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Title: The role of barrier function, autophagy, and cytokines in maintaining intestinal homeostasis

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Abstract:
Intestinal homeostasis is maintained through the interplay of the intestinal mucosa, local and systemic immune factors, and the microbial content of the gut. The cellular processes of autophagy, endoplasmic reticulum stress, the unfolded protein response and regulation of reactive oxygen species production are required to maintain a balance between pro-inflammatory responses against potential pathogens and a tolerogenic response towards commensal bacteria. Intestinally active cytokines regulate innate immune pathways and cellular pathways within the gut mucosa. Disruption of these processes, or alterations in the cytokine milieu, can result in an improper response to the commensal gut microbial community leading to inappropriate inflammation characteristic of conditions such as inflammatory bowel disease.

Keywords:
mucosal immunity, autophagy, intestinal homeostasis, barrier function, innate immune pathways, gut cytokines

Abbreviations:
IEC – intestinal epithelial cell
TJ – tight junctions
IBD – inflammatory bowel disease
ER – endoplasmic reticulum
UPR – unfolded protein response
CD – Crohn's disease
IL - Interleukin
NOD2 – nucleotide-binding oligomerization domain–containing protein 2
TLR – Toll-like Receptor
TNF – tumor necrosis factor
IFN – Interferon
PERK - pancreatic ER eIF2α kinase

IRE1α - inositol-requiring kinase 1α
Xbp1 - X-box binding protein-1

1. Introduction

The gut represents a unique environment with an immense surface area specialized for nutrient and water absorption. The gut epithelium has among the highest mitotic rates in the body and epithelial cells undergo a constant cycle of maturation, apoptosis and renewal, all while maintaining exquisitely controlled barrier function [1]. The close proximity with the dense gut microbial community, which may harbor potential pathogens, requires precise adaptive and innate immunologic responses with a fine balance between tolerance and immune activation. This review will discuss intestinal homeostasis with a focus on intrinsic and innate immune pathways including barrier function, autophagy, endoplasmic reticulum stress and reactive oxygen species production, and their regulation by intestinally active cytokines.

2 Barrier Function

The primary role of the gut mucosa is selective absorption through transcytosis while preserving barrier function and maintaining intestinal homeostasis [2]. The maintenance of barrier function requires the dynamic regulation of multiple tissue and cellular structures and functions as summarised in Figure 1. The intestinal mucosal surface is comprised of a physical outer barrier of intestinal epithelial cells (IECs) joined by tight junctions and arranged into pore-like crypts, and in the small intestine protruding villi, protected by a layer of protective mucus which harbors a variety of secreted factors [3, 4]. The epithelium is comprised of a monolayer of specialized intestinal epithelial cell types that perform distinct functions including absorptive enterocytes, hormone-secreting enteroendocrine cells, mucin-producing goblet cells, and anti-microbial peptide-producing Paneth cells. Intestinal stem cells located at the base of each crypt provide a continual source of cell replenishment [5].

IECs actively maintain intercellular cohesion through tight junctions and desmosomes, which are carefully regulated to prevent diffusion of potential toxic substances or invasion by microbes. Tight junctions (TJ) are multi-protein complexes that regulate paracellular trafficking of macromolecules by engaging in selective permeability [2]. TJ are composed of four transmembrane proteins; occludin, claudin, junctional adhesion molecule, and tricellulin, that form a selective barrier by interactions with the adjacent cells [2, 3]. TJ are also composed of cytosolic scaffold proteins including zonula occludens proteins, which provide the foundation to the actin cytoskeleton by anchoring the transmembrane proteins [6]. Wounding of the mucosa and disruption of the intercellular connections, for example by chemically induced injury or infection, leads to rapid migration of epithelial cells into the denuded area and re-establishment of epithelial integrity [7, 8]. Structural abnormalities or functional perturbations in TJ can compromise the barrier function of the gut and significantly disturb homeostasis leading to intestinal disorders including inflammatory bowel disease (IBD), a condition characterized by chronic relapsing-remitting inflammation of the digestive tract. The resulting increase in permeability leads to internalization of TJ structural proteins (occludin, claudins, and junction adhesion molecule) further contributing to the inflammatory state [2]. Patients

with IBD have reduced expression of occludin and junctional adhesion molecule-A, but cell culture models show a significant increase of claudin-2 [2, 9, 10]. While undetectable in normal colon, claudin-2 is strongly expressed along the inflamed crypt epithelium, and is correlated to increased TJ permeability [9]. Pro-inflammatory cytokines upregulated in IBD, including IL-6, induce the expression of claudin-2 correlating with increased rates of cell division in crypts, a feature characteristic of IBD [9, 11]. Thus the inflammatory state, and particularly the increase in pro-inflammatory cytokines, is characterized by increased paracellular permeability related to alterations in TJ structure and function.

The mucous layer lies atop the epithelial cell layer and plays a critical role in host defense by providing a physical barrier, as well as additional protective mechanisms. The colonic mucous layer in particular plays an important role in separating the numerically abundant luminal bacteria from the epithelial surface [12]. Studies using fluorescence in situ hybridization to visualize bacteria residing in the gut lumen define a 50-micron bacterial-free region above the mucosa termed the bacterial zone of exclusion, a firmly organized physical barrier impeding bacterial access to the epithelial surface [13]. The thick matrix of mucous is produced by intestinal goblet cells through the synthesis and secretion of mucin proteins. Mucins are large highly glycosylated glycoproteins which form a mesh-like gel and are the main constituents of mucous [14, 15]. MUC2 is the most abundant mucin in intestinal mucous and is crucial to the mucous integrity and function [14]. Mice containing a missense mutation in the MUC2 gene, resulting in aberrant MUC2 assembly and a diminished mucus barrier, develop spontaneous distal colitis as well as enhanced susceptibility to chemical induced colitis. Moreover MUC2 mutant mice show evidence of increased endoplasmic reticulum (ER) stress in goblet cells, leading to up-regulation of the unfolded protein response (UPR) and dysregulated expression of genes involved in innate defense [16].

The protective mucosal layer shields contact between gut bacteria and IECs, however emulsifiers, detergent-like agents commonly used in processed foods, can increase bacterial translocation across the epithelium and can cause IBD-like inflammation in mouse models. Emulsifiers commonly found in processed foods promote colitis in genetically predisposed mice, and induce low-grade inflammation in wild-type mice, highlighting the necessity of a fully operative mucosal barrier against disease development [17].

Murine models and human studies have revealed the importance of Paneth cells in protection from intestinal pathogens and influencing the relative abundance of commensal bacteria. Paneth cells are specialized secretory cells of the small intestinal epithelium that reside in small clusters at the base of crypts [18]. Their diverse secretory activity, which includes anti-microbial proteins, is strongly implicated in intestinal innate immunity [19]. Their unique ultrastructural characteristics, including extensive rough endoplasmic reticulum and Golgi complexes, facilitates Paneth cell secretory function and distinctly identifies the cell type [18]. Paneth cells contain large secretory granules rich in anti-microbial peptides, including α-defensins, lysozyme, lipopolysaccharide (LPS)-binding protein, and RegIII-γ, that are secreted into the mucous layer in response to exposure to microbes, or their products, and the pro-inflammatory cytokines TNF-α

and IL1-β [18-20]. Secreted anti-microbial peptides and B-cell derived secretory immunoglobulin A (SIgA) in the mucous layer act as physicochemical shield impairing pathogenic bacteria from penetrating the mucous lining. Anti-microbial molecules bind with high affinity to enteric pathogens, however have low affinity for commensal bacteria, promoting a homeostatic microbial ecology through innate defense [12].

Defects in Paneth cell physiology or secretory activity can lead to increased inflammation by inducing ER stress, the UPR, apoptosis and autophagy (discussed below). These alterations may increase susceptibility to infection and predispose individuals to chronic inflammatory conditions including Crohn's disease (CD), a form of IBD [20, 21]. Reduced Paneth cell expression of the anti-microbial α-defensins HD5 and HD6 has been linked to ileal CD. In mice reduced expression of HD5 leads to a pronounced alteration of the luminal microbiota comparable to the deficiency of PC α-defensins in ileal CD [22]. Another link between Paneth cell dysfunction and CD is the innate immune receptor and susceptibility gene for CD called the nucleotide-binding oligomerisation domain–containing protein 2 or NOD2 (discussed further below), which is highly expressed in Paneth cells. Murine models of NOD2‐deficient Paneth cells demonstrate reduced α‐defensin expression when compared to the wild‐type mice [18]. NOD2‐deficient mice also show dysbiosis of commensal microbiota in the small intestine [23]. Reduced anti-bacterial activity and decreased defensin production by Paneth cells may therefore contribute to the pathogenesis of CD [22].

3.1 Autophagy Overview

Autophagy is a highly conserved catabolic process in eukaryotes required for the maintenance of cellular and tissue homeostasis [24]. It was initially characterized as a pathway required for starvation survival in yeast, but autophagy has since been recognized to be critical for fundamental processes including programmed cell death, proliferation, as well as cell mediated and innate immunity [25]. Autophagy involves the coordinated action of over 30 autophagy-related (ATG) proteins resulting in the characteristic formation of a double-membrane vesicle (autophagosome) engulfing targeted intracellular cargo. Autophagosomes are trafficked to and fuse with lysosomes to form an autolysosome resulting in subsequent degradation of cargo and liberation of amino acids from digested proteins [26]. This catabolic process liberates energy from degraded cargo including aged or damaged organelles and protein aggregates, thereby promoting cellular homeostasis [24]. Autophagy is required for maintaining intestinal cell survival and a normal colonic mucosa, as well as regulating inflammatory responses. Defects in autophagy-related genes are associated with inflammatory diseases of the gastrointestinal tract detailed below [27, 28].

3.2 Autophagy and Intestinal immunity

Studies have revealed the critical interplay that exists between autophagy and both innate and adaptive immunity. Autophagy has been implicated in the restriction of cytoplasmic bacterial replication, including Salmonella and adherent invasive *E. coli*, within epithelial cells [29, 30]. Autophagy is also involved in the secretion of pro-inflammatory cytokines

by epithelial cells in response to bacteria. In macrophages and dendritic cells, autophagy negatively regulates the secretion of interleukin-1β (IL-1β) and IL-18, and disruption of autophagic pathways is linked to the increased release of IL-1β [31]. Autophagy is also involved in the processing and secretion of IL-1β through the non-canonical secretion pathway. Unconventional release of IL-1β is dependent on autophagy machinery and enhanced upon autophagy activation [32]. IL-1β is translocated into the lumen small vesicles which then acquires autophagosome markers with IL-1β accumulating in the space between the outer and inner membranes of the autophagosome, and is later secreted when autophagy is triggered [32]. Therefore autophagy appears to have opposing roles, restricting IL- β production through the conventional pathway, but facilitating its release through the unconventional pathway.

In gut resident phagocytic cells, including macrophages and dendritic cells, autophagy is utilized for antigen presentation [33]. In the setting of macrophage infection by *Listeria monocytogenes* and *Mycobacterium tuberculosis*, autophagy is required to control infection [34]. This involves both recognition of the bacteria by adaptor proteins and killing within autophagolysosomes [35]. Autophagy intersects with innate immune pathways downstream of Toll-like receptor (TLR) interaction [36, 37]. Engagement of TLRs and NOD-like receptors by their ligands is a strong inducer of autophagy and downstream immune defense pathways [31, 36]. Within gastrointestinal macrophages, autophagy induced by TLR activation enhances pathogen degradation [37]. The engagement of TLR1, TLR3, TLR5, TLR6, and TLR7 leads to autophagy induction in macrophages [37]. Complementary data shows that knockdown of the autophagy protein Atg7 attenuates TLR-mediated IL-8 production in intestinal epithelial cells [38].

Pro-inflammatory cytokines and chemokines including IFN-γ, TNF-α, IL-1α, IL-2, and IL-6 have been shown to increase autophagy flux in macrophages [31, 37], while IL-4, IL-13, and IL-10 are inhibitory [39]. Autophagy can also have a direct influence on the production and secretion of cytokines including IL-1 β, IL-18, TNF-α, and Type I interferon [31]. This reciprocal relationship highlights the interdependence of autophagy and immune mediators. Autophagy is also required for B and T-cell survival and proliferation, and mice deficient in ATG5 have reduced numbers of B- and T-lymphocytes. In addition, apoptosis in CD8+ T lymphocytes is dramatically increased in the absence of Atg5 [40]. Autophagy is also required for efficient functioning of antigen cross presentation by delivering endogenous antigens to class II major histocompatibility complex loading compartments for presentation to CD4+ T cells [41]. The requirement of autophagy for survival in immune cells and the prominent interactions between autophagy and cytokine production and response reveal its pivotal role in coordinating innate and adaptive immune responses.

3.3 Autophagy in Intestinal Inflammation

Autophagy facilitates multiple critical cellular functions and therefore alterations in this pathway can lead to pleomorphic effects including disruption of intestinal homeostasis as summarised in Figure 1. Large genome wide association studies have identified a coding polymorphism in the core autophagy gene ATG16L1 as a susceptibility gene for Crohn’s

disease (CD) [33, 42-44]. The ATG16L1 risk polymorphism results in the substitution of an alanine for threonine, ATG16L1T300A, and leads to a modest increase in risk of developing CD. ATG16L1 is part of an enzymatic complex including Atg5 and Atg12 that is required for the formation of autophagolysomes in mammalian cells. In addition to its role in cellular homeostasis, autophagy plays an important role in cellular defense against gastrointestinal invasive bacteria such as Salmonella, Shigella, Listeria and *E. coli* [29, 45-47]. Defects in ATG16L1 are associated with inefficient anti-bacterial autophagy [42, 48], contributing to the pathogenesis of CD. Anti-bacterial autophagy requires bacteria to be tagged for degradation, often through the conjugation of ubiquitin by the E3 ligase LRSAM1, resulting in recruitment of the autophagy machinery via the adaptor proteins NDP52 and p62/SQSTM1 [49-51]. Additional proteins have been implicated in the recruitment of autophagy machinery to invading bacteria including Nod2, which is recruited to sites of bacterial entry [52]. Recognition of cytoplasmic bacteria can be mediated through direct recognition by E3 ligases, or by membrane damage associated with bacterial invasion by galectin 8 [53]. Bacteria engulfed by autophagosomes are trafficked to the lysosome resulting in degradation of invasive bacteria.

The Crohn's disease risk polymorphism in ATG16L1 is associated with altered granule morphology and secretions in Paneth cells, even in the absence of Crohn's disease [42]. Paneth cells are located at the base of intestinal crypts where they produce anti-microbial peptides and support the intestinal stem cell niche [42]. Using mice hypomorphic for ATG16L1 Cadwell *et al* found significant abnormalities in Paneth cells, including irregular, disorganized granules, reduced granule numbers, and decreased secretion of anti-microbial peptides similar to that observed in patients with CD [54]. Additionally, ATG16L1 deficiency in Paneth cells led to mitochondria degeneration and absence of apical microvilli. Nod2 activation in Paneth cells leads to defensin secretion and has also been linked to autophagy activation [25, 52]. Notably, deletion of Atg5 in the intestinal epithelium also showed Paneth cell and granule abnormalities, while other IECs appeared to be normal [42]. Additionally, loss of Atg7 and Atg4B produces similar defects as ATG16L1 hypomorphic mice, leading to Paneth cell abnormalities, once again highlighting this cell type's sensitivity to autophagy disruption [55, 56].

The ATG16L1T300A polymorphism is associated with impaired anti-bacterial autophagy *in vitro* [29, 48]. Recently two independent groups reported on the *in vivo* effect using knock-in mice expressing the Atg16L1 T300A variant [44, 57]. The studies revealed abnormal Paneth cell lysozyme distribution, similar to patients with the CD T300A polymorphism and ATG16L1 hypomorphic mice. Furthermore $CD11b^{+}$ cells isolated from the lamina propria exhibited impaired anti-bacterial autophagy and increased production of the pro-inflammatory cytokine IL-1β. Epithelial cells expressing Atg16L1 T300A showed enhanced bacterial replication compared to wild-type cells. The observed effects were associated with an increased propensity of the T300A variant to be degraded by caspase-3 [44, 57]. Thus, caspase-3 activation in the setting of metabolic stress or infection causes a reduction in the level of the variant ATG16L1 leading to an increase in pro-inflammatory signaling and decreased bacterial clearance resulting in further increased inflammation.

3.4 Pattern recognition receptors: Critical Inducers of Autophagy

Pattern recognition receptors are inducers of autophagy and dysfunction of these detection systems can lead to widespread inflammation, believed to contribute to the exuberant immune response observed in CD [43]. Traditional genetic linkage and positional cloning strategies, as well as genome wide association studies, have identified loss-of-function mutations and polymorphisms in NOD2 as enhancing the susceptibility for the development of CD [24, 58]. Polymorphisms in both the ATG16L1 and NOD2 genes are linked to Paneth cell dysfunction [52]. Mutations in NOD2 are linked to abnormalities in immune function that includes an overexpression of pro-inflammatory cytokines, and decreased expression of IL-10. Murine models of NOD2 deficiency lead to exaggerated intestinal inflammation as a result of this dysfunctional immune response [43]. NOD2 physically interacts with ATG16L1 and recruits the autophagy machinery to the site of bacterial entry into cells [52]. A physical interaction between the two proteins is necessary for autophagy-mediated clearance of pathogens [43]. Patients possessing mutations in both NOD2 and ATG16L1 have dysfunctional autophagy and impaired formation of autophagosomes [43]. Similarly to NOD2, NOD1 also contributes to ATG16L1 recruitment [52]. In addition, independently of its role in autophagy, ATG16L1 negatively regulates NOD1 and NOD2, leading to suppressed pro-inflammatory downstream signaling [21].

Engagement of TLR2, TLR4 and TLR5 by their respective ligands stimulate autophagic clearance of pathogens in macrophages [59]. Furthermore, TLR4 stimulation by LPS results in an overexpression of pro-inflammatory cytokines IL-1β and IL-18 in ATG16L1-deficient macrophages compared to wild-type macrophages. Additionally, chimeric mice lacking Atg16L1 in the haematopoetic compartment are highly susceptible to chemically-induced colitis [59]. The elevated immune response in the context of Atg16L1 deficiency highlights the important interactions between autophagy and TLR-mediated inflammatory response.

3.5 Autophagy and ER stress

The endoplasmic reticulum (ER) is responsible for the proper folding of secretory and membrane proteins. Intestinal cells are specialized for the secretion of proteins into the intestinal lumen and therefore are reliant on ER homeostasis, which can be easily disturbed by physiological and pathological conditions such as oxidative stress or energy deficiencies [60]. This leads to an accumulation of misfolded proteins, a condition known as ER stress, which is associated with inflammatory diseases, such as IBD and type-2 diabetes. ER stress activates the unfolded protein response (UPR) [61], which plays a critical role in restoring ER homeostasis following accumulation of potentially toxic misfolded proteins [62].

Increased ER stress/UPR leads to induction of autophagy [62, 63]. The UPR leads to activation of three ER-resident transmembrane proteins: activating transcription factor 6α, pancreatic ER eIF2α kinase (PERK) and inositol-requiring kinase 1α (IRE1α). The PERK–IRE1α pathway is essential for autophagy induction after ER stress [62].

Furthermore, gut inflammation induces the UPR in IECs leading to activation of the transcription factor X-box binding protein-1 (Xbp1) [62]. Deletion of Xbp1 within IECs results in excessive ER stress, Paneth cell apoptosis, spontaneous enteritis and increased secretion of pro-inflammatory cytokines including tumour-necrosis factor (TNFα) [38]. Selective deletion of Xbp1 in Paneth cells results in ER stress, autophagy induction and spontaneous ileitis [63]. Atg16l1/Xbp1 deficient mice, which lack UPR-induced autophagy in IECs, developed severe spontaneous CD-like ileitis as a result of IEC death, IRE1α -regulated NF-κB activation and TNF signaling [63]. This model reveals the important role of ATG16L1 in suppressing ER stress-induced inflammation, acting as a feedback mechanism by restraining IRE1α, modulating the pathologic overproduction of NF-κB, and preventing IEC death [63].

Autophagy is also linked to ER stress through the NOD-like receptor family [61]. In addition to initiating anti-bacterial autophagy, NOD1 and NOD2 mediate ER-stress-induced inflammation in IECs [61]. In murine models ER stress triggers the production of pro-inflammatory cytokine IL-6, which is dependent on NOD1 and NOD2 [61]. These findings suggest that variants in NOD2 may contribute to inflammatory conditions such as CD, not only through impairment of NOD-mediated inflammatory responses, but also through ER stress-induced autophagy.

3.6 Autophagy and ROS

Reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, play a role in regulating autophagy. ROS are generated in the mitochondria through nicotinamide adenine dinucleotide phosphate oxidases, and are involved in a variety of critical physiological and pathological processes associated with cell survival, death, and immune defenses [64]. Cell starvation induces the production of ROS, which can cause oxidative damage and cell death [65]. However ROS production can also promote cell survival through autophagy-mediated engulfment of damaged mitochondria [66]. In addition to activating starvation-induced autophagy, ROS can also activate anti-bacterial autophagy, and autophagic cell death, while autophagy can play a regulatory role by suppressing ROS production [65]. Along the intestinal epithelium, ROS produced in response to bacteria or pathogens play important roles in IEC signaling to promote epithelial repair and wound healing [67].

4.1 Cytokines: Modulators of Mucosal Homeostasis

Cytokines play a key role in the maintenance of intestinal homeostasis, orchestrating the actions of innate and adaptive immunity imperative to host defense and healing through their pro- or anti-inflammatory actions. In the context of mucosal immunity cytokines regulate mucosal barrier function, influence immune cell migration and activation, and impact cellular metabolism to maintain a homeostatic environment. Cytokine dysregulation underlies the pathogenesis of multiple inflammatory disorders including Coeliac disease, eosinophilic disorders, ulcerative colitis and Crohn’s disease. The main sources of cytokines in the intestine include transient and resident lymphocytes,

mononuclear cells - including macrophages and dendritic cells, polymorphonuclear cells, innate lymphoid cells as well as intestinal epithelial cells and supporting stromal cells.

4.2 IL-1 – driver of inflammation

The IL-1 family of pro-inflammatory cytokines plays a pivotal role in regulating mucosal immunity, particularly in the context of intestinal pathogens. Some members of the IL-1 cytokine family are synthesized as pre-cursor proteins and secreted as mature proteins under inflammatory conditions via caspase-1-mediated cleavage (see Section 3.2) [68]. The IL-1 family is expressed throughout the GI tract where it has been associated with autoimmune and chronic inflammatory diseases [68, 69]. The IL-1 family of cytokines are predominately produced by stimulated macrophages, monocytes, fibroblasts, and dendritic cells, but can also be produced by lymphocytes and epithelial cells [70, 71]. In the gut, various family members including IL- 1α, IL- 1β, IL-18, IL-33 and IL-37 are produced directly by IECs [72]. IL- 1α and IL- 1β bind to two IL-1 receptor types; IL-1 receptor type I and IL-1 receptor type II [71, 73]. Signaling is regulated by the IL-1 receptor antagonist (IL- 1Ra), which competitively binds the IL-1RI. IL ‐ 1α and IL ‐ 1β are predominantly expressed by macrophages in inflamed tissue, whereas IL- 1Ra is produced mainly by IECs [73]. The balance of IL-1 and IL-1Ra secretion within the intestinal mucosa regulates the balance between inflammation and tolerance [74]. Early administration of IL-1Ra in murine models of colitis is associated with decreased tissue injury and reduced inflammation [75].

Upon binding, IL-1RI initiates signal transduction promoting cell proliferation, while IL-1RII acts as a natural inhibitor of IL-1 [73]. Although IL- 1α and IL- 1β signal through the same receptor complex, they have different activities and roles in inflammatory responses, including the pathogenic inflammation observed in ulcerative colitis [75]. IL- 1α acts as a regulatory cytokine, inducing the activation of transcription factors during times of cellular stress or inflammatory conditions promoting the expression of genes involved in cell proliferation and differentiation. IL-1α functions locally in the early stages of the immune response, and induces inflammation through the recruitment of neutrophils. In contrast, IL- 1β acts systemically and is involved in mediating widespread inflammatory responses that may eventuate tissue damage [73, 75]. IL-1β expression is significantly enhanced in the inflamed mucosa of patients with IBD compared with healthy patients [76].

Full length IL-1α is able to facilitate pro-inflammatory gene transcription independent of IL-1R1 binding [77]. IL-1α contains a nuclear localisation sequence within the N-terminal portion of the molecule. Unlike IL-1β, IL-1α can act as a damage-associated molecular pattern (DAMP); molecules or alarmins released by stressed cells undergoing necrosis that function as endogenous signals of danger by activating immune responses. Binding rapidly initiates the production of chemokines and inflammatory cytokines, promoting ‘sterile inflammation’ in the absence of infection [78].

IL-1α and IL-1β may have further implications in intestinal inflammation through autophagy (see Section 3.2). Induction of autophagy is associated with decreased IL-1α

and IL-1β production, and disruption of autophagic flux leads to increased secretion. Working in a negative feedback fashion, the two cytokines exhibit regulatory roles by inducing autophagosome formation [39].

Other members of the IL-1 family have pronounced roles in regulating intestinal inflammation. IL-33 is upregulated within inflamed mucosa in patients with IBD and is associated with delayed wound healing and impaired epithelial barrier function in chemically-induced colitis models. IL-33-deficient mice have reduced intestinal inflammation compared to controls, reinforcing its pro-inflammatory function in the colon [79]. Interesting, recent evidence may suggest a protective role of IL-33 in mucosal immunity against parasites. By promoting the expansion of innate effector leukocytes, called nuocytes, IL-33 facilitates an effective immune response that leads to epithelial and goblet cell proliferation and eosinophilic infiltration in the gut mucosa [80]. Notably the activity of pro-inflammatory IL-18 has been implicated in several autoimmune diseases including IBD [81]. Throughout the GI tract, IL-18 is produced primarily by macrophages and DCs. IL-18 mediates its immunoregulatory role by inducing pro-inflammatory IFNγ release from natural killer cells [81, 82]. IL-18 deficient mice have reduced mucosal inflammation in murine models of CD [83]. Neutralization with the IL-18 antibody leads to a decrease in IFNγ, and reduction of IFNγ in CD is associated with a protective clinical response [82]. Finally, blocking IL-18 with the IL-18 binding protein reduces experimental colitis [81]. These studies highlight the contribution of IL-1 family members in initiating and regulating gut inflammation.

4.3 IL-10 – regulator of inflammation

The IL-10 family of cytokines comprises nine members including the IL-20 subfamily and the type III IFN family [74]. IL-10 itself is a critical anti-inflammatory cytokine in the regulation of mucosal homeostasis [84]. Binding of IL-10 to its heterodimeric transmembrane receptor complex consisting of IL-10R1 and IL-10R, results in the activation of the Jak/STAT signaling pathway and subsequent activation of the transcriptional factors STAT3 and STAT1, and in non-macrophage cells STAT5 [85]. Intestinal sources of IL-10 include T cells, B cells, and activated monocytes [74, 84]. IL-10 attenuates chronic intestinal inflammation by potently inhibiting antigen presentation and the subsequent release of pro-inflammatory cytokines such as IL-1α, IL-1β, TNFα, IFN-γ, and IL-6 [69, 86]. IL-10 dampens acute inflammation caused by activated macrophages and dendritic cells (DC) in response to pathogen detection [87]. IL-10 also limits the proliferation and differentiation of T cells by inhibiting antigen-presentation on antigen presenting cells and down regulating class II major histocompatibility complex (MHC) expression [88].

Several lines of evidence support a protective role for IL-10 signaling in limiting the pathogenesis of IBD. Genome-wide association studies show that polymorphisms in the IL-10 gene are associated with an increased risk for IBD [89-91]. Additionally, studies show that patients with homozygous loss of function mutations in IL-10 and IL10R develop a severe form of IBD with a very early onset (under the age of 5) [92].

In the gut, IL-10 is primarily produced by a subset of CD4+ T cells known as regulatory T (Treg) cells. Treg cells suppress inflammatory responses that may cause tissue damage by producing IL-10 and other anti-inflammatory cytokines [93]. Depletion of Treg cells or disruption of IL10 or its receptor results in spontaneous colitis [94]. T cell-specific IL-10R signaling is important for suppression of inflammation, however IL-10 detection by innate immune cells is also required for regulating intestinal inflammation and preventing colitis, independent of its effects on T cells. In mice, IL-10R-disruption on $CD4^+$ T cells impairs Treg cell differentiation and function and leads to the development severe colitis. The loss of IL-10R signaling also results in the impaired generation of intestinal macrophages and their ability to secrete IL-10. Importantly, IL-10R-dependent signals determine the functional fate of intestinal macrophages as predominantly pro-inflammatory or anti-inflammatory [89].

IL-10 has a critical role in the regulation of intestinal inflammation and maintenance of homeostasis. IL-10-deficient mice develop extensive intestinal inflammation and chronic enterocolitis reminiscent of human celiac disease and ulcerative colitis [95]. IL-10 deficiency results in an accumulation of inflammatory infiltrates in the mucosa and defective expression of MHC class II molecules in IECs which leads to disease pathology [95]. IL-10-deficient mice in germ-free conditions do not develop colitis or immune system activation, demonstrating that resident enteric bacteria are necessary for the development of spontaneous colitis and that IL-10 plays an important role in the generally tolerogenic immune response to commensal gut bacteria [96]. Interestingly, in macrophages it is the loss of IL-10R expression, and not the loss of gut resident macrophage IL-10 production, that leads to spontaneous development of severe colitis, emphasizing the importance of the innate immune response to this cytokine [97].

IL-10 also exerts effects on the intestinal epithelial cells by driving mucin production in goblet cells and maintaining barrier function (see Section 2). Administration of antibodies against IL-10 or IL-10R leads to a rapid increase in ER stress and T-cell-mediated inflammation in mice harbouring a MUC2 point mutation that develop spontaneous colitis. IL-10 antibody administration enhances MUC2 misfolding in the ER of goblet cells, which leads to an accumulation of proteins and subsequent activation of the UPR [98]. This demonstrates the direct effect of IL-10 on modulating cellular homeostasis and consequently mucus production by goblet cells.

Murine IECs deficient in IL-10 show altered interactions with non-pathogenic enteric bacteria resulting in chronic inflammation [99]. Wild type and IL-10-deficient germ-free mice colonized with non-pathogenic bacteria express a completely different set of regulatory proteins as determined by proteomic analysis. The changes in protein levels identify pathways responsible for regulating ER stress, energy metabolism, and apoptosis as contributing to the observed inflammation and loss of tissue homeostasis in the IL-10 deficient mice [100]. The absence of colitis observed in wild-type mice reveals the tolerogenic role that IL- 10 plays in the interplay between commensal enteric bacteria and the intestinal mucosa [99, 100].

IL-10 modulates immune responses in the intestine and is involved in regulation of ER stress, energy metabolism and apoptosis. Murine knockout models, as well as translational studies from patients harboring mutations, suggest that IL-10 and its receptor are key regulators of mucosal homeostasis.

4.4 IL-22 and gut homeostasis

Among the intestinally active cytokines, IL-22 plays a key role in regulating mucosal barrier function and maintaining intestinal homeostasis. This cytokine has both pro-inflammatory and anti-inflammatory functions but studies suggest a protective role for IL-22 in the gut. IL-22 is member of the IL-10 cytokine family, and is produced by innate lymphoid cells, dendritic cells, neutrophils and various subtypes of CD4+ T cells, most notably; Th17, Th1 and Th22 cells [101, 102]. IL-22 is recognized by a heterodimeric receptor complex composed of two subunits, IL-10R2 which is expressed on a broad array of tissues and IL-22R1, which is expressed in a limited number of tissues including the intestine, lung and skin [103]. Therefore the effects of IL-22 are important to barrier surfaces including the mucosal epithelia of the digestive system [104]. IL-22R1 is highly expressed by epithelial cells of the small intestine under normal conditions but only expressed in the colonic epithelium under inflammatory conditions [105]. The binding of IL-22 to its receptor induces Jak-mediated STAT3 phosphorylation and subsequent nuclear translocation, resulting in transcriptional upregulation of various anti-microbial peptides and mucins. IL-22 signalling restores barrier function during inflammation and its protective role for the gut mucosa has also been demonstrated in mouse models of colitis. In the intestine, IL-22 promotes wound healing, tissue regeneration, and epithelial cell proliferation [106, 107]. It regulates epithelial-microbial homeostasis through the induction in IECs of anti-microbial peptides, specifically of the Reg family, including RegIIIβ and RegIIIγ, as well as β-Defensin 2 and β-Defensin 3 [103, 108]. Through the production of these AMPs, IL-22 significantly contributes to innate defense to maintain the epithelial integrity of the colon during colitis.

IL-22 also plays an important role in alleviating oxidative stress, one of the key features of chronic inflammation [109]. IL-22 stimulation induces the transcription of proteins that protect from cellular stress [106]. As stated earlier, conditions of stress initiate the immune response and through the induction of specific proteins, IL-22 can counteract uncontrolled inflammation. In animal models, IL-22 is associated with susceptibility to colonic inflammation [110] and dysregulated IL-22 expression at barrier surfaces is associated with inflammatory human disease, suggesting a critical role in the maintenance of normal barrier homeostasis [106, 111]. An aggressive immune response is associated with an increase in pro-inflammatory cytokines that may impair epithelial TJs and lead to a diminished mucosal barrier. In mice with diet induced colonic inflammation, administration of IL-22 limits excessive inflammation by reducing both ER and oxidative stress, improving the integrity of the mucosal barrier [112].

In vivo IL-22 gene delivery targeting inflamed tissue leads to the rapid amelioration of local intestinal inflammation in a STAT3 dependent manner [105, 106, 110]. Knockout

mice with IEC-specific deletion of STAT3 activity are highly susceptible to experimental colitis [106]. Thus, activation of STAT3 pathways appears to be crucial for attaining the protective benefits of IL-22 against colitis. In the colon goblet cell depletion is a characteristic finding in IBD and IL-22 promotes goblet cell restitution, which correlates with STAT3 activation [110]. Heterologous expression of IL-22 in the colon results in improvement in chronic colitis through restoration of goblet cells and enhanced mucus production, thereby alleviating disease severity [110].

Studies focusing on the protective role for IL-22 show that treatment with anti-IL-22 antibody in the recovery phase of chemically-induced murine colitis results in impaired recovery. Antibody-mediated inactivation of IL-22 is associated with a thin colonic wall and reduced goblet cell restoration, as well as more severe weight-loss and extensive inflammation, demonstrating the importance of endogenous IL-22 in limiting inflammation and maintaining homeostasis [110]. Furthermore, ethanol and burn-induced mucosal injury results in increased intestinal permeability and decreased Reg3γ expression that is rescued by IL-22 administration [113]. These findings suggest that the IL-22 may have therapeutic potential by accelerating recovery following mucosal injury and restoring anti-microbial peptide pathway.

IL-22 binds competitively to the secreted IL-22 binding protein (IL-22BP) also known as IL22RA2, with an affinity of 20- to 1,000-fold higher than the IL22 receptor [104]. Under basal conditions, IL-22BP is highly expressed by dendritic cells in the colon leading to low IL-22 activity, however under inflammatory conditions, and particularly during the recovery phase following chemical injury, IL-22BP is down regulated, promoting IL-22 mediated homeostatic effects [114, 115]. Heterologous overexpression of IL-22BP suppresses IL-22-mediaited goblet cell restitution during the of recovery phase of murine colitis [110]. Although the IL-22-IL-22BP axis remains incompletely understood, the findings to date support a crucial role for endogenous IL-22BP in regulating intestinal tissue repair, and additionally reveal the inverse pattern of effects of IL-22BP with IL-22.

5 Conclusion

The maintenance of intestinal homeostasis relies on the critical interactions of the innate and adaptive immune system acting on critical pathways within the gut mucosa. Barrier function within the epithelium is maintained through intercellular adhesion that limits access to the underlying lamina propria and circulatory system. In addition the mucous layer that is secreted by goblet cells contains secreted anti-microbial peptides and immunoglobulins that protect against potential pathogens. Innate cellular process important for maintaining homeostasis including autophagy, ER stress and ROS, and these processes are involved in bidirectional regulation with secreted cytokines and immune cells. Pro-inflammatory signalling, which is critical for host defense against pathogens, is balanced against anti-inflammatory pathways that promote tolerance to commensal gut flora. Disruption of this balance can lead to pathologic inflammatory conditions of the gut, including inflammatory bowel disease.

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DE wrote the manuscript with JB.

Figure Legends

Figure 1. (A) Overview of the villus crypt architecture. (B) Perturbations underlying the loss of intestinal homeostasis in the setting of pathologic inflammation. (C) Normal crypt homeostasis. (D) Effect of impaired autophagy on crypt homeostasis.

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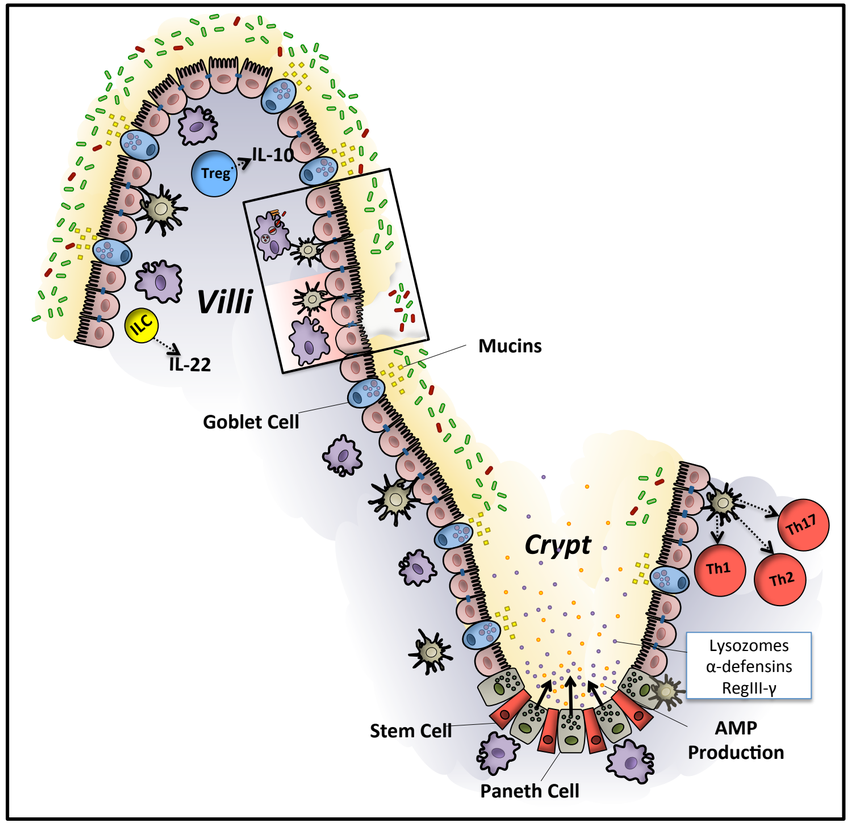
A


IL-10
Treg
Villi
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IL-22
Mucins
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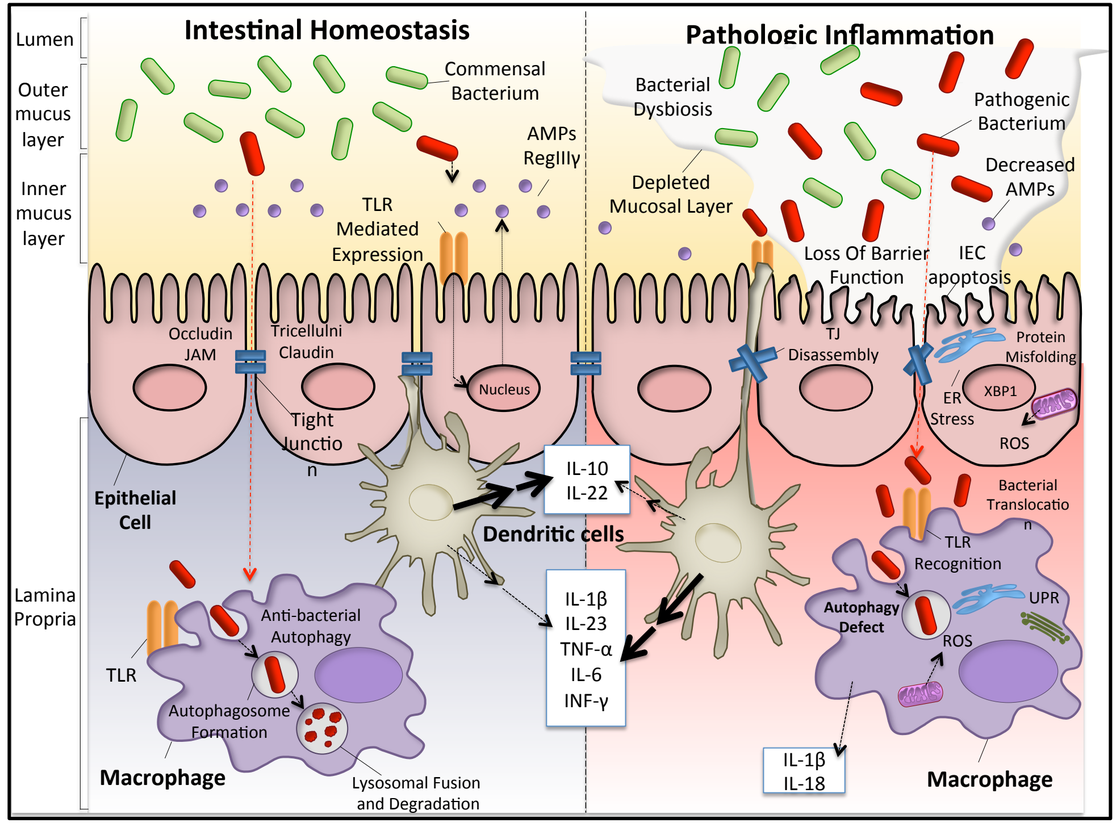
α-defensins
RegIII-γ
Stem Cell
AMP
Production
Paneth Cell

B


Lumen
Outer mucus layer
Inner mucus layer
Lamina Propria
Intestinal Homeostasis
Commensal Bacterium
AMPs
RegIIIγ
TLR Mediated Expression
Occludin
JAM
Tricellulni
Claudin
Tight Junctio n
Nucleus
Epithelial Cell
IL-10
IL-22
Dendritic cells
IL-1β
IL-23
TNF-α
IL-6
INF-γ
TLR
Anti-bacterial Autophagy
Autophagosome Formation
Macrophage
Lysosomal Fusion and Degradation
Pathologic Inflammation
Bacterial Dysbiosis
Pathogenic Bacterium
Decreased AMPs
Depleted Mucosal Layer
Loss Of Barrier Function
IEC apoptosis
TJ Disassembly
Protein Misfolding
ER Stress
XBP1
ROS
Bacterial Translocatio n
TLR Recognition
UPR
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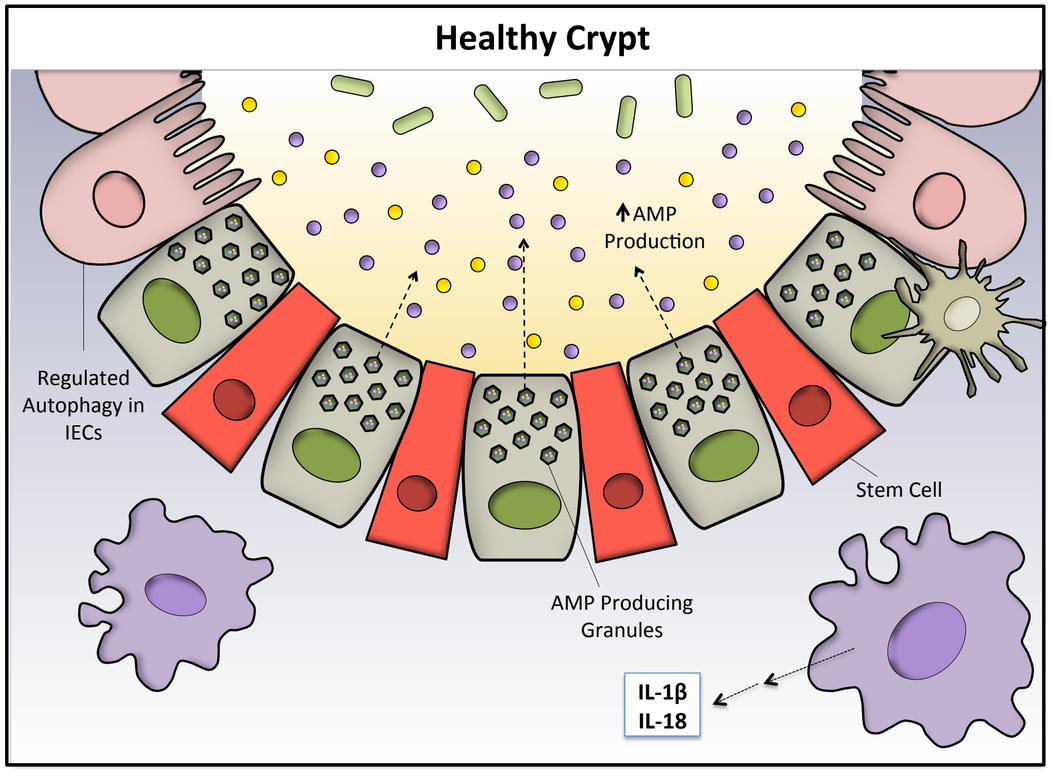
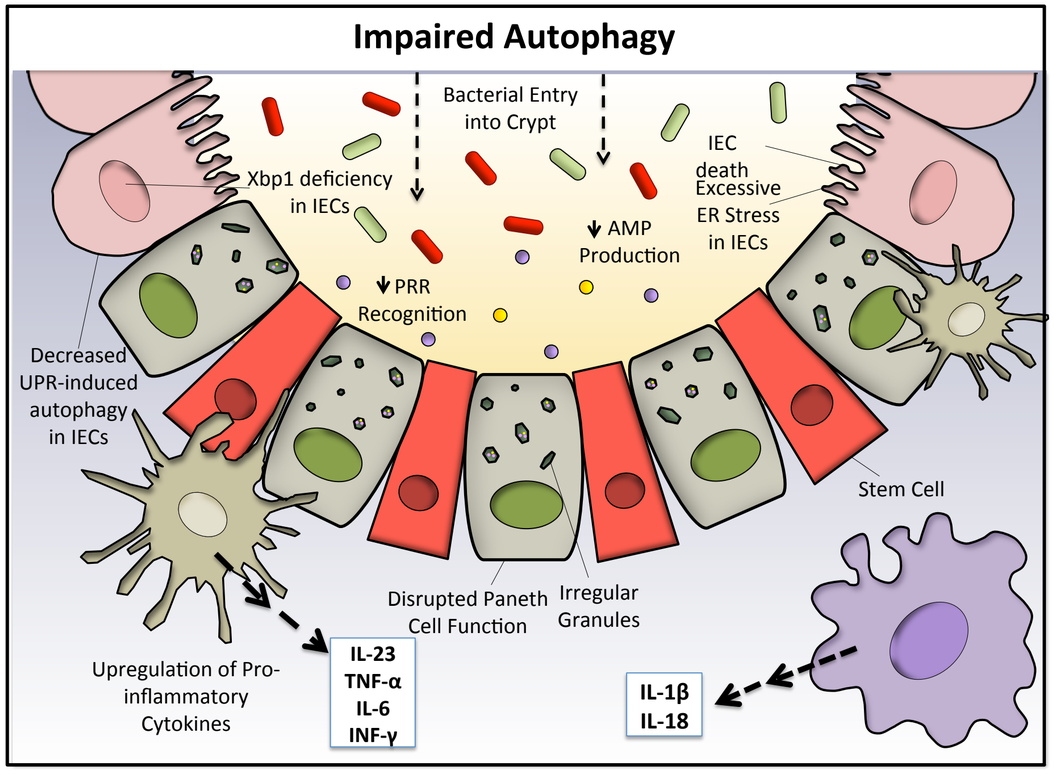
ROS
IL-1β
IL-18
Macrophage

C


Healthy Crypt
AMP Production
Regulated Autophagy in IECs
Stem Cell
AMP Producing Granules
IL-1β
IL-18

D


Impaired Autophagy
Bacterial Entry into Crypt
Xbp1 deficiency in IECs
IEC death
Excessive ER Stress in IECs
AMP Production
PRR Recognition
Decreased UPR-induced autophagy in IECs
Stem Cell
Disrupted Paneth Cell Function
Irregular Granules
Upregulation of Pro-inflammatory Cytokines
IL-23
TNF-α
IL-6
INF-γ
IL-1β
IL-18