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Review

Cell death of intestinal epithelial cells in intestinal diseases

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Abstract: Gut injury continues to be the devastating and unpredictable critical illness associated with increased cell death of intestinal epithelial cells (IECs). The IECs, immune system and microbiome are the interrelated entities to maintain normal intestinal homeostasis and barrier integrity. In response to microbial invasion, IEC cell death occurs to maintain intestinal epithelium function and retain the continuous renewal and tissue homeostasis. But the imbalance of IEC cell death results in increased intestinal permeability and barrier dysfunction that leads to several acute and chronic intestinal diseases, such as intestinal ischemia/reperfusion (I/R), sepsis, inflammatory bowel diseases (IBD), necrotizing enterocolitis (NEC), *etc*. During the pathophysiological state, the excessive IEC apoptotic cell death leads to a chronic inflammatory condition, later switches to necroptotic cell death mechanism that induces more pathological features than apoptosis and may also induce other lytic cell death mechanisms like pyroptosis and ferroptosis to increase the pathogenesis of the intestinal diseases. But still, there remains gaps in the fundamental knowledge about the IEC cell death mechanisms in chronic intestinal diseases. Together, a deep understanding of the specific cell death mechanisms underlying chronic intestinal diseases, including sepsis, IBD, NEC, and intestinal I/R, is desperately needed to develop emerging novel promising therapeutic strategies. This review aims to show how the acute and critical illness in the gut are driven by IEC cell death mechanism, such as apoptosis, necrosis, necroptosis, pyroptosis, and ferroptosis.

Key words: intestinal epithelial cells; apoptosis; necroptosis; pyroptosis; ferroptosis; intestinal diseases

肠道疾病中肠上皮细胞的死亡

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摘 要：目前，由肠上皮细胞死亡引发的肠损伤仍然是危险且难以预测的严重疾病之一。肠上皮细胞、免疫系统和微生物组之间相互关联以维持正常肠道稳态和肠屏障的完整性。在微生物入侵时，肠上皮细胞进入死亡程序以维持肠上皮功能，并且保持其持续更新能力，维护组织稳态。但是脱离稳态的肠上皮细胞死亡会导致肠通透性增加和肠屏障功能障碍，导致多种急性和慢性肠道疾病，例如肠缺血/再灌注、败血症、炎症性肠病(inflammatory bowel diseases, IBD)，坏死性小肠结肠炎(necrotizing enterocolitis, NEC)等。在病理生理状态下，过量的肠上皮细胞凋亡性死亡导致慢性炎症状态，而后转向坏死性凋亡细胞死亡机制，其诱导的病理特征比细胞凋亡的情况下更多，此外还可能诱导其它溶细胞性死亡机制，例如细胞焦亡和铁凋亡，从而增加肠道疾病的病理指征。但是，目前关于慢性肠道疾病中肠上皮细胞死亡机制的研究仍然存在空白。目前亟需对慢性肠道疾病(包括败血症，IBD，NEC和肠缺血/再灌注)中特定的细胞死亡机制进行深入了解，以开发针对此类疾病

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的新型的有效治疗策略。本文旨在综述肠上皮细胞不同死亡机制(例如细胞凋亡，细胞坏死，坏死性凋亡，细胞焦亡和铁凋亡)在急、慢性肠道疾病中的研究进展。

关键词：肠上皮细胞；细胞凋亡；坏死性凋亡；细胞焦亡；铁凋亡；肠道疾病
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1 Introduction

The intestinal epithelium consists of a single layer of tightly linked columnar epithelial cells, providing the intestinal mucosa's first-line defense. It is organized in crypt-villus units encompassing the luminal surface of the intestinal mucosa and is continuously replaced every 4–5 days. The intestinal epithelium has various critical physiological functions beyond absorption and digestion, and it mainly provides a critical barrier to prevent the translocation of destructive intraluminal substances including foreign antigens, microorganisms, and their toxins [1]. In the event of a sensation of danger molecules, enterocytes transmit the signals to underlying cells/tissues and to the body to initiate innate and adaptive immune defense mechanisms together with specialized immune cells [2]. Therefore, the epithelium, the immune system, and the microbiome are the three closely interrelated entities to maintain the balanced homeostasis in the gut [3]. The alteration in these entities, including the dysregulation of the immune system, the perturbation of intestinal epithelial homeostasis, uncontrolled bacterial colonization, and the epithelial barrier dysfunction contribute to the onset of the gut injury [4]. Consequently, the disruption of intestinal membrane permeability by altering cell-cell junctional proteins such as occludin, E-cadherin, and ZO-1, is a pathogenic key factor and an early marker in the development of systemic inflammatory response syndrome and multiple organ failure (MOF).

Now the key questions are, how disruption of intestinal epithelium homeostasis arises and progresses into the acute and chronic gut injury? What is the central risk factor for the enhanced intestinal permeability? In this regard, the growing evidence shows that increased intestinal permeability is highly associated with dysregulated intestinal epithelial cells (IECs) death [3]. The inappropriate IECs apoptosis promotes gut injury, intestinal barrier dysfunction, and translocation of bacteria, which results in chronic gastrointestinal (GI) disorders. Apoptosis is the important event to maintain the function of the intestinal epithelium at normal state, but the excessive cell death leads to the chronic inflammatory condition during the pathophysiological state, as found in the patients with critical GI symptoms [3]. Beyond apoptosis, the other programmed and non-programmed cell death mechanisms including necrosis, necroptosis, pyroptosis, and ferroptosis also play a key role in the pathogenesis of acute and chronic gut injury. We postulate that IECs cell death mechanism plays a key role in the intestinal hyperpermeability seen in the chronic intestinal diseases. This review highlights mechanisms of how IEC death results in acute and chronic GI disorders, as well as preventive approaches to IECs death mechanisms for restoring and maintaining the gut barrier.

2 IECs cell death

The persistent IECs renewal is essential for maintaining tissue homeostasis. As we mentioned earlier, the excessive IECs cell death disrupts intestinal barrier integrity and permits the invasion of luminal antigens into the lamina propria (LP), thereby leading to a chronic inflammatory condition in the LP [5]. IECs undergoes several cell death pathways including apoptosis, necrosis, necroptosis, pyroptosis, and ferroptosis, depending on their stress, inflammatory, and microbial dysbiosis state. Therefore, IECs cell death is a hallmark of intestinal inflammation. To understand the pathophysiology and pathogenesis of acute and chronic GI diseases, as well as the development of therapeutic approaches for GI illnesses, it is therefore essential to focus on the cellular and molecular mechanisms of IECs cell death.

2.1 Apoptosis

Apoptosis is a programmed cell death, characterized by the cell rounding, nuclear fragmentation, and blebbing of the plasma membrane [6]. It occurs spontaneously in IECs as the end-phase of migration and differentiation along the crypt-villus axis to maintain the regular gut morphology and function, including the intestinal homeostatic balance between epithelial cells proliferation and apoptosis [7]. The imbalance of this event turns out to the excessive loss of villi IECs beyond the frequency

of crypt IECs regeneration, which leads to pathological IEC shedding [8]. In the early era of pathogenic microbial translocation, the dysregulated apoptosis is initially mediated by pattern recognition receptors (PRRs) including toll-like receptors (TLRs) and nucleotide-binding domain leucine-rich repeat containing receptors (NLRs) of IECs and other inflammatory cells; These receptors recognize the ligands such as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) of the pathogenic microbes [9]. After receptor-ligand binding occurs, the IECs quickly upregulate the expression levels of pro-inflammatory cytokines and mediators including tumor necrosis factor (TNF)-α, nitric oxide (NO) and pro-inflammatory chemokines to attract the inflammatory cells. Moreover, this chronic inflammatory environment induces the concomitant expression of death receptors such as Fas and tumor necrosis factor receptor-1 (TNFR1) and their ligands such as Fasl, TNF-α, and TNF-related apoptosis-inducing ligand (TRAIL), by the same or adjacent cells to induce the extrinsic cell death mechanism of enterocytes [10]. The binding of ligands to their respective death receptors recruits TNF receptor type 1-associated death domain (TRADD) protein and receptor-interacting protein kinase 1 (RIPK1) to TNFR1 to form a complex I [11]. Subsequently, RIPK1 dissociates from TNFR1 and recruits adaptor proteins such as Fas-associated death domain (FADD) to initiate the Caspases cascade including Caspase-8 to form the death-induced signaling complex (DISC) [12]. Finally, the activation of Caspase-8 can either directly activates the downstream Caspases like Caspase-3, -6, and -7 or cleaves pro-Bid to form Bid which is translocated to mitochondria (intrinsic pathway) and induces cytochrome c along with the activating factor 1 (Apaf-1) to activate Caspase-3 and -9 [12], thereby results in apoptosis. Although the Caspase-mediated apoptosis is essential for IECs turnover and GI tract morphology, growing evidence has shown the pathogenic function of Caspase-mediated IECs apoptosis in the chronic GI disease, such as Crohn's disease (CD) and ulcerative colitis (UC) [13] (Fig. 1).

2.2 Necrosis

Unlike apoptosis, necrosis is an unprogrammed or uncontrolled and accidental form of cell death, characterized by cell and organelles swelling, moderate chromatin condensation, rupture of the plasma membrane,

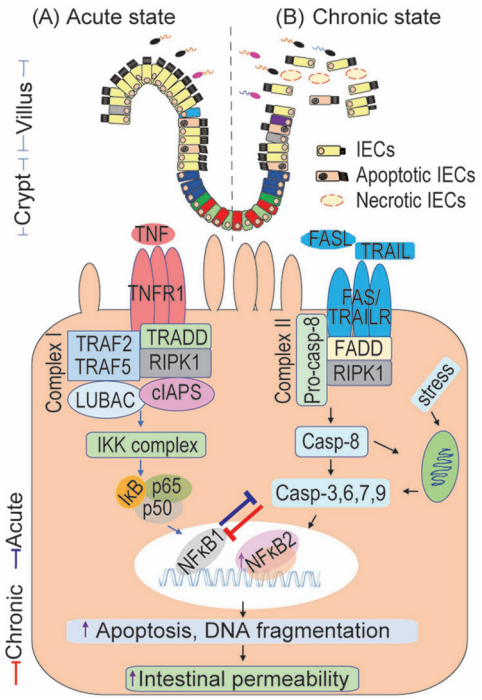

Fig. 1. Intestinal epithelial cells (IECs) apoptosis in an acute and chronic state of intestinal diseases. Apoptosis is a crucial factor for preserving gut homeostasis. The apoptotic pathway is mediated by the stimulation of TNFR1 and FAS receptor with receptive ligands TNF and FASL or TRAIL that leading to the activation of NFκB, which turns in the regulation of cell survival, apoptosis, and pro-inflammatory genes expression. *A*: During the acute intestinal disease state, there is an increase in the activation of NFκB1 survival pathways and its mediated pro-inflammatory mediators, which plays a host defensive mechanism in regulating the Caspase activity and apoptotic program to remove infected and damaged IECs. *B*: In meanwhile, during the chronic intestinal disease state, there is an increase in the activation of NFκB2 pro-apoptotic pathways and Caspase-3, -8 and -9 that inhibit the epithelial survival NF-κB1 pathways, provoke strikingly rapid epithelial apoptosis, reduce epithelial proliferation and result in the pathological IECs shedding. This event results in increased intestinal barrier dysfunction and intestine permeability, leading to several acute and chronic intestinal diseases, including sepsis, inflammatory bowel diseases, necrotizing enterocolitis, *etc*. TNF, tumor necrosis factor; FASL, Fas ligand; TNFR1, tumor necrosis factor receptor 1; TRAIL, TNF-related apoptosis-inducing ligand; TRAILR, TRAIL receptor; RIPK1, receptor-interacting serine/threonine-protein kinase 1; FADD, Fas-associated death domain; TRADD, TNFR1-associated death domain protein; cIAPs, cellular inhibitor of apoptosis proteins; LUBAC, linear ubiquitin chain assembly complex; IKK, the inhibitor of I-κB kinase; NFκB, nuclear factor kappa B; DISC, death-induced signaling complex; Apaf-1, activating factor 1; Casp, Caspase.

and extensive cell lysis [6]. During the presence of various pathological stimuli, the inappropriate release of pro-inflammatory cytokines, including TNF-α, not only mediates IEC apoptotic cell death, but also induces necrotic cell death [14]. Whereas necrosis is highly associated with increased Caspase-independent inflammation and reactive oxygen species (ROS) levels [15]. Once the intestinal integrity disturbance happens, intestinal mucosal T lymphocytes induce the expression levels of Th1 cytokines, such as TNF-α and interleukin (IL)-1, and stimulate IECs to generate ROS that acts as secondary messengers to regulate inflammation and its mediated signaling pathways [16]. As well the phagocytic leukocyte-derived ROS also maintains the chronic inflammatory state in the intestine and worsens the infectious GI diseases [17]. While mitochondrial generation and removal of ROS are dynamically balanced and useful for cells without causing damage, over-generating of ROS in IECs is dangerous [16] and induce intestinal injury (Fig. 2*A*).

2.3 Necroptosis

Necroptosis, a novel manner of cell death modality, is an inflammatory form of programmed cell death mechanism with a necrosis phenotype characteristic, but not of apoptosis [18]. In detail, necroptosis is highly regulated by an intracellular protein platform, including the com-

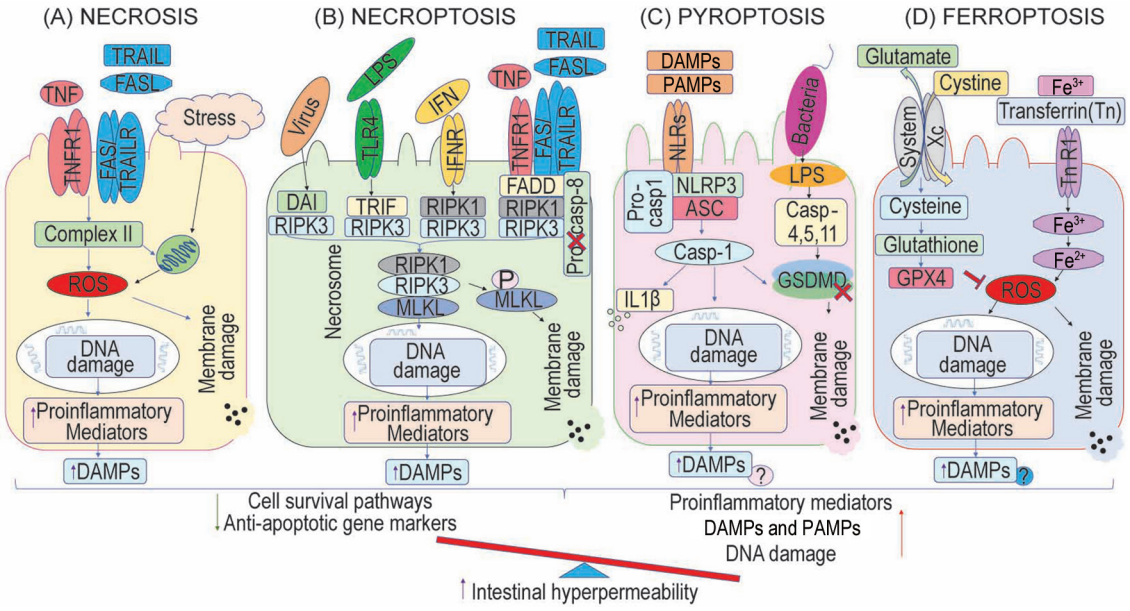

Fig. 2. Lytic cell death pathways of intestinal epithelial cells (IECs) during the intestinal diseases. *A*: The inadequate release of TNF-α not only mediates IECs apoptosis, but also triggers necrotic cell death by inducing ROS, which acts as secondary messengers to regulate inflammation and its mediated signaling pathways during the presence of multiple pathological stimuli and stress condition. *B*: The binding of death ligands with their corresponding receptors (as shown in the figure) together with adapter proteins, but under Caspase-8 or cIAP depletion, promotes the cell death pathways of necroptosis through the formation of necrosome RIPK1/RIPK3/MLKL, which leads to the phosphorylation of MLKL (p-MLKL). This event reduces the cell survival and anti-apoptotic pathways and increases the pro-inflammatory mediators along with intestinal barrier dysfunction and intestine permeability. *C*: Sensing of DAMPs or PAMPs by NLRs promotes the formation of inflammasome complex that involves NLRP3, ASC, and Caspase-1 in the canonical pathway. In the non-canonical pathway, LPS leads to the activation of Caspase-11 and results in cytoplasmic swelling and cytosolic content leakage along with DAMPs. Although pyroptosis occurs in inflammatory cells, it seems that pyroptosis in IECs plays an important role in the pathogenesis of chronic intestinal diseases, but several scientific pieces of evidences are needed to fill this gap. *D*: The increase in iron-dependent lipid peroxidation and lipid ROS accumulation and decrease in GPX4 activation through system Xc^- inhibition lead to the destruction of IECs and intestinal hyperpermeability. However, the evidence for supporting ferroptosis in IECs is very less. DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular pattern molecules; DAI, DNA-dependent activator of IFN-regulatory factors; MLKL, mixed lineage kinase domain-like pseudokinase; NLRs, nucleotide-binding oligomerization domain-like receptors; ASC, an apoptosis-associated speck-like protein containing a CARD domain; NLRP3, nucleotide-binding oligomerization domain-, leucine-rich repeat-, and pyrin domain-containing protein 3; GPX4, glutathione peroxidase 4; LPS, lipopolysaccharides; TLR4, Toll-like receptor 4; IL-1β, interleukin-1β; ROS, reactive oxygen species; GSDMD, gasdermin-D.

bination of death ligands and receptors along with adapter proteins, but under the inhibition of Caspase activation [19]. TNFR1 and Fas are often used as a prevalent upstream signal by both necroptosis and apoptosis, but during necroptosis, suppression of Caspase-8 system [13, 20] and recruitment of receptor interacting protein kinase (RIPK)-3 through the RIPK homotypic interaction motif (RHIM) domain to RIPK1 occur to form a necrosis-inducing complex [11, 21]. The interaction of RIPK1 and RIPK3 kinases through RHIM results in their auto- and trans-phosphorylation, and RIPK3-mediated phosphorylation of the downstream pseudokinase mixed lineage kinase domain-like (MLKL) [22, 23]. The phosphorylation of MLKL in IECs ultimately increases cytokine/alarmin expression such as interleukin 8 (IL-8), IL-1β, IL-33, and high mobility group box 1 (HMGB1), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)-p65 translocation and NACHT, LRR and PYD domains-containing protein 3 (NALP3) inflammasome assembly [24]. Thereby the phosphorylation of RIPK3 and MLKL is essential for necrosis execution [25, 26] (Fig. 2*B*). Taken together, the central function of Caspase-8 [13] and inhibition of RIP3 [27] and MLKL [11] in IECs preserve epithelial barrier integrity, maintains homeostasis and prevents chronic intestinal inflammation by protecting IECs from necroptotic cell death. However, the functions and detailed mechanisms of necroptosis in IECs-mediated chronic GI tract diseases remain largely unknown.

2.4 Pyroptosis

Pyroptosis is also a programmed and inflammatory form of cell death but Caspase cascade dependent mechanism, described morphologically as cell rupture, followed by membrane "re-sealing," and cell swelling with nuclear condensation [6]. In response to microbial infection, the subset of NLRs is triggered by detecting a variety of DAMPs and PAMPs, promoting to form a multimeric protein complex known as the inflammasome through oligomerize with an adaptor protein known as ASC (an apoptosis-associated speck-like protein containing a CARD domain), and a proenzyme, Caspase-1 [28]. The inflammasome formation stimulates the protease activity of Caspase-1 (canonical pathway) that cleaves the pore-forming effector protein, gasdermin-D, and results in the release of IL-1β and IL-18 to a form of pyroptosis-mediated inflammatory cell death [28]. The influence of lipopolysaccharides (LPS) from gram-negative bacterial components leads to the activation of Caspase-11 and pyroptosis of enterocytes [29] (Fig. 2*C*). Recent studies show that pyroptosis acts as a central role in intestinal immune defense and pathology by regulating microbial infections and secretion of IL-18, ROS production, or lysosomal damage [15, 28]. However, luminal content occasionally carries pathogenic microbes or toxic elements proficient of producing mucosal damage, pyroptosis-mediated NLRP3 inflammasome [30], Caspase-1 [31] and cytokines, such as IL-1 and IL-18 [32], results to the chronic inflammatory state in GI tract diseases. Thus, it is important to understand the debate in the protective and destructive function of pyroptosis mechanism in IECs during the acute and chronic GI diseases.

2.5 Ferroptosis

Ferroptosis is a new form of iron-dependent, Caspase-independent, lipid oxidation-mediated programmed cell death, differing from traditional apoptosis, necroptosis, and classic necrosis [33]. It is characterized by morphologically shrinkage of mitochondria and increases in mitochondrial membrane density, biochemically accumulation of iron and lipid ROS (L-ROS), and genetically involvement of a unique set of genes [34]. The increased levels of lipid peroxidation (LPO) by deficiency of glutathione peroxidase 4 (GPX4) activation through system Xc^- inhibition, and oxidation of arachidonic acid (AA) and its esterifiable phosphatidylethanolamine (PE) production [34, 35], subsequently lead to the destruction of IECs and intestinal mechanical barrier [36] (Fig. 2*D*). Recently, the finding of reduced activity of GPX4 in the lesioned areas of the gut of the CD patients in IECs suggests that reduction of GPX4 activity in IECs induces ferroptosis [37]. It appears that dietary derived compounds, pathogenic microbial mediated metabolites, such as fatty acids, could trigger ferroptosis that contributes to the pathogenesis of the GI diseases. However, the evidence for supporting ferroptosis in IECs is very less. Thus, this review brings up an interesting research topic related to IECs ferroptosis mechanisms in chronic GI disease.

3 Cell death in IECs-mediated acute and chronic GI injury

Apoptosis is a crucial factor for usual intestinal mucosal turnover. The balance between proliferation and apoptosis of IECs partially depends on the microenvironment, host state, and stress categories in the intestine;

the defect in balance is strongly connected with several intestinal diseases and GI injuries [38]. It is essential to characterize the duration of intestinal diseases as an acute injury that lasts only for a few (7–14) days, and chronic GI injuries that persist for months or longer [4]. Several studies have been performed to evaluate the pathological features of IEC apoptosis during the pathogenesis of acute and chronic GI injury. The clinical patients with acute and chronic intestinal diseases, *in vitro* IEC models, and *in vivo* animal models are mostly accessible to study the IEC cell death mechanism (Table 1). Here we target the pathogenic impact of cell death in IECs to the acute and chronic diseases, as well as the available therapeutic approach to prevent IEC cell death to regulate intestinal diseases.

3.1 IEC cell death in sepsis

Sepsis is a serious and life-threatening dysfunction of the organs, and it is secondary to a dysregulated host response to infection, affecting the intestine intensely [38]. There is increased apoptosis in the colon and ileum of septic patients in whom focal regions of columnar epithelial apoptosis occurred in crypt or villus [39]. In another clinical study, trauma patients who are much more prone to sepsis disclosed the increased severity of crypt epithelial apoptosis in the colon specimens, compared to control patients. Simultaneously, these trauma patients experienced a statistically significant increase in the number of cytokeratin-18 positive IECs [40]. In patients with septic shock, norepinephrine uses to maintain adequate blood pressure at ICU admission is associated with more enterocyte damage and higher intestinal fatty acid-binding protein (I-FABP), a marker for the early diagnosis of intestinal damage [41]. Thereby, the greater degree of apoptosis in the intestinal villi and crypts of septic patients suggested the enhanced IEC apoptosis in sepsis [39].

Several preclinical models of sepsis also suggested the key role of IEC apoptosis in the pathophysiology of sepsis. Mostly, cecal ligation and puncture (CLP)- and pneumonia-induced sepsis murine models have been used to evaluate the role of IEC apoptosis. There is a decrease in intestinal proliferation and an increase in gut epithelial cells apoptosis observed in both murine models of pneumonia-induced sepsis and CLP-induced sepsis [42–44]. Whereas, methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia-induced sepsis showed intestinal apoptosis that highly associated with increased proapoptotic Bid and Bax proteins and the antiapoptotic Bcl-xL protein in the mitochondrial pathway, and FasL in the receptor-mediated pathway. This study shows that MRSA pneumonia-induced sepsis alters intestinal integrity by increasing IEC apoptosis and decreasing crypt proliferation and villus length, through apoptosis mechanism mediated by the mitochondrial pathway [45]. At the same time, mice lacking intestinal epithelium functional NFκB (Vil-Cre/Ikkβ$^{f/\Delta}$) showed an increase in IEC apoptosis and intestinal permeability along with decreased villus length and atrophy in CLP-induced sepsis animals, compared to septic wild-type mice. During ongoing sepsis, Vil-Cre/Ikkβ$^{f/\Delta}$ mice showed increased serum levels of pro-inflammatory, including TNF and monocyte chemoattractant protein-1 (MCP-1) and anti-inflammatory cytokines, such as IL-10, compared to septic wild-type mice. This result indicates that inhibition of IEC specific NFκB worsens the sepsis-induced intestinal injury and aggravates mortality in CLP mice [46]. TNFR1 is crucial for LPS-induced IEC apoptosis and shedding, and the destiny of IECs is also dependent on NFκB signaling, via NFκB1 favoring cell survival or NFκB2 favoring apoptosis [8]. Neutralizing TNF activity with anti-TNF antibody in CLP-induced septic Vil-Cre/Ikkβ$^{f/\Delta}$ and septic wild-type mice, prevented intestinal hyperpermeability by increasing claudin-2 gene expression. This outcome suggests that TNF is the main mediator of the dysfunction of the intestinal barrier in sepsis, where anti-TNF antibody significantly decreases sepsis-induced IECs apoptosis and hyperpermeability [46].

In order to enhance our understanding to highlight the link of IECs apoptotic cell death in sepsis, Hu *et al*. showed that increased STING signaling that promotes the phosphorylation of STAT3, STAT6, IRF3, and NFκB, is highly associated with intestinal inflammation and induction of IEC apoptosis in patients with sepsis, as well as in CLP-induced septic mice [47]. The mice with STING genetic depletion showed a reduced inflammatory response, intestinal permeability, and bacterial translocation. The treatment of DNase I protects the intestinal injury by decreasing mtDNA levels in CLP-induced septic animals. These findings indicate that mtDNA-STING pathway regulation can be a promising therapeutic approach to improve mucosal healing in patients with sepsis and protect the intestinal barrier [47]. In a very current study, both genetic [hepcidin-1 knockout (HKO)] iron overload and iatrogenic (intravenous) iron overload mice developed sepsis after

Table 1. Intestinal epithelial cell death in the acute and chronic intestinal diseases

Model	Subjects	Cell death	Findings
A) Sepsis			
Clinical	Patients with sepsis	Apoptosis	Increase in active Caspase-3 [39]
Clinical	Patients with trauma injuries	Apoptosis	Increase in cytokeratin 18 and active Caspase-3 [40]
Clinical	Patients with sepsis	Apoptosis	Increase in I-FABP [41]
In vivo CLP induced sepsis	Transgenic mice that overexpress Bcl-2 (Fabpl-Bcl-2)	Apoptosis	Decrease in apoptosis and active Caspase-3 [44]
In vivo pneumonia-induced sepsis	Fabpl-Bcl-2 mice	Apoptosis	Decrease in apoptosis and active Caspase-3 [42]
In vivo pneumonia-induced sepsis	Fabpl-Bcl-2 mice	Apoptosis	Decreases in apoptosis and active Caspase-3 associate with increase in S-phase cells proliferation [43]
In vivo MRSA pneumonia-induced sepsis model	Wild-type FVB/N mice	Apoptosis	Increase in Bid and Bax, and Bcl-xL in the mitochondrial pathway [45]
In vivo MRSA pneumonia-induced sepsis model	*Bid*$^{-/-}$ mice Fabpl-Bcl-2 mice	Apoptosis	Regulate the mitochondrial apoptotic pathway [45]
In vivo CLP induced sepsis model	Lacking functional NF-κB in IECs (Vil-Cre/Ikkβ$^{f/\Delta}$)	Apoptosis	Increase in mortality, apoptosis with pro-inflammatory cytokines [46]
In vivo CLP induced sepsis model	STING-knockout mice	Apoptosis	Decrease in apoptosis, inflammation, intestinal permeability and bacterial translocation [47]
In vivo LPS induced sepsis model	*Tnfr1*$^{-/-}$, *Tnfr2*$^{-/-}$, *Nfκb1*$^{-/-}$, and *Nfκb2*$^{-/-}$ mice	Apoptosis	Dependent on NFκB signaling, via NFκB1 favoring cell survival or via NFκB2 favoring apoptosis [8]
In vivo LPS induced sepsis model	Co-expressed both Bcl-2 and TAg to Fabpl	Apoptosis	Bi-transgenic animals had reduced crypt apoptosis, but had a paradoxical increase in the markers of apoptosis such as Caspase-3, BAX and cytochrome c in villus [38]
B) Intestinal ischemia/reperfusion (I/R)			
Clinical	Jejunum from patients undergoing pancreaticoduodenectomy	Apoptosis	Increase in apoptosis and I-FABP during ischemia and gradually decrease during reperfusion [51]
Clinical	Jejunum from patients undergoing pancreaticoduodenectomy	Apoptosis	Increase in apoptosis and I-FABP associate with inflammatory markers such as C3c complement activation, IL-6, IL-8, and TNFα [52]
In vivo I/R rat model	Ischemia (15–90 min) and I/R (15 min of ischemia followed by 15–75 min of reperfusion)	Apoptosis or necrosis	Death cells exhibit apoptosis (80%) and necrosis (20%) characteristics; increase in DNA fragmentation [53]
In vivo I/R rat model	Ischemia clamping the SMA (30 or 60 min), after reperfusion various time points up to 4 days.	Apoptosis	Increase in apoptosis and decrease in intestinal ALP and lactase after ischemia, and returned normal with reperfusion [54]
In vitro model of ischemia	2-deoxyglucose and oligomycin-A treated HT-29 and Caco-2 cells	Apoptosis	Greater apoptosis in differentiated cells than undifferentiated cells [54]
In vivo I/R rat model	Underwent occlusion of both SMA and PV for 20 min followed by 48 h of reperfusion	Apoptosis	Increase in apoptosis along with inflammatory markers upregulation of TLR-4, MyD88, and TRAF6 [49]
In vivo I/R rat model	Underwent occlusion of both SMA and PV for 20 min followed by 24 h or 48 h of reperfusion	Apoptosis	Increase in apoptosis inversely associates with SHh signaling pathways [50]
In vivo I/R rat model	1 h of ischemia followed by reperfusion	Necroptosis or necrosis	Increase in necroptotic markers such as RIP-1, -3 and MLKL [19]

To be continued

Continued

Model	Subjects	Cell death	Findings
In vitro model of ischemia	Oxygen and glucose deprivation model in IEC-6	Necroptosis or necrosis	Increase in RIP-1, -3 and MLKL together with HMGB1-TLR4/RAGE signaling [19]
In vivo I/R rat model	SMA occlusion (1.5 h) of ischemia and 6 h of reperfusion	Necroptosis	RIP1/3 mediated necrosome formation [55]
In vivo I/R murine model	$Ikbkb^{F/\Delta}$ Vil-Cre; SMA occlusion for 30 min followed by reperfusion	Apoptosis	Increase in apoptosis and pro-inflammatory markers such as TNF, IL-1, IL-6 and ICAM. Probably dual function of NFκB signaling [56]
In vivo I/R murine model	Fabpl-Bcl-2 mice; SMAO for 20 min followed by reperfusion	Apoptosis	Decrease in p53-dependent death [57]
C) Inflammatory bowel diseases (IBD)			
Clinical	patients with UC	Apoptosis	Increase in apoptosis, active Caspase-3 and PUMA expression [59, 62]
Clinical; *In vivo* TNBS induced colitis murine model	Patients with CD and UC; Wild-type balb/c mice	Apoptosis	up-regulation of TRAIL in IEC [60]
In vitro model	TRAIL, TNF-α and IFN-γ treatment in HIEC, HT-29 or Caco-2 cells	Apoptosis	NFκB-dependent (TNF-α) or NFκB-independent (IFN-γ) pathway to induce TRAIL mediated apoptosis [60]
In vivo DSS or TNBS induced colitis murine model	Wild-type, $PUMA^{-/-}$, $Bid^{-/-}$, and $p53^{-/-}$ mice	Apoptosis	PUMA inhibition can provide an efficient way of protecting IEC apoptosis and serve as a new anti-IBD approach [59]
In vivo model	$TAK1^{IE\text{-}KO}$ mice	Apoptosis	Enhance in cleaved Caspase-3 and reduction in claudin-3 and antioxidant-genes and transcription factor Nrf2, and ROS accumulation, like the IBD pathology [61]
In vivo anti-CD3 or DSS induced colitis murine model	Wild-type, *p53*, $Bid^{-/-}$, *Bim*, $Bax3^{-/-}$, $Bak^{-/-}$, *PUMA*, and $Noxa^{-/-}$ mice	Apoptosis	p53-dependent and -independent mechanisms; PUMA mediated intrinsic apoptosis pathway [62]
Clinical *in vivo* TNF induced apoptosis model	Patients with CD and UC transgenic mice that overexpress A20 in IECs A20-Tg mice	Apoptosis	RIPK1-dependent IEC death [63]
In vivo DSS induced colitis murine model	Villin knockout mice	Apoptosis	Anti-apoptotic function of villin is regulated by PI3-kinase and Akt [64]
In vivo DSS induced colitis murine model	$TLR4^{-/-}$mice	Apoptosis	Increase in apoptosis with reduced Cox-2 and PGE-2 levels [65]
In vivo LPS induced injury model	Epithelial cell-specific deletion of Caspase $8^{\Delta IEC}$ mice TLR stimulation	Necrosis or necroptosis	RIP3-dependent epithelial necroptosis [66]
In vivo spontaneous model	Epithelial cell-specific deletion of $FADD^{\Delta IEC}$	Necrosis or necroptosis	RIP3-dependent epithelial necroptosis [27]
In vivo TNBS induced colitis murine model; *In vitro* necroptosis model	Wild-type mice; TNF-α and Z-VAD-fmk induced Caco-2 cells	Necrosis or necroptosis	Increase in TUNEL-positive, Caspase-3 negative cells along with p-RIPK3 [11]
Clinical; *In vivo* model; *In vitro* model	Patients with CD; Caspase-1/IL-10 double knockout; T84 monolayers	Pyroptosis	Increase in the activated Caspase-1 [67]
Clinical	Patients with CD	Ferroptosis	Reduction in GPX4 levels [37]

To be continued

Continued

Model	Subjects	Cell death	Findings
D) Necrotizing enterocolitis (NEC)			
Clinical	Infants with NEC	Apoptosis	Increase in NO and apoptosis through peroxynitrite formation [70]
In vivo NEC model	Formula feeding, and cold/asphyxia stress induced neonatal rat	Apoptosis	Increase in Caspase-3 and DNA fragmentation [71]
In vitro NEC model	H_2O_2 induced rat IECs (RIE)-1	Apoptosis	Increase in intracellular ROS generation activates PI3-K pathway [72]
In vivo; *In vitro* NEC model	Formula feeding/hypoxia followed by *Enterobacter sakazakii* (ES) mediated NEC; ES administration to IEC-6 *in vitro*	Apoptosis	Increase in active Caspase-3 and pro-inflammatory cytokines such as IL-6 [73]
In vivo; *In vitro* NEC model	Formula feeding/hypoxia followed by *Cronobacter sakazakii* (CS) mediated NEC; CS administration to HT-29 *in vitro*	Pyroptosis, Apoptosis	Increase in NLRP3 inflammasome, Caspase-3 and Caspase-1 levels [74]
In vivo; *In vitro* NEC model	Rat pups collected by caesarian section, followed by hand fed; TNF-α and IFN-γ induced IEC-6 cells	Apoptosis	Increase in Bax/Bcl-w ratio, cleaved Caspase-3 and COX-2 levels; these events were reverted by *Bifidobacterium bifidum* [75]
In vivo NEC model	NEC induced by asphyxia and cold stress, and followed by hand fed milk	Apoptosis	Increase in pro-apoptotic Bax, cleaved Caspase-3, and decrease in anti-apoptotic Bcl-2; this effect was attenuated by EGF administration [76]
In vivo NEC model	NEC induced by hypoxia, hypothermia, hypertonic formula feeding plus enteral administration of LPS	Apoptosis	Increase in TUNEL and active Caspase-3 levels; these changes were inhibited by HB-EGF [77]

I-FABP, intestinal fatty acid-binding protein; CLP, cecal ligation and puncture; MRSA, methicillin-resistant *Staphylococcus aureus;* Bcl-2, B-cell lymphoma 2; Bid, BH3 interacting domain death agonist; Bax, BCL2 associated X, apoptosis regulator; Bcl-xL, B-cell lymphoma-extra-large; IKK, the inhibitor of I-κB kinase; NFκB, nuclear factor kappa B; STING, stimulator of interferon genes; LPS, lipopolysaccharides; TAg, viral protein large T-antigen; TNF, tumor necrosis factor; IL, interleukin; I/R, ischemia/reperfusion; SMA, superior mesenteric artery; PV, portal vein; SHh, sonic hedgehog; TLR, Toll-like receptor; TRAF6, tumor necrosis factor receptor (TNFR)-associated factor 6; MyD88, myeloid differentiation factor 88; RIPK, receptor-interacting serine/threonine-protein kinase; MLKL, mixed lineage kinase domain-like pseudokinase; RAGE, receptor for advanced glycosylation end product; ICAM, intercellular cell adhesion molecule; UC, ulcerative colitis; CD, Crohn's disease; PUMA, p53 upregulated modulator of apoptosis; TRAIL, TNF-related apoptosis-inducing ligand; TNBS, 2,4,6-trinitrobenzene sulfonic acid; IFN, interferon; HIEC, human intestinal epithelial cells; IBD, inflammatory bowel disease; DSS, dextran sodium sulfate; TAG1, TGF-β activated kinase 1; COX-2, cyclooxygenase-2; PGE2, prostaglandin E2; FADD, Fas-associated death domain; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; NEC, necrotizing enterocolitis; ROS, reactive oxygen species; NO, nitric oxide; NLRs, nucleotide-binding oligomerization domain-like receptors; NLRP3, NLR family pyrin domain containing 3; EGF, epidermal growth factor; HB-EGF, heparin-binding epidermal growth factor.

administration of clinical isolates *E. coli* within 24 h and associated with high bacterial multiplication and dissemination [48]. Hence host and pathogenic microbes are using iron as an essential micronutrient, it seems that iron-dependent ferroptosis cell death mechanism in IECs may have an impact on the severity of the pathogenesis of sepsis during the pathogenic microbial infection. But still, scientific evidence is needed to fill this gap.

Inhibition of cell death mechanism by blocking apoptotic markers or over-expressing anti-apoptotic markers prevents the pathogenesis of sepsis and enhances sur-

vival rate. Transgenic mice Fabpl-Bcl-2 (intestine-specific overexpression of Bcl-2, linked to Fabpl) showed decreased gut epithelial apoptosis in both sepsis model by decreasing the levels of active Caspase-3. Therefore, preventing IEC apoptosis by overexpression of Bcl-2 was related to a survival advantage in sepsis [42, 44]. *Bid*$^{-/-}$ mice and Fabpl-Bcl-2 mice had decreased intestinal apoptosis, thereby, inhibited MRSA pneumonia-induced sepsis, compared to wild-type animals. It is now highlighted that the potential to genetically manipulate the mitochondrial pathway could have theoretically therapeutic benefit in more lethal sepsis models whose high apoptosis of the gut is associated with increased mortality [45]. In the continuation of the previous study, Lyons *et al*. co-expressed both the genes such as Bcl-2 and TAg (large T-antigen, is limited to villus enterocytes) to Fabp that resulted in the expression of both genes in their villus enterocytes, but Bcl-2 alone in the crypt. As anticipated, bi-transgenic sepsis animals had reduced crypt apoptosis, but had a paradoxical increase in the markers of apoptosis such as Caspase-3, BAX and cytochrome c in villus, compared with septic *fabpi*-TAg (TAg alone) mice, associated with decreased proliferation in both compartments [38]. Other than programmed cell death, the non-programmed cell death mechanism also plays a significant role in the pathogenesis of sepsis. But still, scientific evidence is needed to fill this knowledge gap.

3.2 IEC cell death in intestine ischemia/reperfusion (I/R)

Intestinal I/R injury is a complex and multifactorial pathophysiological process triggered by ROS formation and lipid mediator synthesis alteration [49]. The nonspecific damage caused by I/R increases the levels of several inflammatory cytokines and attracts the inflammatory cells, such as polymorphonuclear leukocytes and mast cells, into the intestinal wall, leading to IEC apoptosis, intestinal hyperpermeability and intestinal barrier dysfunction which could result in MOF and death [50]. Thus, dysregulated apoptosis and inflammation are the main mediators in the pathogenesis of I/R-induced intestinal injury. The clinically relevant intestinal I/R models have resulted in a deeper understanding of the pathophysiology of human small intestinal and colon I/R over the past years. It has been shown that isolated jejunum from the patients undergoing pancreaticoduodenectomy was subjected to ischemia followed by reperfusion [51]. After ischemia, there is a significant increase in I-FABP across the jejunum, revealing the progression of epithelial cell damage. But at the same time, a decrease in I-FABP staining after reperfusion reveals the endogenous clearing mechanism for damaged enterocytes that results in normal epithelial lining [51]. The causative agents of the human intestinal I/R-induced inflammation were characterized by complement activation, cytokines production, and release into the systemic circulation, endothelial activation, and neutrophil influx into intestinal I/R-damaged tissue [52].

In preclinical studies, briefly, Ikeda *et al*. [53] exhibited that ischemia and I/R injury leads to apoptosis, which becomes the main mediator of cell death to intestinal epithelium after I/R. Rats subjected to ischemia and I/R showed increased mucosal injury along with detached epithelial cells that exhibited majority with characteristic morphological features of apoptosis and rest of the cells with necrosis features [53]. It is evident that villus tip epithelial cells are more susceptible to ischemia and loss of intestinal alkaline phosphatase and lactase instantly following ischemia and returned with reperfusion, confirming that differentiated cells are particularly sensitive to ischemic injury [54]. I/R rats underwent laparotomy and vascular occlusion of both superior mesenteric artery (SMA) and portal vein (PV) for 20 min followed by reperfusion showed a significant decrease in the enterocyte proliferation index in both jejunum and ileum [49, 50]; this reduction in cell turnover is highly associated with SHh signaling pathway inhibition [50].

Few studies have investigated the efficacy of regulated IEC necrosis after intestinal I/R. I/R group rats received 1 h of ischemia followed by reperfusion showed an increase in the levels of necroptosis mediated markers RIP1/3 and MLKL, and these levels are inhibited by RIP1 kinase inhibitor necrostatin-1. In meanwhile, blocking both apoptotic and necroptotic cell death mechanism, using the pan-caspase inhibitor Z-VAD and necrostatin-1 respectively, confers better protection against intestinal I/R injury. But at the same time, in the presence of either only one of the inhibitor, these two pathways can be converted to one another. In *in vitro* (IEC-6) oxygen and glucose deprivation-induced I/R model, necrostatin-1 decreases cell death and pro-inflammatory cytokine gene expression and confers IEC-6 protection via inhibiting HMGB1-TLR4/RAGE signaling activation [19]. Rats subjected to SMA occlusion consisting of 1.5 h of ischemia and 6 h of reperfu-

sion, showed the activation of poly (adenosine diphosphate-ribose) polymerase 1 (PARP-1) and RIP1/3 mediated necrosome formation. The pretreatment of PARP-1-specific inhibitor PJ34 and the RIP1-specific inhibitor necrostatin-1 resulted in a decrease in IEC cell death and optimal protection of the intestine. Thus, in the development of I/R-induced intestinal injury, PARP-1 could function as a RIP1 downstream signaling molecule, and the RIP1/PARP-1-dependent cell death signaling pathway functioned independently of Caspase-3 inhibition [55]. IEC specific ablation of IκB kinase (IKK)-β resulted in the prevention of the systemic inflammatory response triggered by I/R and in severe apoptotic damage to the re-perfused intestinal mucosa, suggesting that NFκB system has a dual role for both tissue safety and systemic inflammation which could be inhibited by using NFκB and IKK inhibitors [56]. The forced overexpression of Bcl-2 inhibits I/R-induced p53-dependent apoptosis pathways in the intestinal epithelium of transgenic mice Fabpl-Bcl-2 [57]. Overall, targeting the IEC cell death mechanisms could be beneficial to alleviate intestinal I/R tissue injury.

3.3 IEC cell death in inflammatory bowel diseases (IBD)

IBD, which involves CD and UC, is the result of the breakdown of the symbiotic relationship between the commensal microbial/host intestinal immunity [58]. IEC death is a prevalent pathological characteristic of IBD causing inflammation by altering the integrity of the intestinal barrier. Despite this, in response to intestinal inflammation, little is known about the molecular mechanisms of IEC apoptosis. Increased apoptosis of IECs was identified in patients with UC and CD at the involved inflammatory sites/tissues as well as in colitis animal models with the disruption of intestinal mucosal integrity and barrier function [59]. Stimulation of TNF-α, TLR and platelet-activating factor (PAF) can lead to a stable or transient rise in IECs shedding with loss of epithelial barrier function. TRAIL is significantly up-regulated during intestinal inflammation in IECs and LP lymphocytes to detect molecular events causing IEC destruction during inflammatory processes such as IBD. The increase in TRAIL-induced IEC apoptosis is mediated via increasing the levels of proinflammatory cytokines such as TNF-α and interferon (IFN)-γ, the expression of the pro-apoptotic receptor TRAIL-R2 and the functional levels of Caspase-3 [60]. Intestinal epithelial-specific deletion of transforming growth factor (TGF)-β-activated kinase 1 (TAK1) leads to enhanced apoptosis (cleaved Caspase-3), disturbance of tight junctions (claudin-3) and reduced antioxidant-responsive genes through transcription factor Nrf2, resulting in ROS accumulation. These observed pathological scenarios are very similar to the IBD pathology. Targeting TAK1-Nrf2 pathway could, therefore, control the ROS levels and enhance the survival and integrity of enterocytes [61].

A key apoptotic molecule p53-upregulated modulator of apoptosis (PUMA) is significantly increased in colonic epithelial cells in colitis induced by either dextran sulfate sodium salt (DSS; 5%) or 2,4,6-trinitrobenzene sulfonic acid (TNBS; 100 mg/kg). At the same time, PUMA knockout relieved DSS- and TNBS-induced colitis and inhibited IEC apoptosis in mice. These findings indicate that by promoting IEC apoptosis, PUMA induction contributes to colitis pathogenesis [59]. In both mice and human, colon inflammation induces IEC apoptosis through p53-dependent and -independent mechanisms and PUMA mediated intrinsic apoptosis pathway [62]. A latest study demonstrates that enhanced expression of the TNFAIP3 gene encoding A20 is expressed in IECs from patients with IBD in the areas of apoptosis. TNF-induced cell death is extremely prone in transgenic mice that overexpress A20 in IECs. In these mice, by activation of Ripoptosome/RIPK1, A20 potentiates TNF-induced mucosal erosion and IEC apoptosis. Whereas, RIPK1 inhibitors can prevent A20-enhanced IEC damage and intestinal inflammation, suggesting a new strategy for IBD therapy [63]. Wang *et al*. [64] showed that villin, an actin regulatory protein, acts as an anti-apoptotic function. The overexpression of villin in the Madin-Darby canine kidney Tet-Off epithelial cell line protects the cells from apoptosis by inhibiting the activation of Caspase-9 and -3 and activating the pro-survival proteins such as phosphatidylinositol 3-kinase and phosphorylated Akt, thereby maintaining IEC mitochondrial integrity. Increased apoptosis in DSS induced villin knockout mice, suggesting the possible anti-apoptotic role in the development and progression of IBD [64].

As stated previously, TNF and TLRs can trigger Caspase-dependent apoptosis through the FADD and pro-Caspase-8. TLR4-deficient mice exhibit significantly lower IEC proliferation and increase apoptosis with reduced Cox-2 and PGE-2 levels in DSS-induced injury. Although short term TLR4 signaling is useful, per-

sistent TLR4 signaling may lead to colitis-associated cancers [65]. TLR's stimulation in IECs are highly associated with activated Caspase-8 and increased shedding of IECs. Epithelial cell-specific deletion of Caspase-8 triggered RIP3-dependent epithelial necroptosis that resulted in serious tissue damage and death instead of apoptosis [66]. Furthermore, this study emphasized that the release of TNF-α from non-epithelial cells is responsible for TLR4-mediated epithelial necroptosis [66]. In another study, IEC-specific FADD knockout spontaneously developed epithelial cell necrosis, loss of Paneth cells, enteritis and severe erosive colitis. Prevention of these changes by RIP3 inhibitor suggests that intestinal epithelial permeability and inflammation is caused by RIP3-dependent death of FADD-deficient IECs [27]. Recently the increase in TUNEL-positive, Caspase-3 negative cells along with p-RIPK3 is found in TNBS-induced colitis mice. At the same time, the increasing levels of p-RIPK3 and p-MLKL in IECs Caco-2 cells under the stimulation of TNF-α and Z-VAD-fmk, a novel *in vitro* necroptosis model that mimics IBD, confirmed the regulated necroptosis cell death; these effects are reversed by necroptosis inhibitor necrosulfonamide [11]. Therefore, RIP3-mediated IECs necroptosis is critical for maintaining intestinal homeostasis and indicate that programmed IECs necrosis may be involved in the pathogenesis of IBD.

A recent study demonstrates that IEC pyroptosis is crucial to the development of mucosal barrier dysfunction and intestinal inflammation using cell culture, animal model (IL-10 and Caspase-1 knockout mice) and patients with IBD. Specific Caspase-1 inhibitor YVAD and IBD therapeutic agents, such as mesalamine and dexamethasone, considerably inhibit IEC pyroptosis cell pathway, the pyroptosis cell mechanism in IECs [67]. To fully investigate the pathogenic impact of IECs pyroptosis in IBD, more studies are warranted. Recently, the reduced levels of GPX4, a key mediator of ferroptosis, were shown in the colon tissue of patients with CD, suggesting the role of IEC ferroptosis in the pathogenesis of IBD [37]. Still, it is essential to bring more scientific evidence to confirm the ferroptosis cell death in IBD. It is urgent to develop the pharmacological target for apoptosis as a therapy due to increasing rate of apoptosis in intestinal pathologies like IBD. Mostly, in patients with IBD, anti-TNF therapy has been found to inhibit IEC apoptosis. Treating mice with infliximab suppressed DSS- and TNBS-induced colitis and IEC apoptosis via suppressing PUMA expression [59]. Overall, a better understanding of the role of cell death machinery in the epithelial cell might aid the design of better therapeutic or preventive strategies for IBDs.

3.4 IEC cell death in necrotizing enterocolitis (NEC)

NEC is a devastating and life-threatening inflammatory GI disease in 2%–5% of all premature infants, characterized by intestinal inflammation, ischemia, apoptosis and necrosis [68]. Although evidence shows that various risk factors have been involved in the pathogenesis of NEC, including prematurity, hypoxemia, formula feeding, bacterial exposure, and intestinal ischemia, the provocative events leading to NEC remains unclear [69].

IEC apoptosis is considered as one of the prominent pathological features in NEC. It has been shown that elevated levels of NO by enterocytes of infants with NEC, leading to apoptosis in IECs through peroxynitrite formation at apical villi [70]. Indeed, it remains unclear whether the observation of epithelial apoptosis is due primarily to gross tissue necrosis or corresponds only with extensive tissue destruction in NEC. IEC apoptosis and tissue morphology were assessed to test the hypothesis that enhanced epithelial apoptosis is a preliminary event that underlies the gross histologic modifications in formula feeding and cold/asphyxia stress (FFCAS) induced neonatal rat model of NEC. In this model, the increased coincidence of morphologic damage and apoptosis in the respective tissue sections along with Caspase-3 and DNA fragmentation levels in FFCAS compared to mother-fed (MF), suggested that IEC apoptosis preceded gross morphologic changes for subsequent gross tissue necrosis [71]. ROS-mediated IECs apoptosis plays a significant role in the pathogenesis of NEC in premature infants. Induction of H_2O_2 in rat IECs (RIE)-1 resulted in enhanced IEC apoptosis with intracellular ROS generation and depolarization of the mitochondrial membrane [72]. As we discussed earlier, the pathogenic microbial invasion is also one of the risk factors in the pathogenesis of NEC. *Enterobacter sakazakii* (ES), a prevalent contaminant in milk-based powdered infant formula, was found to bind to enterocytes in rat pups at the tips of villi, and exposure to ES resulted in apoptosis and enhanced IL-6 levels in IEC-6 cells and the animal model [73]. *Cronobacter sakazakii* (CS), a major pathogen, relates to NEC, induce dual pyroptosis and apoptosis cell death mechanism including NLRP3 inflammasome, Caspase-3 and -1 levels in HT-29 IECs and neonatal rat model, result-

ing in increased intestinal permeability [74].

Interestingly, the findings of this study [74] have shown the probiotic, *Bacteroides fragilis* ZY-312f suppresses CS-induced NEC by modulating apoptosis and pyroptosis dual cell death. *Bifidobacterium bifidum* can reduce apoptosis in both *in vivo* and *in vitro* (IEC-6) NEC models by a COX-2-dependent matter, which suggests a molecular mechanism by which this probiotic preserves intestinal integrity [75]. Whereas, EGF reduces the incidence of NEC in a formula milk fed-neonatal rat model by controlling the presence of Caspase-3-positive epithelial cells and altering the balance between pro-apoptotic BAX and anti-apoptotic Bcl-2 proteins in the site of NEC injury to maintain intestinal integrity and protect intestinal epithelium [76]. Insulin-like growth factor (IGF)-1 activates PI3-K pathway to promote IECs survival in H_2O_2 induced *in vitro* NEC model [72]. In meanwhile, with the administration of heparin-binding EGF (HB-EGF), the median TUNEL and active Caspase-3 scores are significantly decreased in the incidence of NEC in the group of hypoxia, hypothermia, hypertonic formula feeding plus enteral administration of LPS [77]. Erythropoietin (Epo), a breast milk component is shown to reduce the incidence of NEC and maintain the function of intestinal barriers. Yu *et al*. [78] demonstrated that Epo protects the intestinal epithelium from excessive apoptosis by decreasing the number of total cleaved Caspase-3 positive ileal epithelial cells and upregulating Bcl-2 expression through MAPK/ERK pathway in both *in vitro* TNF-α-induced IEC-6 cells and *in vivo* traditional rat neonatal NEC model. Lactoferrin, a milk supplement, administration modulates intestinal injury by reducing inflammatory cytokines such as IL-6 secretion and upregulating cell proliferation through the Wnt/β-catenin pathway in H_2O_2-induced IEC-18 and Caco-2 IECs NEC *in vitro* model [79]. In this regard, focusing on both programmed and non-programmed cell death pathways of IECs may shed light on novel therapeutic approach for the NEC. Overall, these findings highlight the pathological features of IECs cell death and pharmacological intervention for the prevention and recovery of IECs injury in NEC.

4 Future directions and concluding remarks

Here remain several questions regarding how pathological IECs cell death mechanism interconnects and induces intestinal permeability? Is targeting one of the cell death mechanisms as a therapeutic approach provides promising treatment to patients with chronic intestinal diseases? Thus, understanding the mechanism of IECs cell death brings new insights to explore a novel therapeutic approach for the disease. In this review, we highlight the vital role of IECs death mechanisms in the acute and chronic intestinal disorders such as sepsis, I/R, NEC, and IBD, and address the potential therapeutic approach to such intestinal diseases by preventing the IECs cell death mechanism.

Several studies have revealed that the extended IEC apoptosis progresses intestinal injury and permeability via Caspase mediated inhibition of the epithelial survival NFκB pathways, provokes epithelial apoptosis and reduces proliferation, which results in pathological IEC shedding and chronic GI disorders. Simultaneously, deficient in Caspase-8 or FADD leads to RIPK1-RIPK3-MLKL mediated IEC necroptosis cell death [27, 66] which has more pathological features than apoptosis. A few studies showed that the pyroptosis cell death mechanism is highly associated with mucosal barrier dysfunction and intestinal inflammation in intestinal diseases such as NEC and IBD [67, 74]. But still, the IECs pyroptosis cell death mechanism to induce intestinal permeability remains unclear. Another rapidly expanding cell-death mechanism in IECs is ferroptosis. The findings of the reduction of GPX4 activity in CD patients [37], iron supplementation influenced bacterial dysbiosis to NEC [80], and iron overload worsened the sepsis pathogenesis [48], suggest us the contribution of the IECs ferroptosis cell-death mechanism in the development and progression of chronic intestinal diseases. But more scientific evidence is warranted to fill this gap.

A recent study shows that blocking both apoptotic and necroptotic mechanism using Z-VAD and necrostatin-1 delivers better protection than any one of these inhibitors [19]. This result suggests that pathogenic events may switch over the cell-death mechanism and escape from the therapeutic inhibitors in the chronic state of the intestinal diseases. Therefore, it is important to understand what the exact cell-death mechanisms are underlying chronic intestinal diseases to develop emerging novel promising therapeutic approaches. This review enlightens the need for new therapeutic activators or inhibitors in a disease-specific and IECs cell-death mechanism-specific manner. However, further investigations are needed to explore a novel therapeutic approach for the development and clinical testing in patients with intestinal diseases.

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