressing killer-cell lectin-like receptor G1 (KLRG1) [50]. KLRG1 distinguishes an exclusive subset of T_{reg} cells showing proinflammatory and senescence features, mitochondrial alterations, and DNA damage that accumulate in mouse tissues and human blood during ageing [100]. Interestingly, KLRG1$^+$ T_{reg} cells fail to restrain colon inflammation in a T cell transfer model of colitis [100]. Therefore, these studies depict an age-related expansion of T_{reg} cells with altered anti-inflammatory and pro-repair features in the intestine. Future in-depth scRNA-seq analyses could help us delve into the different T_{reg} cell subsets residing in the intestines in homeostasis and during ageing.

CD8 T cells in the intestinal LP remain poorly characterised in scRNA-seq studies. Nevertheless, analyses of these cells using spectral flow cytometry have shown a reduction in the naïve pool, alongside an increase in subpopulations displaying exhaustion and cytotoxicity markers in naturally aged mice [50]. Similarly, several subsets of CD8 T cells exhibit an age-associated proinflammatory and cytotoxic signature in the intestines of aged individuals, with particular emphasis on GzmK [99]. In contrast to human studies, the presence of GzmK-producing CD8 T cells appears minimal in ageing mouse intestines, which seem to be dominated instead by granzyme B ($GzmB^+$) CD8 T cells [57]. Furthermore, a subset of regulatory CD8 T cells expressing FoxP3 is increased in the colonic LP of mice during ageing [50], as previously observed in the lymphoid tissues of mice and the peripheral blood of older individuals [101,102]. Similar to their CD4 T_{reg} cell counterparts, aged CD8 T_{reg} cells exhibit weakened immunosuppressive activities [103].

Although the IEL fraction is highly dominated by γδ T cells in the intestine of young mice, this population shows a drastic age-related contraction [91]. Some works indicate the accumulation of interleukin (IL)-17–producing γδ T cells in secondary lymph organs and barrier tissues during ageing, potentially reinforcing immune surveillance while also contributing to inflammageing [104–106]. Nevertheless, data on the γδ T cell compartment in the aged intestines of mice remain scarce. The age-associated decline of γδ T cells in the small intestine is also observed in humans [107]. Both children and older adults are enriched in $Vδ1^+$ over $Vδ2^+$ γδ T cells in the small intestine, yet these populations are phenotypically distinct between them and during ageing [107]. In children, $Vδ1^+$ γδ T cells display a diverse, naïve, tissue-adaptive phenotype consistent with tissue repair functions, whereas $Vδ2^+$ γδ T cells exhibit effector-like profiles [107]. By contrast, both $Vδ1^+$ and $Vδ2^+$ subsets become more clonally restricted and acquire tissue-resident and cytotoxic/effector features in older adults [99,107]. These findings indicate that, with age, γδ T cells transition from adaptive, tissue-protective functions during the formative period of childhood antigenic exposure to more specialised roles in tissue immunosurveillance. In line with this, some reports show that human circulating $Vγ9^+$ $Vδ2^+$ γδ T cells selectively detect and eliminate senescent cells [108], but manifest impaired cytotoxic activities in frail older adults compared with nonfrail age-matched counterparts [109], proposing a protective role of γδ T cells in healthy ageing.

The age-associated skewing of T cells towards highly inflammatory profiles in the intestine can influence tight junction architecture, which enhances the permeability of the intestinal epithelium. Proinflammatory mediators originating from T cells could contribute to ISC depletion [83,110], disruption of intercellular junctional complexes [50,87], and subsequent gut hyperpermeability [16,50]. CD4 CTLs are the topmost producers of interferon (IFN)-γ in the aged intestine of mice, which is one of the main drivers of ISC ageing [11]. T_H1 and T_H17 cytokines, such as IFN-γ and IL-17A, respectively, promote ISC differentiation over self-renewal [83], and IFN-γ can induce ISC apoptosis in murine and human organoids [110], which could ultimately deplete the ISC pool under sustained inflammatory conditions. Furthermore, cytokines such as tumour necrosis factor (TNF), IFN-γ, or IL-6 not only downregulate the expression of proteins involved in the stability of tight junctions but also remodel their distribution within IECs, thereby widening the intercellular space [87]. On top of this, IFN-γ triggers the global upregulation of MHC class II in IECs, which then become non-conventional antigen-presenting cells [11,90]. This could further activate immune cells in a positive feedback loop, amplifying the unresolved inflammatory response observed in the ageing process. Accordingly, blocking IFN-γ restores the regenerative capacity of the intestine [11], and genetically ablating TNF re-establishes intestinal integrity during ageing [16].

Altogether, mouse and human studies indicate that age-associated T cells manifest terminally differentiated profiles in the intestine, with highly proinflammatory and cytotoxic features that could impinge on host–microbiota mutualism and the physical integrity of the intestinal barrier.

Intrinsic and extrinsic alterations of T cells in the aged intestines

Ageing of T cells is defined by the acquisition of a series of molecular hallmarks that directly cause the functional decline of these cells, including (epi)genetic alterations, failure in the proteostasis network, and metabolic alterations [111]. Interrogation of T cell molecular alterations, as well as variations occurring in the intestinal microenvironment with age, may help to understand cell-intrinsic and extrinsic factors favouring T cell ageing at this barrier site.

scRNA-seq analysis of CD4 T cells isolated from the colonic LP of aged mice shows enrichment of gene pathways related to glycolysis and oxidative stress, as well as downregulation of genes supporting oxidative phosphorylation [10]. These data suggest a scenario of mitochondrial dysfunction coupled with the proinflammatory profile of intestinal T cells during ageing. In line with this, mimicking an age-associated mitochondrial decline in T cells, owing to depletion of mitochondrial transcription factor A (TFAM), accelerates immunosenescence in the intestinal mucosa [50]. *Tfam*$^{fl/fl}$*Cd4*^{Cre} mice display a notable decrease in T_{FH} and T_{FR} cells, diminished GC B cell reactions, and aberrant IgA responses against the gut microbiota, which is accompanied by bacterial dysbiosis [50]. In addition, the intestinal CD4 and CD8 T cell compartments show a reduction of the naïve pools and acquisition of enhanced proinflammatory and cytotoxic activities that correlate with disruption of the intestinal barrier and systemic dissemination of bacteria [50]. Interestingly, gut dysbiosis and intestinal barrier dysfunction due to prematurely aged T cells trigger early-onset inflammageing, tissue senescence, and the ageing-related phenotype in this mouse model [50]. Another context of premature T cell ageing can be found in people living with HIV. Even under antiretroviral therapy, chronic HIV infection accelerates immune ageing by almost 2 decades in humans, which is characterised by a marked decline in naïve T cell pools and expansion of memory-like subsets when compared with age-matched HIV-negative counterparts [112]. People living with HIV also show increased bacterial translocation and premature inflammageing, which are important features for the development of age-related comorbidities in this population [113,114]. Interestingly, CD8 T_{RM} cells in the colon display alterations in fatty acid metabolism during HIV infection due to downregulation of peroxisome proliferator-activated receptor-γ (PPARγ) [115]. These immunometabolic defects disturb intestinal barrier integrity by means of lipid scavenging from adjacent IECs, which then undergo apoptosis [115]. Overall, these works suggest an active role of metabolically impaired T cells in the disruption of the intestinal barrier and consequent ageing phenotypes. In addition to the loss of PPAR signalling, the intrinsic immunometabolic failure of T cells may be owing to the increasing mutation burden in mitochondrial DNA [116] and/or the inefficient recycling of malfunctioning mitochondria with age [66]. Therefore, the compensatory enhancement of the glycolytic pathway could sustain persistent proinflammatory and cytotoxic phenotypes in T cells during ageing [117].

Intestinal T cells dwell in a highly dynamic microenvironment, with age-related shifts in the gut microbiota, as well as molecular alterations in the intestinal mucosa that could account for the age-related decline of these cells. Microbiota transplantation assays uncovered the role of the gut microbiota in the age-related immune dysfunction of mice, namely myeloid skewing of haematopoietic precursors [118], defective GC reactions [43], and activation of T cells [46]. Intriguingly, enhanced bacterial translocation correlates with proinflammatory and senescent phenotypes in circulating T cells of aged adults [119] and people living with HIV [114], suggesting that the accumulation of bacterial byproducts could chronically stimulate T cells in the intestine and peripheral tissues, driving dysfunction, as detected in GC B cells [93]. Accordingly, aged germ-free mice, which lack microbiota and do not develop leaky gut, seem to be protected from signs of thymic ageing [119], depicting a gut–thymus axis that could delay T cell ageing. In addition, decreased levels of butyrate are associated with T cell senescence in older adults [120]. These works propose a causal role of gut dysbiosis in T cell immunosenescence through

heightened microbial translocation and loss of beneficial microbiota-derived metabolites. Moreover, age-related upregulation of MHC class II in IECs [11,90] and imbalanced eicosanoid metabolism by surrounding innate immune cells in the intestine [121] could continuously activate T cells, fostering unresolved inflammatory reactions in this barrier tissue during ageing.

Overall, cell-intrinsic and environmental factors cooperate to drive aged phenotypes in the T cell compartment of the intestine.

Interventions to restore intestinal homeostasis during ageing

The exponential increase in the ageing population has prompted the design and development of new therapeutic avenues that foster healthier ageing. In recent years, research has highlighted the prominent role of T cells in the onset of age-related disorders [50,65,122,123]. Specifically, age-associated metabolic failure in T cells at barrier sites causes microbial dysbiosis and intestinal barrier disruption, which trigger inflammageing, tissue senescence, and health decline in mice [50]. Therefore, the interplay between T cells, the intestinal mucosa, and its resident microbiota could be exploited to establish beneficial host–microbiome interrelations and to preserve the physical integrity of the intestinal barrier during ageing (Figure 3).

Evidence for the microbiota basis of healthy ageing stems from the screening of microbial genes associated with longevity through the regulation of host metabolism [124]. Given the profound changes that the gut microbiota undergoes during ageing [28–31], rejuvenation of this ecosystem of microbes could postpone the development of age-related disorders. Antibiotic-induced microbiota depletion experiments suggest that eradication of Enterobacteriaceae could strengthen the intestinal barrier and delay inflammageing and peripheral senescence [50], in line with observations made in geriatric germ-free mice [16,93]. Given the potential resistance and off target issues arising from the use of broad-spectrum antibiotics, more sophisticated strategies are emerging to target specific members of the aged intestinal microbiota. For instance, a research group has designed a narrow-spectrum molecule with high specificity against pathogenic Enterobacteriaceae that spares the commensal microbiota [125]. This pharmacological approach may avoid gastrointestinal complications associated with the inappropriate use of antibiotics in the elderly (e.g., diarrhoea and *Clostridioides difficile* infections) [126]. Moreover, the development of **phage therapy**—in other words, the therapeutic use of bacteriophages—could also make progress in the field of Geroscience with the aim of targeting specific disease-associated bacteria in the commensal microbiota [127].

Nutraceutical interventions are emerging as geroprotective approaches to restore health-promoting members of the gut microbiota during ageing [29,30]. Transplantation of faecal microbiota from young healthy donors (yFMT) appears to be a feasible tool to extend health and lifespan in experimental models [22,48,128]. Beyond improving intestinal barrier integrity [21], yFMT successfully rebalances the lymphoid/myeloid differentiative potential of aged haematopoietic precursors [118] and enhances GC reactions in aged mice [43,129]. This strategy also mitigates ovarian ageing [130], neuroinflammation [21,131], as well as age-related alterations in the hippocampus that attenuate behavioural deficits in old mice [132]. Considering the lack of consensus regarding the description of a healthy donor microbiota, another potential solution may involve the use of **probiotics** to mitigate the hallmarks of ageing. Some strains of *Lactobacillus* improve physical, metabolic, and cognitive functions in naturally aged mice [133,134]. Supplementation with *Bifidobacterium* sp. limits osteoporosis and neurodegeneration, extending lifespan in a mouse model of premature ageing [135]. Intriguingly, analysis of the microbiome in the world's oldest living person revealed elevated abundance of *Bifidobacterium* compared with a cohort of non-supercentenarian older adults, which correlates with the daily

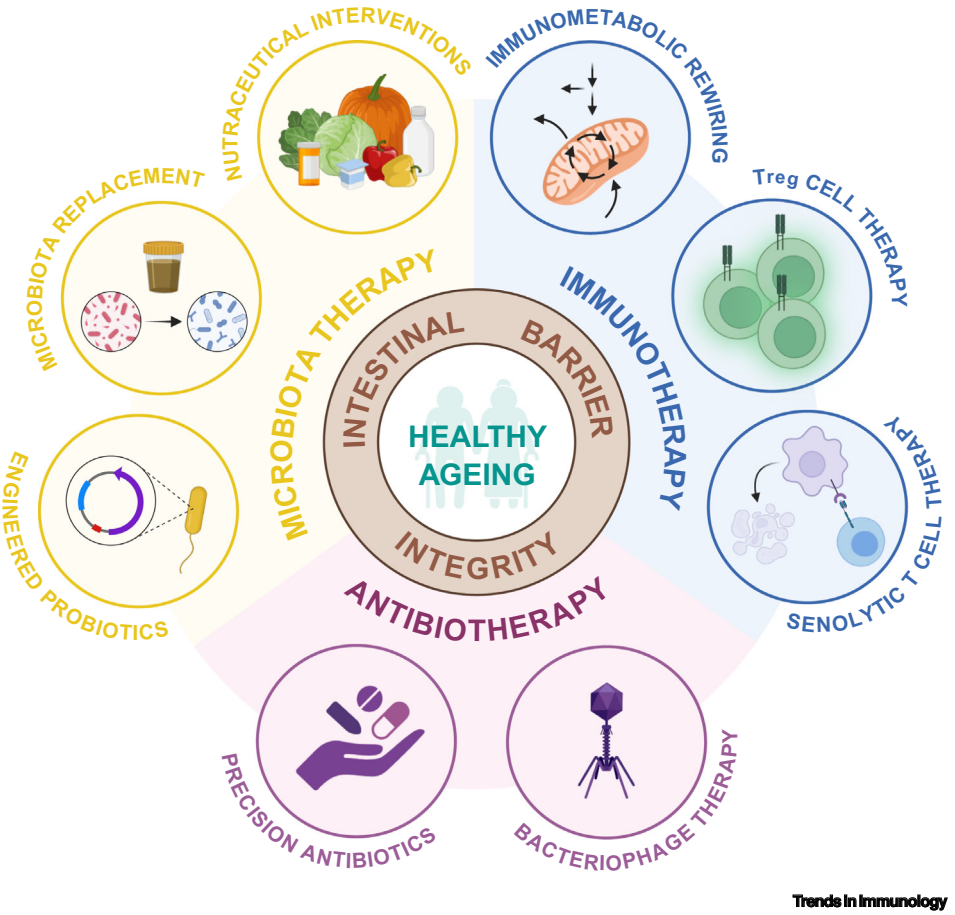

Figure 3. Intestine-targeted strategies to enhance resilience against ageing-related conditions. The microbial and immune components of the intestine could be leveraged to safeguard the intestinal barrier, which could mitigate inflammageing, tissue senescence, and health decline during ageing. The intestinal microbiota could be modulated from general approaches, such as dietary and nutraceutical interventions or transplantation of a youthful microbiota, to more specific strategies such as the use of naïve or synthetically engineered probiotics. Moreover, defined members of the gut microbiota could be eliminated by using narrow-spectrum antibiotics or targeted phage therapy. Lastly, the immune system, in particular T cells, could be rejuvenated through manipulation of their metabolism, or could be used as tools to dampen the highly inflammatory and senescent burden found in age-related diseases. This figure was created using BioRender (https://biorender.com/). T_{reg} cell: regulatory T cell.

ingestion of probiotic-containing yoghurts [41]. In addition, research has shed light on the use of synthetically designed bacteria that could express specific genes to modulate host longevity [124]. Next-generation engineered probiotics can generate self-tuneable responses to neutralise intestinal inflammation and dysbiosis [136], which could open new therapeutic avenues to reinforce intestinal barrier integrity in age-related pathologies.

Interrogation of the gut microbiota in long-lived individuals has unveiled biochemical pathways that could boost metabolic resilience in ageing hosts. For instance, some centenarian cohorts show an enrichment of metabolic routes involved in the synthesis of short-chain fatty acids (SCFAs) [39,40]. The administration of these metabolites as **postbiotics** can bypass the need for the successful engraftment of a healthy microbiota into a hostile intestinal environment during ageing. Several preclinical studies have shown that supplementation with SCFAs, particularly

butyrate, improves age-related phenotypes in mice, including neurodegeneration, renal failure, sarcopenia, and even T cell senescence [22,120,137]. Interventional evidence in older humans remains limited, however. Mechanistically, SCFAs provide alternative energy sources to sustain cellular bioenergetics, ensure intestinal hypoxia, regulate host metabolism and inflammation through activation of G protein- coupled receptors and PPARs, and modulate the host epigenetic landscape [22,138,139], all of which enhance gut barrier function. In addition, centenarian microbial signatures show a high abundance of bile-acid-modifying bacteria that participate in the preservation of secondary bile acid pools in the host [42]. Accordingly, the restoration of secondary bile acids correlates with improved health outcomes in a mouse model of accelerated ageing [128]. The anti-ageing potential of these microbiota-derived molecules may open avenues for translating bile-acid-related therapies to alleviate age-related pathologies. Therefore, elucidating the compositional and metabolic fingerprint of the gut microbiota during ageing could establish diagnostic tools and medical interventions to support a tailored geriatric medicine [140].

T cells offer compelling therapeutic opportunities to rejuvenate mucosal sites. Interventions to rewire T cell metabolism during ageing can bolster immune competence while preventing detrimental outcomes derived from aged T cells [115,141,142]. The technology of T cell-based immunotherapies is an expanding universe in the context of ageing. For instance, transplantation of T_{reg} cells from young mice shows beneficial outcomes in mouse models of age-related neurodegenerative disorders [143–145]. Adoptive transfer of competent CD4 T cells—particularly T_{reg} cells—from young mice reinvigorates gut mucosal immunity, boosting GC reactions in PPs to maintain host–microbiota symbiosis while restoring a well-balanced CD4 T cell compartment that prevents inflammation and tissue damage in the intestine in a mouse model [50]. As a result, this strategy mitigates inflammageing and senescent phenotypes, ultimately delaying signs of ageing-related multimorbidity, including sarcopenia, locomotor dysregulation, and metabolic alterations [50]. The senolytic nature of γδ T cells may soon be exploited to address age-associated diseases, since adoptive transfer of γδ T cells has shown promising results in the elimination of senescent cells in a mouse model of pulmonary fibrosis [108,146]. In addition, the knowledge of chimeric antigen receptor (CAR) T cells in oncologic treatments is being leveraged as an alternative to directly target damaging cells in age-associated pathologies. Amor *et al*. revolutionised this field by designing CAR T cells aimed at urokinase-type plasminogen activator receptor, a surface protein that is upregulated in senescent cells during ageing [147,148]. These senolytic CAR T cells restore the regenerative potential of aged ISCs and prevent MHC class II upregulation in the intestinal niche, thereby promoting intestinal barrier integrity in aged mice [149]. On top of this, combined therapy using engineered probiotics not only could restore symbiotic bacteria in the intestine but also enhance the efficacy and specificity of T cell therapies in aged hosts [150,151]. Overall, these works highlight the anti-ageing potential and therapeutic applicability of T cell-based therapies through modulation of the gut microbiota and reinforcement of the intestinal barrier.

Concluding remarks

The ageing process threatens the balance between T cells, the intestinal mucosa, and its commensal microbiota. The intricate cause–effect relationship between these players is a challenging topic to address (Figure 4). Chronologically, T cells are affected earlier due to the atrophy of the thymus at puberty and the concomitant decline in the output of naïve T cells [59,111]. The accumulation of extremely differentiated T cells interferes with gut-associated GC reactions, contributing to pathological shifts in the gut microbiota with age [43,64,93]. Microbiota transplantation experiments and analyses of geriatric germ-free mice have unveiled a causal role of gut dysbiosis in the age-related deterioration of the intestinal epithelium and persistent activation of T cells [16,21,119]. Perturbations in the intestinal barrier can also lead

Outstanding questions

What are the specific roles of T cells through their secretome and cytotoxic activities in intestinal stem cell dynamics and the epithelial barrier of the intestine during ageing?

While there is an increased frequency of regulatory T cells in the gut-associated lymphoid tissue, the intestine displays inflammatory signatures in aged mice. Do intestinal regulatory T cells still retain their anti-inflammatory and pro-resolving features in the context of ageing?

How do T cells interact with other immune-cell networks (e.g., macrophages and innate lymphoid cells) at barrier sites such as the intestine in aged mammals?

Metabolic alterations seem to correlate with age-related changes in intestinal T cells. What are the metabolic profiles of the different T cell subsets in the intestine at the steady state and during ageing?

Do T cells manifest other primary hallmarks of ageing (i.e., epigenetic alterations, loss of proteostasis, or DNA damage) in the gut-associated lymphoid tissue?

Could current T cell ageing clocks predict alterations in the gut microbiota or the integrity of the intestinal barrier to prevent age-related diseases?

Could we dissect extrinsic (e.g., diet, exercise, and polypharmacy) and intrinsic factors (e.g., impaired peristalsis and T cell ageing) to design personalised strategies to shield the intestinal barrier as we age?

Given the geroprotective actions of transferring competent T cells in mouse models, are these cellular therapies promising tools to foster healthier ageing in older individuals?

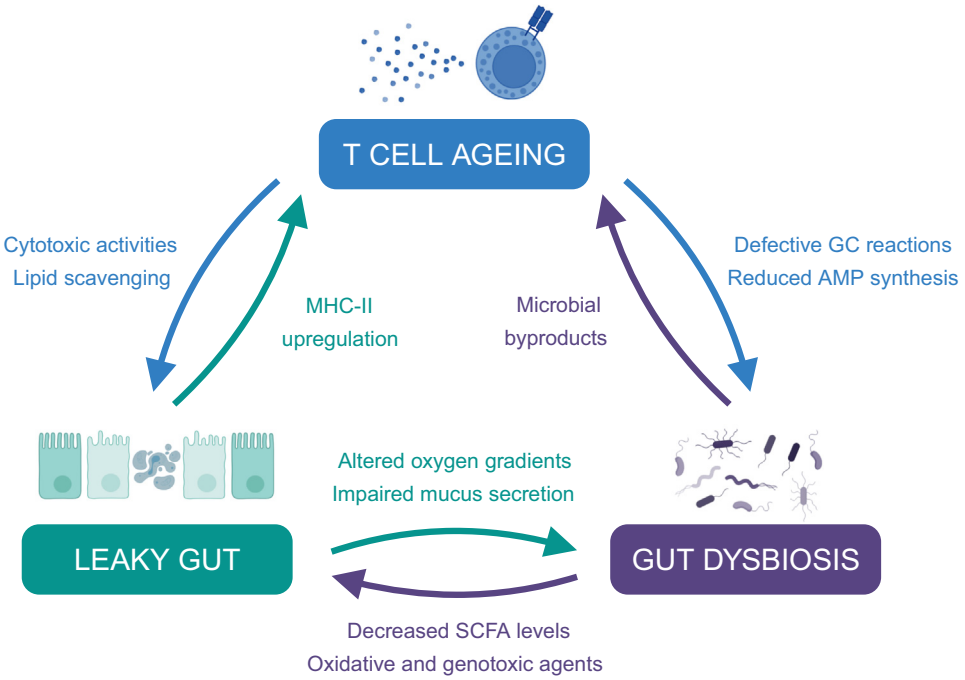

Figure 4. Disentangling cause–effect relationships between T cells, leaky gut, and dysbiosis during ageing. The intricate interplay between T cells, the intestinal epithelium, and its resident microbiota depicts a pathological vicious cycle that disrupts the integrity of the intestinal barrier during ageing. Age-related defects in T cells impair germinal centre (GC) reactions and synthesis of antimicrobial peptides (AMPs) at barrier sites, which correlate with perturbations in the gut microbiota. Dysbiotic bacteria markedly damages the intestinal epithelium through overproduction of pro-oxidative and genotoxic molecules, and decreased synthesis of beneficial short-chain fatty acids (SCFAs). The highly cytotoxic and proinflammatory activities of T cells, together with their excessive scavenging of cell-membrane lipids from intestinal epithelial cells (IECs), deteriorate the intestinal barrier and further exacerbate leaky gut. Consequent alterations in mucus layers and the oxygen gradients provided by the intestinal epithelium could drastically aggravate microbial dysbiosis. On top of this, systemic dissemination of bacterial byproducts, and the proinflammatory environment favoured by upregulation of MHC class II in IECs could drive activated and senescent profiles in the T cell compartment. This figure was created using BioRender (https://biorender.com/).

to reduced mucus secretion and alterations in luminal oxygen gradients [22–24,139], which aggravate microbial dysbiosis. On top of this, memory-like T cells can further damage the intestinal epithelium through indiscriminate cytotoxic and proinflammatory activities, which dismantle cell-to-cell junctions and alter ISC behaviour [50,83,110], and through excessive scavenging of lipid membranes from IECs that then undergo apoptosis [50,115]. Finally, enhanced bacterial translocation drives senescent phenotypes in T cells [112,113,119], suggesting a pathological vicious circle between T cells, gut dysbiosis, and leaky gut, deteriorating intestinal barrier function during ageing. Altogether, microbial dysbiosis, gut barrier dysfunction, and the subsequent translocation of bacteria to the periphery actively fuel inflammageing, tissue senescence, and health decline, which is associated with the onset of age-related cardiometabolic and neuropsychiatric diseases [16,21,50,152].

In recent years, research has placed T cells at the core of unhealthy ageing [67,68]. While this is a highly dynamic area of research, further efforts are required to fully underscore the role of T cells in the intestine during ageing (see Outstanding questions). Current analyses lack sufficient sensitivity to study age-related variations in T cell subsets that are instrumental for gut homeostasis, such as

T_{reg} cells, as well as other unconventional subpopulations such as natural IELs, invariant natural killer T cells, and mucosal-associated invariant T cells [153]. Therefore, panoramic scRNA-seq analyses could not only reveal hierarchies and networks in intestinal immune cells during ageing and upon therapeutic interventions but also help dissect intrinsic and extrinsic factors that compromise T cell functions with age.

Given the importance of gut homeostasis in health and disease, interventions to strengthen the intestinal barrier hold promise for delaying age-related conditions and fostering healthier ageing. Microbiota-based therapeutics, such as nutraceutical interventions, transplantation of a youthful microbiota, or naïve/synthetic probiotics, could restore health-promoting symbionts in the intestine [21,22,29,30,43,128,135]. Direct translation of microbiota findings from mouse models to humans requires careful validation in human cohorts. Gut architecture and physiology, as well as the taxonomic composition of the intestinal microbiota in rodents, differ from those in humans. Furthermore, the controlled environment of laboratory mice cannot fully capture the interindividual variation (i.e., genetics, lifestyle, diet, and polypharmacy) and the cumulative impact of lifelong exposures seen in human populations. Moreover, future research should focus on the compositional and functional characterisation of nonbacterial microorganisms, such as fungi and viruses, in the aged microbiota. Comprehensive analyses of the intestinal bacteriobiota, mycobiota, and virome of long-lived individuals may soon unmask the secrets to healthier longevity [41,154,155]. On top of this, strategies to rejuvenate the immune system through metabolic rewiring of aged T cells or adoptive transfer of competent or engineered T cells [50,149] could broaden therapeutic avenues to ensure host–microbe symbiosis and reinforce the intestinal barrier in aged individuals.

Therefore, advancing knowledge of the interplay between T cells, the intestinal barrier, and its commensal microbiota will ensure healthier ageing and longevity.

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Declaration of interests

The authors declare no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation and revision of this work, the authors used Perplexity in order to improve the language and readability. After using this tool, the authors carefully reviewed and edited the content as needed and take full responsibility for the content of the publication.

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