Review

Novel Insights into the Interaction between Enteropathogenic Bacteria, Pyroptosis and IBD

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Abstract

Inflammatory bowel disease (IBD) is a chronic and recurrent inflammatory disease of the intestinal tract. The complex pathophysiological mechanisms of IBD include genetic susceptibility, environmental factors, and abnormal immune response of the gut microbiota. Gut microbiota forms a metabolic organ that contributes to human health by performing various physiological functions. The development of IBD is closely linked to the imbalance of gut microbiota. In IBD patients, this imbalance is mainly characterized by an increased abundance of pro-inflammatory microorganisms, specifically enteropathogenic bacteria. Pyroptosis is a form of programmed cell death that can be initiated by microbial infection or host factors. It occurs mostly after intracellular infection with bacteria or pathogens. Other than cell death, its primary effect is to release inflammatory mediators that trigger an inflammatory response in the host. Pyroptosis is an important component of innate immunity and can protect against intracellular risk factors via the inflammatory response. However, excessive activation can cause disease. Previous studies of IBD have indicated a complex relationship between gut microbiota and pyroptosis. Some enteropathogenic bacteria can activate the host’s immune system to clear infected cells. This inhibits the proliferation of enteropathogenic bacteria by inducing pyroptosis and restoring the balance of gut microbiota. However, the initial inflammatory response and damage to the integrity of the intestinal barrier are crucial factors that elicit the onset of IBD and favor its progression. This review summarizes research on the role of several common enteropathogenic bacteria in the development of IBD through their induction of host cell pyroptosis. A better understanding of the complex interactions between gut microbiota and pyroptosis should lead to the identification of new targets and treatment options for IBD.

Keywords: inflammatory bowel disease (IBD); gut microbiota; pyroptosis; enteropathogenic bacteria

1. Introduction

Inflammatory bowel disease (IBD), which includes Crohn’s disease and ulcerative colitis, is a chronic, relapsing inflammatory disorder of the gastrointestinal tract [1]. The pathogenesis of IBD is multifactorial and involves genetic susceptibility, environmental factors, and an aberrant immune response to the gut microbiota [2]. The gut microbiota is pivotal in maintaining intestinal homeostasis and influencing host immune responses. Dysbiosis, or an imbalance in the gut microbiota, is increasingly recognized as a critical factor in the development and progression of IBD [3].

Pyroptosis is a form of programmed cell death that leads to swelling and rupture of the cell and the release of molecules that promote inflammation [4]. Due to its involvement in the immune response, pyroptosis is a key factor in the innate immune response to microbial infection. The dysregulation of pyroptosis is likely to play a contributory role in the pathogenesis of chronic inflammatory disorders such as atherosclerosis, cancer and IBD [5–7]. Expression of the inflammatory molecule caspase was found to be significantly increased in various IBD models, suggesting the involvement of pyroptosis in the occurrence and development of IBD [8]. Recent studies have highlighted the interplay between the gut microbiota and pyroptosis in the pathogenesis of IBD. Certain gut bacteria can induce pyroptosis in intestinal epithelial cells (IEC), leading to increased intestinal permeability, translocation of bacteria across the intestinal barrier, and activation of the immune response to exacerbate intestinal inflammation [5,9]. On the other hand, pyroptosis can also alter the composition of the gut microbiota, potentially leading to dysbiosis. A better understanding of the complex interactions between the gut microbiota and pyroptosis may therefore provide new insights into the pathogenesis of IBD and allow the identification of novel therapeutic targets.

2. Pyroptosis

Pyroptosis is a unique form of programmed cell death that distinguishes itself from other forms of cell death such...
as apoptosis or necrosis. It is characterized by cell swelling and bursting, leading to the release of cellular contents that can initiate an inflammatory response. Pyroptosis typically serves as a defense mechanism against bacterial, viral, and parasitic infections [8].

The process of pyroptosis begins when the cell senses infection signals, thereby activating a protein complex known as the inflammasome. This subsequently activates caspase-1 or 4/5 (murine caspase-11), which then cleaves gasdermin D to form pores in the cell membrane [10–12]. The formation of these pores leads to osmotic lysis due to the influx of water and ions, causing the cell to swell and eventually burst, releasing its contents into the surrounding environment [13,14]. The released contents, including damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), are recognized by the immune system and trigger an inflammatory response. DAMPs and PAMPs serve as signals to alert the immune system of the presence of an infection or tissue damage, thereby initiating the body’s defensive response [15,16].

Pyroptosis not only eliminates infected cells but also helps to recruit and activate the immune system to further combat infection [17]. However, if not properly regulated, pyroptosis can contribute to pathological inflammation. Excessive or prolonged inflammation can lead to tissue damage and may contribute to the development of chronic inflammatory diseases [18].

Therefore, understanding the underlying mechanism of pyroptosis and its regulation is crucial for the development of therapeutic strategies that control inflammation and treat inflammatory diseases.

2.1 Canonical Inflammasome Pathway

Over the past decade, numerous studies have highlighted the ability of specific inflammasomes to detect distinct microbial stimuli and internal threats [19]. The NOD-like receptor 3 (NLRP3) is a member of the nucleotide-binding domain-like receptor (NLR) family. The NLRP3 inflammasome responds to DAMPs and PAMPs [20,21]. It was the first inflammasome to be discovered and is also the best known. Activation of the NLRP3 inflammasome occurs through the NLRP3 nucleotide-binding and oligomerization structural domains and leads to the recruitment of apoptosis-associated speck-like proteins (ASC) through Pyrin Domain (PYD) - PYD interactions [22]. These cleave downstream pro-caspase-1 to generate active caspase-1, which then mediates the maturation and secretion of IL-1β and IL-18. Active caspase-1 also cleaves Gasdermin-D (GSDMD) to generate the GSDMD N-terminal (N-terminal, NT), thereby forming the plasma membrane pore and inducing cell death [23]. The canonical inflammasome pathway dominated by the NLRP3 inflammasome is considered to be the major mode of pyroptosis and is essential for host immune defenses against bacterial and viral infections [24,25]. Moreover, pyroptosis is thought to play an important role in tumor development, atherosclerosis, and IBD [4,5].

2.2 Non-Canonical Inflammasome Pathway

Distinct from the canonical inflammasome pathway, human caspase-4/5 and murine caspase-11 can directly recognize lipopolysaccharide (LPS) of bacterial or other origin, active caspase activation and recruitment domain (CARD), as well as lipid A moiety [9]. Unlike typical signaling pathways, the pyroptosis process mediated by caspase-4/5/11 does not release IL-18, but instead releases IL-1α and high mobility group box 1 (HMGB1) [26]. Caspase-4/5/11 is activated by the NLRP3/ASC/caspase-1 pathway rather than direct maturation of pro-IL-1β to regulate IL-1β secretion. Recent studies have shown that after intracellular LPS stimulation, caspase-11 is involved in the cleavage of pannexin-1 channels and release of Adenosine triphosphate (ATP), which ultimately activates the NLRP3/ASC/caspase-1 pathway [27].

3. Pathogenesis of IBD

IBD is usually divided into ulcerative colitis (UC) and Crohn’s disease (CD). It is a chronic and recurrent inflammation of the intestine mediated by environmental and genetic factors, microorganisms, and aberrant mucosal immunity. The pathological mechanism of IBD can be summarized as follows. The combination of specific intestinal flora, genetic susceptibility and environmental factors can initiate intestinal immune responses. These chronic relapsing and remitting events damage the intestinal mucosal barrier and cause persistent ulcers, inflammation and cell proliferation. However, IBD is not caused by a single factor, and the specific pathological mechanisms can be divided into several aspects [28]. A recent review highlights the fact that GSDMD in goblet cells provide a physical barrier that reduces pathogen colonization and infection. In addition, GSDMD in IEC also participates in the secretion of the intestinal mucus layer, and the composition and distribution of gut microbiota. Therefore, dysfunction of the mucosal barrier is closely related to intestinal disease, and GSDMD could represent a novel potential therapeutic target for the prevention of intestinal mucosal damage [29].

3.1 Adherent-Invasive Escherichia coli

Escherichia coli (E. coli), a predominantly facultative anaerobic Gram-negative bacterium, swiftly colonizes the human intestinal tract post-birth and plays a pivotal role in maintaining normal intestinal homeostasis [30]. E. coli strains are grouped into three main categories based on genetic and clinical factors: commensal strains found in the human and animal gut, intestinal pathogenic E. coli strains, and extraintestinal pathogenic E. coli strains [31]. Intestinal pathogenic E. coli strains are further divided into six types, including enterotoxigenic E. coli (ETEC) and
adherent-invasive *E. coli* (AIEC), both of which have been associated with IBD’s such as CD and UC [30,32].

Clinical studies have shown that patients with CD, UC, and other intestinal pathologies have a higher proportion of *E. coli* strains associated with the intestinal mucosa compared to controls [33]. AIEC characteristics were only identified in isolates from IBD patients and included virulence genes, invasiveness, and survival inside macrophages. AIEC induce IL-1β through an NLRP3-dependent mechanism, although their elimination by macrophages was independent of the NLRP3 inflammasome [33]. This suggests that invasion of AIEC into the intestinal mucosa and the production of IL-1β in an NLRP3-dependent manner may contribute to the pathogenesis of CD and UC.

A study in IL-10−/− mice confirmed that macrophage pyroptosis induced by AIEC was involved in the IBD process [34]. Singh and coworkers reported that non-steroidal anti-inflammatory drugs (NSAID) exacerbate colitis in AIEC-colonized animals. This was accompanied by activation of the NLRP3 inflammasome, caspase-8, apoptosis, and pyroptosis in macrophages, all of which promote inflammation and cell death. Conversely, inhibition of NLRP3 inflammasome activation or caspase-8 activity alleviates colitis. Significant upregulation of colonic NLRP3, caspase-1, and its downstream target IL-1β was observed at the gene expression and protein level in the combined AIEC/NSAID group, but not in mice colonized only with AIEC, or administered NSAID alone [34]. This reveals a synergistic effect between AIEC and NSAID in exacerbating IBD.

Triggering receptor expressed on myeloid cells 2 (TREM2) is a macrophage phagocytic receptor. It inhibits NLRP3-mediated pyroptosis in macrophages while enhancing the clearance of a variety of pathogenic bacteria, including *E. coli*, thereby protecting mice in sepsis models. TREM2 inhibits inflammasome activation by reducing mitochondrial reactive oxygen species (ROS) production [35]. Concurrently, TREM2 inhibits assembly of the NLRP3 inflammasome complex by regulating phosphorylation of the downstream protein β-catenin [36]. TREM2 may be a potential therapeutic target for IBD, but further studies are needed to confirm this.

Several toxins produced by *E. coli*, such as hemolysin-coregulated protein 1 (Hcp1), LPS, and Shiga toxin 2/LPS complexes, have been shown to cause pyroptosis and to promote inflammation by activating the NLRP3 inflammasome [37–39]. Additionally, deoxyxynivalenol is a common mycotoxin that is generally not considered harmful to healthy humans at low doses. However, deoxyxynivalenol exacerbates *ETEC*-induced intestinal inflammation in mice by activating macrophages and the NLRP3 inflammasome [40]. Fortunately, new drugs and targets for these inflammatory mechanisms have been discovered. Dihydromyricetin, a natural flavonoid compound, has been demonstrated to improve injury to the ileum in chickens by inhibiting the NLRP3 inflammasome and the TLR4/NF-κB signaling pathway [38]. Moreover, Bo et al. [41] described tea tree oil nanoliposomes (TTONL) that can inhibit and kill *E. coli* and significantly reduce the mRNA expression of NLRP3 and NF-κB (p65) in the duodenum and cecum of *E. coli*-infected chickens. These drugs require further clinical trials to test their efficacy in IBD [42].

In summary, NLRP3-mediated pyroptosis caused by *E. coli* infection can aggravate host intestinal inflammation, thereby preventing the clearance of some pathogenic microorganisms.

### 3.2 Bacteroides fragilis

*Bacteroides fragilis* (*B. fragilis*) is a quintessential commensal bacterium in the gut that has a dual role in modulating the NLRP3 inflammasome within the intestinal microenvironment. This Gram-negative, rod-shaped, obligate anaerobic bacterium manifests as two molecular subtypes: enterotoxigenic *B. fragilis* (ETBF) and nontoxigenic *B. fragilis* (NTBF) [43,44]. ETBF is strongly implicated in acute diarrheal disease, IBD and colorectal cancer (CRC) [45,46], whereas NTBF is considered a potential candidate for probiotics due to its powerful immunoregulatory benefits and health-promoting effects [47].

The NTBF strain *B. fragilis* ZY-312 lacks potential virulence factors and exhibits high genetic stability [47]. Hecht’s co-culture model was used to show that NTBF colonization reduced ETBF load. This suggests a competitive interplay between strains that limit toxin exposure and those that protect the host against IBD [48]. Similarly, in a multicellular model comprising Caco-2 cells, HT29-MTX, and M cells differentiated by Raji B lymphocytes, *B. fragilis* exhibited weak translocation compared to the high efficiency of *Salmonella* Heidelberg. When this multicellular model was exposed to both bacteria, the cell-free supernatant of *B. fragilis* inhibited *Salmonella* Heidelberg translocation [49], indicating that NTBF confers health benefits to the host by inhibiting the exposure to toxin and translocation of gut pathogens.

As a classic representative of gut commensal bacteria, *B. fragilis* is a double-edged sword. Depending on the experimental research model and stimulus, *B. fragilis* can have both anti- and pro-inflammatory roles by modulating the NLRP3 inflammasome in the intestinal microenvironment. The abundance of *B. fragilis* is significantly lower during the development of colitis-associated cancers. Broad-spectrum antibiotics (BSAB) accelerate the development of CRC in mice by interfering with the intestinal flora, and gavage transplantation of *B. fragilis* can effectively reverse this effect. Butyric acid is a short-chain fatty acid secreted by *B. fragilis* that inhibits the activation of macrophages and the secretion of pro-inflammatory mediators such as IL-18 and IL-1β. It does this by negatively regulating the NLRP3-mediated inflammatory signaling path-
way, thereby reducing the level of intestinal inflammation and limiting the development of CRC [50].

As previously described, the *B. fragilis* strain ZY-312 has been used as a probiotic. In the Cronobacter sakazakii-induced necrotizing enterocolitis model, ZY-312 inhibited the invasion of Cronobacter sakazakii both in *vivo* and *in vitro*, significantly altered the composition of the gut microbiota in neonatal rats, and decreased the expression of NLRP3, caspase-3 and caspase-1. ZY-312 attenuated Cronobacter sakazakii-induced necrotizing enterocolitis by regulating pro-inflammatory and dual cell death (apoptosis and NLRP3-independent pyroptosis) [51]. Conversely, LPS and interferon-γ (IFN-γ) were used as exogenous stimuli to establish a model of intestinal inflammation using enteric glial cells (EGCs). Activation of EGCs with *B. fragilis* supernatant upregulated the expression of NLRP3, IL-18, IL-1β, and caspase-1, thereby promoting intestinal inflammation [52].

**ETBF** is an important source of chronic inflammation in gastrointestinal disease and has been regarded as a risk factor for CRC. However, **ETBF** rarely induces intestinal inflammation by activating the NLRP3 inflammasome. Spermine oxidase (SMO) is a polyamine catabolic enzyme that can be highly induced by inflammatory stimuli. **ETBF**-induced colitis in mice is associated with SMO and the toxin can up-regulate the expression of SMO in HT29/c1 and T84 colon epithelial cells. This results in increased ROS and DNA damage, thus promoting the development of intestinal tumors [53]. Although it is well known that ROS is an important activator of NLRP3, whether **ETBF** can motivate intestinal inflammation and intestinal tumor development through NLRP3-dependent pyroptosis is unclear.

The pro-inflammatory effect of **ETBF** and the anti-inflammatory effect of **NTBF** in the intestinal microenvironment can be achieved by regulating the NLRP3 inflammasome. However, the precise mechanisms by which *B. fragilis* regulates diseases via the NLRP3 inflammasome pathway remain to be elucidated and warrant further investigation. Pyroptosis of the NLRP3 pathway has a negative effect on intestinal inflammation and can be exacerbated by **ETBF** or attenuated by **NTBF**. This suggests it may be possible to treat IB by regulating the level of pyroptosis of intestinal cells with probiotics.

### 3.3 *Citrobacter rodentium*

*Citrobacter rodentium* (*C. rodentium*) is a Gram-negative, extracellular murine-specific bacterial pathogen that serves as a model to study human pathogenic *E. coli* infections and IBD. The rodent model of colitis induced by *C. rodentium* is useful for analyzing the host response to intestinal bacteria, thereby providing insights into the potential mechanisms of IBD pathogenesis [54,55].

Following tissue injury or bacterial infection, NLRP3 initiates the formation of a multiprotein inflammasome complex comprising NLRP3, ASC, and caspase-1. This complex is responsible for the maturation and secretion of downstream IL-1β and IL-18 [55]. The NLRP3 inflammasome and its downstream production of IL-1β and IL-18 play pivotal roles in host defense against intestinal infection caused by *C. rodentium* [56]. In a mouse model of acute *C. rodentium* infection, exogenous administration of IL-1β reduced bacterial colonization and dissemination in the mesenteric lymph nodes of NLRP3−/− mice, preserved epithelial cell integrity, and enhanced macrophage activity to protect against *C. rodentium* infection [55]. Compared to wildtype mice, NLRP3−/− and ASC−/− mice infected with *C. rodentium* exhibited severe colitis with leukocyte infiltration and increased expression of inflammatory cytokines [57]. These studies highlight the importance of the NLRP3 inflammasome in *C. rodentium* infection and IBD, providing a link between NLRP3 polymorphisms and IBD susceptibility.

In *C. rodentium*-induced colitis, a variety of cytokines or proteins in the pyroptosis pathway are involved in activation of the NLRP3 inflammasome and in the host defense response against bacterial infection. The interferon (IFN) signaling pathway is considered to be a central regulator of inflammasome activation during bacterial infection. In *C. rodentium*-infected mouse bone marrow-derived macrophages (BMDM), interferon regulatory factor 8 (IRF8) significantly increased caspase-11 activation and GSDMD cleavage, thereby promoting NLRP3 inflammasome activation [58]. Loss of NOD2 or its downstream adaptor protein receptor interacting protein 2 (RIP2) increases the production of ROS and enhances the N-terminal kinase (JNK) signaling pathway, thereby promoting activation of NLRP3 and caspase-11-dependent noncanonical inflammasomes. The activation of inflammasomes reduces the abundance of *C. rodentium* in the colon and plays a protective role in the colonic epithelium [59]. During infection with *C. rodentium*, caspase-8 and its upstream Fas-associated death domain (FADD) are the apical mediators of NLRP3 inflammasome priming and activation [60]. Cooperation between caspase-8 signaling and GSDMD-independent canonical inflammasome signaling plays an important role in establishing gut and systemic host defense against gastrointestinal *C. rodentium* infection [61]. Wang *et al.* [62] reported that β2 integrin expression on intestinal macrophages is required for Rac1/ROS-mediated, noncanonical NLRP3 inflammasome-dependent IL-1β production. This promotes innate lymphoid cell 3 (ILC3)-derived IL-22, which in turn attenuates the effects of *C. rodentium*-infected colitis.

Furthermore, Rathinam *et al.* [63] identified a novel Toll/IL-1R domain-containing adaptor-inducing IFN-β (TRIF) pathway that allows all Gram-negative bacteria to activate the NLRP3 inflammasome. This was also validated in a *C. rodentium* infection model. The TRIF pathway is initiated by TLR4 and mediated by type I interferon. Moreover, the activation of caspase-11 through the TLR4-TRIF-
IFNβ pathway acts in concert with the NLRP3 pathway to coordinate caspase-1-dependent secretion of IL-1β and IL-18, leading to caspase-1-independent cell death. This study explored the molecular mechanism of inflammation in bacteremia induced by Gram-negative bacteria. Whether the TLR4-TRIF-IFNβ pathway co-regulates the NLRP3 pathway to activate pyroptosis in C. rodentium-induced enteritis or IBD is still unknown and needs further study.

In conclusion, the NLRP3 inflammasome plays a crucial role in defending against C. rodentium-induced intestinal infections. Various cytokines and proteins contribute to activation of the NLRP3 inflammasome during infection. The interferon signaling pathway is a central regulator, and the novel TRIF pathway has been identified as another potential regulator. Further investigation is needed to understand the role of the TLR4-TRIF-IFNβ pathway in C. rodentium-induced enteritis or IBD.

Significantly, while infection-induced NLRP3-mediated pyroptosis has a detrimental impact on intestinal inflammation in E. coli and B. fragilis, the absence of NLRP3 and ASC exacerbate IBD in the C. rodentium-infected mouse model. Hence, the involvement of pyroptosis in infection-associated IBD is complex and multifaceted.

3.4 Salmonella

Salmonella, a Gram-negative bacterium, is increasingly recognized for its complex relationship with IBD. Studies by Raffatellu and Baumler have revealed how Salmonella leverages iron to combat the host and its microbiota, thereby playing a significant role in the pathogenesis of intestinal inflammation [64]. Keestra-Gounder et al. [65] confirmed the interaction of Salmonella with innate immune receptors and its significance in gastrointestinal dysbiosis in the inflamed intestine, emphasizing the role of Salmonella in this process. Recent studies therefore suggest the potential involvement of Salmonella-induced pyroptosis in the pathogenesis of IBD.

Toll-like receptors (TLRs) play a pivotal role in recognizing PAMPs and initiating the host immune response. TLR4 recognizes LPS present on the outer membrane of Salmonella, while TLR5 detects bacterial flagellin, a major component of the flagellar structure [65]. Of note, TLR is also an important upstream receptor for pyroptosis. Salmonella infection has been shown to activate various inflammasomes, including NLRP3, NLRC4, and Absent in melanoma 2 (AIM2), which are key components of the innate immune response against intracellular pathogens [67–69]. The activation of these inflammasomes results in the production of pro-inflammatory cytokines, such as IL-1β and IL-18, and the induction of pyroptosis [69,70].

Knodler et al. [71] examined the noncanonical inflammasome activation of caspase-4/caspase-11 in mediating epithelial defenses against enteric bacterial pathogens. They described how Salmonella infection can induce non-canonical inflammasome activation and subsequent pyroptosis in IEC. Building on this, Rauch et al. [72] further probed the function of the NAIP-NLRC4 inflammasome. They found that it coordinates the expulsion of IEC with the release of eicosanoid and IL-18, achieved through the activation of caspase-1 and caspase-11. However, they also cautioned that over-activation of this coordinated response could result in significant epithelial destruction and systemic pathology [72]. In contrast, Sellin et al. [73] explored a different facet of the NAIP/NLRC4 inflammasome. They found that it drives the expulsion of infected enterocytes, thereby restricting Salmonella replication within the intestinal mucosa. This implies that in addition to inducing pyroptosis, inflammasome activation can also trigger a broad defense mechanism against early stages of mucosal infection.

Several studies have investigated the role of Salmonella-induced pyroptosis in host cells, albeit without direct reference to IBD. Man et al. [68] found that interferon-inducible protein IRGB10 is involved in the liberation of bacterial ligands such as DNA and LPS, which are then sensed by the AIM2 and caspase-11-NLRP3 inflammasomes, respectively. The results showed that IRGB10-deficient mice were more susceptible to Salmonella infections than wild-type mice [68]. In another study, Guan et al. [74] reported that Siruin3 (SIRT3)-mediated deacetylation of NLRC4 enhances its oligomerization, leading to the activation of inflammasomes in macrophages and increased release of pro-inflammatory cytokines such as IL-1β and IL-18. They also found that a deficiency in SIRT3 impairs NLRC4 inflammasome activation and weakens the host defense against bacterial infections. SIRT3 knockout mice show reduced NLRC4 inflammasome activation, decreased cytokine production, and increased susceptibility to bacterial infections [74]. Further, Naseer et al. [75] reported that NAIP/NLRC4 and NLRP3 inflammasomes recognize the activity of the Salmonella type III secretion system. This recognition restricts bacterial replication within host cells by activating inflammasomes in human macrophages [75]. The host defense mechanism against Salmonella infection may be the rapid sensing by NLRC4 of bacteria-derived outer membrane vesicles (OMVs)-binding flagellin [76]. This series of studies highlights the complexity of the immune response to Salmonella infection and the potential dual role of inflammasomes in defending against, but also exacerbating the effects of such infections.

Although not all of the abovementioned studies have directly linked Salmonella-induced pyroptosis to IBD, the activation of inflammasomes and the production of pro-inflammatory cytokines during pyroptosis may contribute to the inflammatory response and exacerbate IBD symptoms. Further research is needed to better understand the
molecular mechanisms underlying Salmonella-induced pyroptosis and its potential impact on IBD, which could in future lead to the development of novel therapeutic strategies.

Consistent with previous findings in a mouse model of C. rodentium infection, the pyroptosis of immune cells in the intestine plays a positive role in combating Salmonella infection while aggravating inflammation. Such pyroptosis is not restricted to the NLRP3 inflammasome, or even to the classical pathway.

3.5 Fusobacterium nucleatum

Fusobacterium nucleatum (F. nucleatum) is a Gram-negative, obligate anaerobic bacterium commonly found in the oral cavity. It possesses potent pro-inflammatory capacity, leading to destruction of the mucosal barrier, impairment of immune cells, and damage to the immune system, thereby triggering or exacerbating infections [77]. F. nucleatum is found in abundance in IBD and CRC patients. It has been identified as an early marker of gut microbial dysbiosis in IBD and serves as an early detection and prognostic marker for CRC, thus making it a potential target for prevention and treatment [78–80].

Research has identified that NLRP3 has a crucial role in the pathogenesis of oral infectious diseases, oral cancer, and esophageal squamous cell carcinoma. F. nucleatum is thought to be a key microorganism that mediates pyroptosis by activating inflammasomes. In gingival epithelial cells (GECs), F. nucleatum infection triggers the NLRP3 inflammasome, which subsequently activates caspase-1 and upregulates DAMPs, including high mobility group box1 (HMGB1) and ASC. This leads to activation of the NF-κB signaling pathway and secretion of mature IL-1β [81]. It was also reported that NLRR1, a member of the NOD-like receptor family located in mitochondria, can positively regulate ATP-mediated NLRP3 inflammasome activation in GECs through ROS. This mechanism potentially contributes to the antimicrobial response of cells in oral health or disease states [82].

Bioinformatics analysis of multiple large public databases has revealed that alterations in pyroptosis-related genes (PRG) are associated with prognosis and immune cell infiltration in CRC, as well as the response to immunotherapy and chemotherapy. Based on these findings, researchers have suggested a potential link between pyroptosis, F. nucleatum, and resistance to 5-fluorouracil chemotherapy in CRC. This hypothesis was tested in vitro, with the results suggesting that F. nucleatum may influence the progression of CRC through pyroptosis [83]. However, the specific molecular mechanism remains unclear, and further experimental data is required for clarification. It is noteworthy that IBD is a risk factor for CRC, and the abundance of F. nucleatum is also significantly increased in IBD patients. Whether F. nucleatum infection contributes to the development of IBD by regulating the pyroptosis pathway should be explored in future research, thereby providing new insights into the pathogenic mechanism of IBD.

3.6 Campylobacter jejuni

Campylobacter jejuni (C. jejuni) belongs to the epsilon proteobacteria class and the Campylobacteriales order. It is generally acknowledged as a primary bacterial instigator of gastroenteritis [84,85]. Many studies have confirmed the potential role of C. jejuni infection in precipitating or exacerbating IBD [86–88]. Interestingly, while C. jejuni can readily colonize poultry symbiotically without inducing disease [89], it triggers an inflammatory response in humans. This suggests that C. jejuni may be a pivotal link in the onset of IBD in humans. Consistent with this, some studies have proposed that the C. jejuni-linked pyroptosis of IEC and macrophages may be implicated in the pathologic progression of IBD.

Before pyroptosis was widely recognized and studied as a form of cell death, it was reported as early as 2004 that C. jejuni could stimulate macrophage caspase-1 to release leucine-1β. However, inhibiting caspase-1 did not impede the “apoptosis” of macrophages during this process [90]. This implied that C. jejuni induced macrophage death and leucine 1β release using a different mechanism to other gastrointestinal pathogens such as Salmonella. Subsequent research found that C. jejuni could activate the NLRP3 inflammasome in human and mouse macrophages, but did not induce the same pronounced cytotoxic phenomenon as E. coli or Salmonella [91]. Whether C. jejuni inhibits macrophage pyroptosis through distinct mechanisms warrants further investigation. However, the situation may differ when C. jejuni infects IEC, resulting in significant pyroptosis. Along with LPS and flagellum, C. jejuni produces cytolethal distending toxin (CDT), which is a key pathogenic mechanism. Recent studies have confirmed that CDT cleaves GSDME by activating caspase-9 and caspase-3, ultimately leading to cell swelling and membrane rupture [92]. This differs from the activation of GSDMD in the classical pyroptosis pathway, and the involvement of GSDME may explain why blocking caspase-1 failed to prevent macrophage death in earlier studies.

Toll-like receptors (TLRs) are significant membrane surface receptors in the pyroptosis pathway that are associated with IBD [93]. Increased TLR4 expression has been observed in the intestinal epithelium of many IBD patients [94]. Moreover, increased TLR2 expression was reported in the peripheral blood mononuclear cells of IBD patients [95]. A recent study using a mouse model also confirmed that TLR2 and TLR4 play crucial roles in intestinal inflammation during C. jejuni infection. In this model, knockout of the Single IgG IL-1 Related Receptor (SIGIRR), a negative regulator of MyD88-dependent signaling, resulted in widespread colonization of C. jejuni and severe gastroenteritis in mice. Furthermore, TLR4 knockout mice showed almost no response to C. jejuni infection, whereas TLR2
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<td>mice, BMDMs</td>
<td>NOD2, RIP2</td>
<td>NLRP3, caspase-11</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>mice, BMDMs</td>
<td>β2 integrin, Rac1/ROS</td>
<td>NLRP3, IL-1β</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td>mice, BMDMs</td>
<td>TLR4-TRIF-IFNβ</td>
<td>NLRP3, caspase-11, IL-1β</td>
<td>[45]</td>
</tr>
<tr>
<td>Salmonella</td>
<td>mice, BMDMs</td>
<td>Interferon-inducible protein (IRGB10)</td>
<td>NLRP3, AIM2, caspase-11, IL-18, IL-1β</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>mice, BMDMs</td>
<td>Sirtuin3 (SIRT3)</td>
<td>NLRP3, AIM2, caspase-11, IL-18, IL-1β</td>
<td>[47]</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>gingival epithelial cells</td>
<td>High mobility group box 1 (HMGB1), ASC</td>
<td>NLRP3, caspase-1, IL-1β</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>gingival epithelial cells</td>
<td>NLRX1, ROS, ATP</td>
<td>NLRP3, ASC, caspase-1, IL-1β</td>
<td>[49]</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>human colorectal cancer cell line HCT116 cells human colon cancer cell line HFC cells</td>
<td>Cytotoxic distending toxin (CDT)</td>
<td>caspase-9, caspase-3, gassericin-E (GSDME)</td>
<td>[50]</td>
</tr>
</tbody>
</table>

THP-1, human acute monocytic leukemia cells; IPEC-J2, porcine small intestinal epithelial cells; RAW264.7, mouse leukemia macrophages; HCT116, human colorectal cancer cells; FHC, human colonic epithelial cells; NSAID, nonsteroidal anti-inflammatory drugs; LPS, lipopolysaccharides; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; NOD2, nucleotide-binding oligomerization domain 2; RIP2, receptor interacting protein kinase 2; TLR4 (toll-like receptor 4)-TRIF (Toll/IL-1R domain-containing adaptor-inducing IFN-β); NLRX1, nucleotide-binding domain and leucine-rich repeat-containing X1; ROS, reactive oxygen species; ATP, adenosine triphosphate; AIM2, Absent in melanoma 2.
knockout mice showed serious inflammatory and pathological reactions [96]. This suggests that TLR4 is pivotal in the inflammation following C. jejuni infection, while TLR2 plays a more protective role.

*Campylobacter concisus* belongs to the same genus as *C. jejuni* and is also closely related to the incidence of IBD [97–99]. Whether it participates in the pathological process by causing pyroptosis of host cells is still unclear and warrants further investigation.

*C. jejuni*-related toxins activate macrophage pyroptosis through different pathways, highlighting the complexity of pyroptosis in IBD. As the upstream signal for pyroptosis, the differential effects of various TLRs during *C. jejuni* infection further confirm the intricate nature of pyroptosis during IBD. Further study of TLRs may provide novel insights into the contradictory effects of pyroptosis in infection-associated IBD.

**4. Conclusion and Perspectives**

Pyroptosis is a pro-inflammatory form of programmed cell death that has been increasingly studied in many different diseases over recent years. Although its pro-inflammatory function may help to remove pathogenic bacteria during intestinal infection, the initial inflammation may also cause or exacerbate intestinal inflammatory diseases. This review provides a summary of relevant studies on the effect of several common intestinal pathogens on the course of IBD through their induction of pyroptosis in host cells (Table 1, Ref. [34–41,50,57–59,62,63,68,74,81,82,92]).

TLRs are important cell surface initiation signals for pyroptosis. Different TLRs are activated depending on their recognition of different pathogen components. For the same pathogenic infection, different types of TLRs are up-regulated in different cells. For example, during *C. jejuni* infection, TLR4 expression is up-regulated in IEC, while TLR2 expression is up-regulated in monocytes. A mouse model of *C. jejuni* infection showed that TLR4 drives inflammation, whereas TLR2 relieves inflammation. This suggests the existence of distinct regulatory roles among TLRs to balance the degree of inflammation during intestinal infection. Hence, the inflammasome is activated differently depending on the type of pathogen. *AIEC, C. rodentium, Salmonella, F. nucleatum, C. jejuni* and other bacteria can activate the NLRP3 inflammasome, while *Salmonella* can also activate the NLR4 inflammasome. In addition to the classical pyroptosis pathway mediated by caspase-1, caspase-11-mediated pyroptosis (caspase-4,5 in humans) has also been implicated in the intestinal inflammation caused by *C. rodentium* and *Salmonella* infections. Furthermore, not all pathogen-induced pyroptosis is executed through GSDMD. GSDME appears to play a major role in intestinal infection by *F. nucleatum* and *C. jejuni* (Fig. 1).

The above examples highlight the complexity of pyroptosis activation during intestinal microbial infection, and further studies are needed to clarify the mechanism of activation of pyroptosis by different pathogens in intestinal cells.

The systemic effects of some commonly used drugs, such as NSAIDs, and some substances that are generally considered to be of limited harm during infection with certain pathogens, may actually induce or exacerbate IBD by increasing pyroptosis. For the treatment of IBD, it is important to identify factors that have synergistic effects on pathogenic bacterial infection. The effects of various bacterial pathogenic factors on intestinal cell pyroptosis is also worthy of further in-depth study.

The pyroptosis of intestinal cells has two sides in IBD. Macrophase pyroptosis helps to eliminate pathogenic bacteria, but the resulting inflammation may also induce or exacerbate IBD. Similarly, pyroptosis of IEC contributes to the release of pathogenic bacteria and activates the immune system to defend against them. At the same time, destruction of the intestinal lamina integrity is also an important part of the pathogenesis of IBD. It is worth noting that some pathogenic bacteria can reproduce faster in an inflammatory environment, and thus pyroptosis can further aggravate the damage caused by infection. Therefore, appropriate regulation of pyroptosis in intestinal cells by different pathogenic bacteria may be of great significance for the prevention and treatment of infection-induced IBD. *E. coli* infection and its toxins can act on IEC and macrophages to activate the NLRP3 inflammasome and induce pyroptosis, thus promoting inflammation and aggravating tissue damage. However, during *Salmonella* infection the activation of inflammasomes triggers a broad defense against mucosal infection at an early stage. Activation of the NLRP3 inflammasome and production of the downstream proinflammatory cytokines IL-1β and IL-18 effectively reduces the abundance of *C. rodentium*. This protects IEC and plays a key role in the host’s defense against *C. rodentium*-induced intestinal inflammation. Inflammasome-induced pyroptosis therefore potentially has a dual role in preventing pathogen infection and exacerbating inflammation, depending mainly on the stage and progression of pyroptosis. While maintaining homeostasis of the gut microbiota, proper control of the duration and progression of pyroptosis is crucial to avoid prolonged inflammatory stimuli and excessive activation of the immune response. Future work should aim to accurately detect the progression of pyroptosis, as well as to further study the interaction between pyroptosis and IBD.

Additional animal models are needed to study IBD during bacterial infection. Although the chicken model is often used to study intestinal infections, it may be less representative of the role of pyroptosis in human IBD than the mouse model, despite the obvious differences between the intestinal microenvironment of mice and humans. Furthermore, further statistical analysis of clinical patient specimens and ethical studies is also required.
The impact of pyroptosis on infection-associated IBD varies across studies. Some findings appear to be contradictory, reflecting the intricate interplay between pyroptosis and the immune system. Different types of cell death caused by pyroptosis have distinct consequences. When IEC are affected, their destruction directly impairs the integrity of the intestinal tissue. Conversely, when immune cells undergo pyroptosis, the effective control of this process can reduce the risk of infection. A prolonged inflammatory response is undoubtedly detrimental for IBD, whereas transient inflammation can enhance gut health by eliminating certain pathogenic microorganisms. Furthermore, the activation of pyroptosis via different upstream signals, including TLR2 and TLR4, causes divergent effects on the gut. This variation in outcomes may be attributed to the simultaneous activation or inhibition of other inter-related pathways by the specific upstream signals. Due to the complexity of these factors, the involvement of pyroptosis in IEC can oscillate between aggravating IBD, and functioning as a protective mechanism against infection (Fig. 1). Thus, the ability to control the core process of pyroptosis, including its modulation and timing, holds promise as an innovative approach for the treatment of IBD.

**Author Contributions**

ZB and YZ conceived the opinion and wrote the draft manuscript. ZQ and YD collected references and drew the figure. All authors contributed to the article and approved the final version. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors contributed to editorial changes in the manuscript.

**Ethics Approval and Consent to Participate**

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Conflict of Interest
The authors declare no conflict of interest.

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