Neutrophil–Epithelial Crosstalk During Intestinal Inflammation

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SUMMARY

Neutrophil–epithelial crosstalk functions as a double-edged sword in intestinal inflammation. Our review summarizes the molecular basis and functional consequences of these interactions during intestinal inflammation, aiming to improve the clinical treatment of intestinal inflammation.

Neutrophils are the most abundant leukocyte population in the human circulatory system and are rapidly recruited to sites of inflammation. Neutrophils play a multifaceted role in intestinal inflammation, as they contribute to the elimination of invading pathogens. Recently, their role in epithelial restitution has been widely recognized; however, they are also associated with bystander tissue damage. The intestinal epithelial provides a physical barrier to prevent direct contact of luminal contents with subepithelial tissues, which is extremely important for the maintenance of intestinal homeostasis. Numerous studies have demonstrated that transepithelial migration of neutrophils is closely related to disease symptoms and disruption of crypt architecture in inflammatory bowel disease and experimental colitis. There has been growing interest in how neutrophils interact with the epithelium under inflammatory conditions. Most studies focus on the effects of neutrophils on intestinal epithelial cells; however, the effects of intestinal epithelial cells on neutrophils during intestinal inflammation need to be well-established. Based on these data, we have summarized recent articles on the role of neutrophil–epithelial interactions in intestinal inflammation, particularly highlighting the epithelium-derived molecular regulators that mediate neutrophil recruitment, transepithelial migration, and detachment from the epithelium, as well as the functional consequences of their crosstalk. A better understanding of these molecular events may help develop novel therapeutic targets for mitigating the deleterious effects of neutrophils in inflammatory bowel disease. 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Abbreviations used in this paper: A2B (Adora2b), adenosine receptor A2b; Ado, adenosine; CAR, Cxoxaseckie and adenosin receptor; DSS, dextran sulfate sodium; HIF, hypoxia-inducible factor; HX3A3, hepoxilin A3; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule-1; IEC, intestinal epithelial cell; IL, interleukin; JAML, junction adhesion molecule-like molecule; LTB4, leukotriene B4; MIP-2, macrophage inflammatory protein-2; MMPs, metalloproteinases; NETs, neutrophil extracellular traps; PHD, prolyl hydroxylase; ROS, reactive oxygen species; SIRP-α, signal-regulatory protein-α; TJs, tight junctions; TNF, tumor necrosis factor.

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addition to their favorable effects, neutrophils can directly
destroy the intestinal epithelium by releasing a series of
proteases, such as matrix metalloproteinases (MMPs) and
neutrophil elastase, and by producing a large amount of ROS
from respiratory bursts. Moreover, neutrophil-derived
secretagogues are closely related to goblet cell depletion,
which is one of the histological features of intestinal
inflammation. It is well-accepted that uncontrolled
accumulation of hyperactivated neutrophils leads to the
deforation of crypt architecture and crypt abscess for-
mation accompanied by an excessive enzymatic reaction;
the production of pro-inflammatory cytokines such as tumor
necrosis factor (TNF)-α and interleukin (IL)-1β; and the
release of non-cytokine inflammatory mediators such as α-
defensins and calprotectin, which have been demonstrated
to recruit monocytes, T lymphocytes, and more neutrophils
to the sites of inflammation and to be closely related to the
pathogenesis of inflammatory bowel disease (IBD). IBD,
including ulcerative colitis and Crohn’s disease, is a group of
chronic and recurrent gastrointestinal inflammatory condi-
tions. These conditions are characterized by massive
neutrophil infiltration, mucosal damage, increased
epithelial permeability, and transepithelial translocation of
commensal microorganisms into the underlying tissues.

As highlighted above, neutrophils function as a double-
edged sword in intestinal inflammation. Recent studies
have shown that the intestinal epithelium plays a vital role
in the recruitment, maintenance, and elimination of neu-
traphils at sites of inflammation. Accordingly,
neutrophil–epithelial crosstalk determines the prognosis of
intestinal inflammation. This review aims to determine the
role of neutrophil–epithelial interactions in intestinal
inflammation, particularly the effects of IECs on neutrophils
during inflammatory processes. An in-depth understanding
of neutrophil–epithelial interactions in intestinal inflamma-
tion, particularly the molecular basis, is imperative in ulti-
mately identifying precise therapeutic targets of IBD.

The Intestinal Epithelium Recruits Neutrophils to Sites of Inflammation

At the onset of intestinal inflammation, neutrophils are
rapidly recruited from microcirculation to the gut through a
series of chemotactic gradients. Numerous studies have
revealed that immune cells, such as macrophages and Th17
cells, release chemotactic cytokines (IL-1β, IL-6, TNF-α, and
chemokines [CXCL-8, CXCL-10], and macrophage inflamma-
tory proteins [MIP-2]) and growth factors (granulocyte-
macrophage colony-stimulating molecule and granuloctye
colony-stimulating factor) which can influence roles in the
recruitment of neutrophils during IBD. Moreover, bac-
terial products (formyl-methionyl-leucyl-phenylalanine and
short-chain fatty acids) have also been shown to attract
neutrophils into the gut. However, little is known about
the function of the epithelium in attracting neutrophils to
the site of inflammation. Based on the few studies con-
ducted to date, we summarized the chemotactic substances
that are released by the intestinal epithelium to enhance
neutrophil migration.

Cytokines are key regulators of neutrophil recruitment
during intestinal inflammation. It is widely accepted that
IL-8, a powerful chemoattractant released by IECs, effect-
ively attracts neutrophils to the basolateral surface of the
epithelium. Notably, the gene expression and protein
concentration of IL-8 in the intestinal mucosa are signifi-
cantly increased during IBD. Moreover, under chronic
inflammatory conditions, Prevotella stimulates IECs to
secrete IL-6, which facilitates neutrophil migration across
the lamina propria to the intestinal epithelium. In addi-
tion, Chen et al demonstrated that the intestinal epithelial-
derived IL-33 indirectly recruits neutrophils to sites of
inflammation via enhanced platelet activity (Figure 1).

In addition to cytokines, epithelium-derived chemokines
are potent chemoattractants of neutrophils. Previous
studies have indicated that compared with that in healthy
tissues, the expression of neutrophil-activating protein-78
(ENA-78/CXCL5) is elevated in the colonic tissues of pa-
ients with ulcerative colitis and have established that
enterocytes are the source of ENA-78. Furthermore,
CXCL10 is abundantly secreted by murine IECs. In a previ-
ous study, the genetic deletion of CXCR3, which is the re-
ceptor of CXCL10, could alleviate dextran sulfate sodium
(DSS)-induced colitis; however, CXCR3-knockout mice had
significantly fewer intestinal neutrophils than wild-type
mice did, which confirmed that CXCL10 is a vital neutro-
phil chemotactic molecule. Moreover, other epithelial-
derived chemokines, such as CXCL7 and CCL20, have been
reported to play important roles in neutrophil recruitment
and infiltration during IBD and experimental colitis
(Figure 1).

Leukotriene B4 (LTB4) belongs to the eicosanoid family
and is an important epithelium-derived lipid mediator that
attracts neutrophils to the sites of intestinal inflammation.
LTB4 is biosynthesized from arachidonic acid. Notably, the
activities of the 3 key enzymes participating in the leuko-
triene pathway, namely, 5-lipoxygenase, 5-lipoxygenase-
activating protein, and leukotriene A4 hydrolase, were
markedly increased in the lesions of patients with active
IBD, indicating that an elevated LTB4 release may
contribute to neutrophil accumulation during IBD. In addi-
tion, genetic depletion of 5-lipoxygenase or neutraliza-
tion of 5-lipoxygenase with zileuton is associated with sig-
nificant amelioration of experimental colitis. Another
arachidonic acid metabolite, hepoxilin A3 (HXA3), a well-
known neutrophil chemotactic agent, is released from the
apical surface of the epithelium to drive neutrophils to
migrate across the epithelium into the gut lumen. Previous
studies have shown that the suppression of key enzymes
essential for HXA3 synthesis inhibits HXA3-mediated
neutrophil infiltration. These observations indicate that
HXA3 is a potent lipid mediator of epithelial origin that
promotes neutrophil chemotaxis (Figure 1).

Epithelium-derived MMPs are also important regulators of
neutrophil recruitment. MMPs are zinc- or calcium-
dependent endopeptidases that potently degrade the extra-
cellular matrix to form channels for neutrophil trans-
migration. In addition, previous studies have revealed that
MMPs can indirectly regulate neutrophil migration through
Figure 1. Epithelium-derived recruitment factors for neutrophils that enable neutrophil migration to inflammation sites. Schematic diagram outlining the recruitment signals released by inflammatory epithelial cells that attract neutrophils to migrate from blood vessels to sites of inflammation across the lamina propria. Epithelium-derived chemokines include cytokines (IL-8, IL-6, IL-33), chemokines (ENA-78/CXCL5, CXCL10, CXCL7, CCL20), lipid mediators (LTB4, HXA3), and matrix metalloproteinases (MMPs): MMP-3, MMP-7. Figure created with BioRender.

processing chemokines. For instance, MMP-3 can enhance neutrophil attraction via proteolytic processing and activation of the IEC-released CXCL7 precursor. Interestingly, in lesions of patients with ulcerative colitis, the expression of CXCL7 is increased. Furthermore, epithelium-derived MMP-7 is important for neutrophil recruitment. Compared with wild-type mice, MMP-7-knockout mice show less neutrophil infiltration in the gut after DSS treatment, and this phenotype is likely to be related to the proteolytic effect of MMP7 on CXCL8 and macrophage inflammatory protein-2. Nevertheless, the specific mechanisms of the interactions between MMP7 and these 2 chemokines remains unclear and needs to be further verified (Figure 1).

Taken together, these examples suggest that intestinal epithelial cells are also an important source of neutrophil trafficking to the intestinal epithelium, and these epithelium-derived neutrophil recruitment factors are promising novel targets for the treatment of IBD (Figure 1).

Adhesion Molecules Act as “Rungs on the Ladder” for Neutrophils Squeezing Through IECs

Neutrophils need to cross the epithelium into the gut lumen in a process termed transepithelial migration, and they participate in the pathogenesis of intestinal inflammation. In this multistep process, neutrophils coordinate with the epithelium through a series of molecules to enable adhesion and transmigration. First, neutrophils adhere to the basolateral surface of the epithelium, and this process exclusively relies on CD11b/CD18 (also known as β2 integrin). Although the ligand for CD11b/CD18 on IECs remains ambiguous, fucosylated glycoproteins are speculated to be potential ligands. In addition, it is well-established that CD11b/CD18 is an important mediator of the transepithelial migration of neutrophils; however, CD11b/CD18 antibodies can only partially inhibit neutrophil migration across the T84 cell monolayer, suggesting the existence of CD11b/CD18-independent mechanisms of neutrophil epithelial trafficking into the gut lumen (Figure 2).

After the adhesion step, neutrophils squeeze through adjacent IECs, where they challenge desmosomes and adherent and tight junctions (TJs) in the paracellular route; this process requires broad cooperation between the epithelium and neutrophils. Although little is known about neutrophil transepithelial migration, a few candidate molecules have been identified. For instance, a transmembrane glycoprotein termed CD47, which is predominantly expressed on the lateral membrane of IECs binds to the signal-regulatory protein-α (SIRP-α) expressed on neutrophils and mediates the post-adhesive events required for neutrophil transepithelial migration in the gut (Figure 2). In contrast, Azcutia et al. indicated that the recently discovered neutrophil-derived CD47, and not the CD47 expressed by IEC, modulated the passage of CD11b/CD18-dependent neutrophils through the intercellular space of the epithelium (Figure 2).

As neutrophils continue to rise to the TJ level, the Cox sackie and adenovirus receptor (CAR) expressed on IECs interact with the JAM-like (JAML) molecules expressed on neutrophils to help them migrate across TJs. Moreover, the recombinant ectodomain of CAR suppresses the transepithelial migration of neutrophils (Figure 2). It is well-known that TJs play a central role in controlling the epithelial barrier function; however, it should be noted that the JAML/CAR interaction creates gaps between IECs, which results in reduced transepithelial resistance, leading to the
**Figure 2. Molecular basis and functional consequences of neutrophil–epithelial crosstalk in transepithelial migration.**

The initiation of the neutrophil adhesion step is mediated by CD11b/18 binding to the fucosylated proteins of the IEC basolateral membrane. The movement of neutrophils through the IEC intercellular space is regulated by neutrophil–epithelial cell (SIRP-α/CD47 and JAM-L/CAR) molecular interactions; in addition, CD47 expressed by neutrophils can promote neutrophil migration in a CD11b/18-dependent manner. Neutrophil-produced enzymes such as elastase and MMP-9 can disrupt cell junctions by cleaving E-cadherin (the core protein of adhesion junctions) and desmoclone 2 (a key protein of desmosomes), respectively. Neutrophil transepithelial migration can stabilize HIF in IECs by promoting epithelial restitution through increased oxygen consumption. When neutrophils rise to the apical surface of the epithelium, their retention is regulated by the interaction of neutrophil-expressed CD11b/18 and epithelium-derived ICAM-1. In contrast, epithelial CD55 (which binds to the CD97 expressed on neutrophils) and CD44v6 are responsible for the detachment of migrating neutrophils. Neutrophils produce a large amount of ATP that is converted into Ado and adenosine by epithelial CD39 and CD73, respectively. Subsequently, adenosine binds to its receptor A2B (expressed on the apical membrane of IECs), which results in the secretion of chloride and water, CL⁻/HCO₃⁻ exchange, and IL-6 production by IECs. Figure created with BioRender.

**Neutrophil Transepithelial Migration Regulates Epithelial Barrier Function**

For transepithelial migration, neutrophils not only directly interact with the above-mentioned adhesion molecules but also target structural molecules called cadherins and catenins by producing elastase, proteinase 3, and MMPs. For example, the core adhesion junction protein E-cadherin maintains mucosal homeostasis by binding to the adapter proteins β-catenin and p120-catenin and is a target of neutrophil elastase (Figure 2). Indeed, anti-elastase treatment has been shown to ameliorate DSS-induced colitis in BALB/c mice. Intriguingly, genetic depletion of p120-catenin in the mouse gut results in massive neutrophil infiltration and dysfunction of the epithelial barrier, which induces a phenotype similar to that of patients with IBD. In addition, neutrophil-derived microparticles containing MMP-9 disrupt epithelial adhesion by cleaving desmoglein-2 rather than E-cadherin, thereby enhancing neutrophil trafficking (Figure 2). It should be emphasized that the marked enhancement of epithelial permeability increases leukocyte exposure to invasive antigens and stimulates immune responses, whereas the persistent transepithelial migration of neutrophils is associated with mucosal damage and altered barrier function, which are the characteristics of IBD.

In addition to increasing the epithelial permeability, neutrophil transepithelial migration also influences the proliferation, apoptosis, and function of IECs. For instance,
the JAML/CAR combination has been demonstrated to disrupt mucosal wound healing by inhibiting IEC proliferation.\textsuperscript{72} Moreover, the binding of neutrophils to intercellular adhesion molecule-1 (ICAM-1) activates β-catenin and Akt signaling to promote IEC proliferation and mucosal wound healing.\textsuperscript{73} In contrast, neutrophil-derived microparticles hinder intestinal wound healing by suppressing epithelial cell proliferation.\textsuperscript{74} Furthermore, the neutrophils in lesions from patients with IBD and experimental mice impede mucosal wound closure by releasing pro-inflammatory miR-23a and miR-155.\textsuperscript{75} Recently, NETs have been shown to induce apoptosis of IECs, and they destroy the integrity of cell junctions in experimental colitis. NETs have been shown to positively correlate with disease activity, as demonstrated by their increased presence in the lesions of patients with IBD and their direct role in perpetuating intestinal inflammation in IBD.\textsuperscript{76-79} However, the underlying mechanism remains poorly understood. Additionally, transepithelial migration of neutrophils has been shown to increase the expression of MMP ADAM17 in IECs, which promotes epithelial migration of neutrophils has been shown to increase the expression of MMP ADAM17 in IECs, which upregulates ADAM17 expression has been found in lesions of patients with IBD in the active phase but not in the chronic phase. Based on this, transmigration of HIF triggers the transcription of a set of genes that induce apoptosis of IECs, and they destroy the integrity of cell junctions in experimental colitis. NETs have been shown to positively correlate with disease activity, as demonstrated by their increased presence in the lesions of patients with IBD and their direct role in perpetuating intestinal inflammation in IBD.\textsuperscript{76-79} However, the underlying mechanism remains poorly understood. Additionally, transepithelial migration of neutrophils has been shown to increase the expression of MMP ADAM17 in IECs, which induces the pro-inflammatory cytokine TNF-α by cleaving its precursor. This upregulated ADAM17 expression has been found in lesions of patients with IBD in the active phase but not in the chronic phase. Based on this, transepithelial migration can lead to colitis by promoting the production of inflammatory cytokines by IECs.\textsuperscript{80-82}

Recent studies have revealed that IECs regulate hypoxia-inducible factor (HIF), an important transcription factor that regulates gene expression in hypoxic microenvironments.\textsuperscript{83-85} HIF targets various genes, such as those encoding mucins, antimicrobial peptides, and others associated with inflammation resolution, which are critical for maintaining the mucosal barrier.\textsuperscript{86,87} Numerous experimental colitis studies have shown that during acute intestinal inflammation, transmigrating neutrophils consume oxygen within the epithelial microenvironment in a reduced nicotinamide adenine dinucleotide phosphate-oxidase-dependent manner, thereby significantly stabilizing HIF.\textsuperscript{88,89} Subsequently, the stabilization of HIF triggers the transcription of a set of genes that enable IECs to promote mucosal restitution (Figure 2).\textsuperscript{80,89} Given that HIF stabilization is an essential component of mucosal recovery during inflammation, a growing number of pharmacological molecules have been developed to achieve HIF stabilization; currently, this is primarily achieved by inhibiting prolyl hydroxylase (PHD).\textsuperscript{69,90} A PHD inhibitor, FG-4497, has been shown to significantly promote inflammatory resolution in experimental colitis, with similar pro-resolution effects observed in dimethyloxalylglycine-treated mice.\textsuperscript{91,92} However, HIF stabilizers need to be developed for clinical translation in the treatment of IBD.

**Neutrophil–Epithelial Interactions at the Lumen Surface of the Gut**

Normally, neutrophils undergo cell death after completing their tasks and detach from the intestinal epithelium to resolve inflammation and restore tissue function. Otherwise, excessive accumulation of neutrophils in the intestinal epithelium leads to crypt abscess formation and tissue damage, a hallmark of IBD, through the release of numerous pro-inflammatory mediators, ROS, and enzymes.\textsuperscript{93} Although infiltrated neutrophils mainly undergo apoptosis and are phagocytosed by macrophages, recent studies have shown that IECs also aid in the clearance of neutrophils after migration.\textsuperscript{71,72} As neutrophils ascend to the apical surface of the intestinal epithelium, an important epithelial-expressed adhesion molecule, termed ICAM-1, interacts with CD11b/CD18 on neutrophils. Furthermore, ICAM-1 is thought to be involved in neutrophil retention on the apical membranes of IECs.\textsuperscript{94} In contrast, another adhesion molecule, CD44v6, is specifically expressed on the apical surface of the epithelium in the inflammatory state and is responsible for the detachment of neutrophils into the gut lumen.\textsuperscript{95,96} Likewise, the increased expression of CD55 (known as a decay-accelerating factor) also facilitates the apoptosis of migrated neutrophils through direct contact with neutrophil-derived CD97.\textsuperscript{97} These adhesion molecules (ie, CD44v6 and CD55) appear to play contrasting roles to ICAM-1 in the release of post-migrated neutrophils, and the balance between CD44v6/CD55 and ICAM-1 is critical in crypt abscess formation (Figure 2).

Furthermore, migrating neutrophils are known to release large amounts of ATP, which are in turn converted into adenosine (Ado) by CD39 and CD73, 2 ectonucleotidases expressed on the apical membrane of the intestinal epithelium.\textsuperscript{98,99} Ado subsequently activates the cyclic adenosine monophosphate signaling pathway by binding to A2B (Adora2b), an adenosine receptor expressed on IECs.\textsuperscript{100,101} The combination of Ado and A2B plays a key role in wound healing, inflammation resolution, and maintenance of intestinal mucosal homeostasis, and it has received extensive attention in recent years.\textsuperscript{102,103} For instance, their interaction with the lumen surface of the gut leads to electrogenic chloride secretion into the gut lumen and passive water transport. This passive water flux helps to remove noxious antigens from the surface of the intestinal epithelium. Colitis severity is significantly increased in mice lacking A2B in the gut.\textsuperscript{100} However, passive water transport is also the pathological basis of diarrhea in patients with IBD.\textsuperscript{31,50,104} Except for their direct role in releasing ATP at the apical membrane of the intestinal epithelium, the translocated neutrophils can indirectly alter fluid homeostasis by facilitating the transepithelial migration of platelets, which produce even higher levels of ATP in the intestinal lumen than neutrophils do.\textsuperscript{105} In addition, previous studies have shown that the combination of Ado and A2B helps maintain pH homeostasis in the gut.\textsuperscript{56,106,107} In murine and human colonic organoids, neutrophil-derived Ado can significantly upregulate the expression of SLC26A3, a major CL-/HCO3- exchanger on the apical membrane of IECs via CREB/cAMP signaling, which subsequently regulates the local pH to facilitate the adaptation of IECs to the inflammation-induced acidity.\textsuperscript{106} Furthermore, it has been shown that stimulation of the intestinal epithelial cell line T84 monolayer with adenosine induces increased polarized secretion of IL-6 (a pro-inflammatory cytokine associated with neutrophil degranulation) into the intestinal lumen. Similar to SLC26A3, Ado-induced IL-6 secretion is also
dependent on CREB/cAMP signaling, which induces an intracellular calcium flux in neutrophils and subsequently promotes neutrophil degranulation to enhance the bactericidal effect of neutrophils upon entry into the intestinal lumen (Figure 2).108

Collectively, the interaction between neutrophils and IECs on the apical surface of the epithelium plays a key role not only in inflammation resolution but also in tissue damage. More research is needed in the future to gain a deeper understanding of how this interaction affects the prognosis of intestinal inflammation, which will facilitate clinical translation (Figure 2).

Conclusion

Our understanding of the double-edged sword role of neutrophil–epithelial interactions in intestinal inflammation has increased significantly in recent years. It is well-known that neutrophils are essential for mucosal immunity; however, excessive recruitment and activation of neutrophils is a direct cause of intestinal mucosal crypt abscesses and extensive mucosal damage. Numerous studies have revealed the effects of neutrophils on IECs; however, the effects of IECs on neutrophils in colitis need to be well-elucidated. Our review provides insights into the recent research on the neutrophil–epithelial crosstalk in colitis and highlights the important roles of IECs in regulating neutrophil recruitment, transepithelial migration, cell death, and clearance. Despite mounting evidence showing that neutrophil–epithelial crosstalk can determine the prognosis of intestinal inflammation, the molecular events modulating their interactions and the underlying regulatory mechanisms are only partially understood, and they should be the major targets in further research. More appropriate methods, such as live-cell imaging and 2-photon microscopy, should be utilized to track neutrophil transepithelial trafficking in vivo. In vitro, an air–liquid interface culture of colonic organoids, similar to the normal monolayer cell culture system but comprising diverse intestinal epithelial cells, can be used to investigate how neutrophils interact with various epithelial cells rather than with enterocytes alone. New technologies such as single-cell RNA sequencing and spatial transcriptomics can improve our understanding of the distribution of cells during neutrophil transepithelial migration, reveal cell-to-cell communication, and predict the potential signals that mediate the interaction between neutrophils and IECs. In addition, exosomes containing functional components, such as proteins and RNAs, have been shown to play an important role in mediating intercellular communication in IBD. They are worth investigating in further studies on the crosstalk between neutrophils and IECs. In conclusion, a better understanding of the complex interactions between neutrophils and IECs during intestinal inflammation may provide new avenues for tissue-specific treatment of IBD.

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Conflicts of interest

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