Review Article

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Mechanisms of anastomotic leakage in colorectal cancer surgery: unraveling the roles of inflammation, immunity, and microbiota – a narrative review

Ke Ma, MD[a], Tingfei Gu, MD[a], Zhouqiao Wu, MD, PhD[a,b,*], Ziyu Li, MD, PhD[a,b,*]

Abstract

Anastomotic leakage (AL) is a major complication of colorectal surgery that can lead to abdominal infection and systemic inflammatory response. Meanwhile, the microbiota and immune microenvironment at the anastomosis are closely related to the formation of AL. Presently, a considerable volume of research is directed toward investigating the colorectal microenvironment in AL. These studies offer a robust foundation for probing etiological factors contributing to the AL. This is the first review to comprehensively depict the bidirectional effects of intestinal immunity and microbiota on anastomotic healing. Furthermore, this study also elaborates on the association between the clinical risk factors and micro-mechanisms of AL, and provides a novel perspective for its early diagnosis.

Keywords: anastomotic leakage, colonic microflora, colorectal cancer, surgical complications

Introduction

Anastomotic leakage (AL), defined as the leakage of luminal contents through a compromised surgical bowel connection, typically results from anastomotic dehiscence or impaired healing processes[1,2]. As one of the most severe postoperative complications, AL occurs in 6–22% of cases and is associated with prolonged hospitalization durations and significantly increased healthcare expenditures[3–10].

HIGHLIGHTS

- **Pathological Basis of Leakage Formation:** Prolonged and exacerbated inflammation, along with tissue damage at the anastomosis, constitutes the pathological basis for the formation of anastomotic leaks.
- **Regulatory Role of Immune Response and Microbiota:** The immune response and microbiota play crucial regulatory roles in the healing processes of the anastomosis.
- **Understanding Interactions for Biomarker Exploration:** A deep understanding of the interactions among inflammation, immune response, and microbiota in patients with anastomotic leaks is significant for the exploration of novel biomarkers.
- **Current State of Biomarker Research:** The exploration of biomarkers is currently in its early stages but holds potential value in the early diagnosis of anastomotic leaks.

Several clinical factors, including poor preoperative nutritional status, neoadjuvant therapies, and inadequate anastomotic blood supply, have long been recognized as impairing anastomotic healing[11–14]. While the clinical mechanisms are well-established, the cellular and molecular mechanisms remain poorly understood[15]. This review is the first to systematically synthesize evidence on how intestinal immunity and gut microbiota synergistically regulate anastomotic healing, thereby bridging the gap between clinical observations and molecular mechanisms. Furthermore, we elaborate on the relationship between the micro-level mechanisms of anastomosis and clinical risk factors, and propose prospective approaches for its early diagnosis. This work has been reported in line with the TITAN criteria[16].

[a]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Gastrointestinal Cancer Center, Peking University Cancer Hospital & Institute, Beijing, China and [b]Beijing Institute for Cancer Research, Beijing, China

Ke Ma and Tingfei Gu contributed equally to this work.

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*Corresponding authors. Address: Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Gastrointestinal Cancer Center, Peking University Cancer Hospital & Institute, China; Beijing Institute for Cancer Research, No. 52 Fu-Cheng Road, Beijing 100142, China.Tel.: +8615011515628. E-mail: z.wu@hsc.pku.edu.cn (Z. Wu); Tel.: +8613901009767.
E-mail: ziyu_li@hsc.pku.edu.cn (Z. Li).

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The healing process of anastomoses

Acute inflammation is rapidly induced following colorectal cancer surgery, as evidenced by the rapid accumulation of neutrophils and macrophages that release pro-inflammatory cytokines. As the intestinal barrier commences healing, immune cell populations alter and anti-inflammatory cytokines are secreted, advancing the anastomosis into the repair phase[15]. Successful healing requires the reconstruction of the intestinal barrier and the reconnection of the mucosa, submucosa, muscularis, and serosa[17].

Anastomotic healing occurs through four overlapping phases that require temporal and spatial coordination. Initially, blood clot formation prevents bleeding and pathogen entry into intestinal tissue and circulation[18,19]. In this phase, the submucosa, abundant in blood vessels, initiates preparations for the following stage. As inflammation resolves, angiogenesis intensifies, with vascular endothelial growth factor and other growth factors stimulating the formation of new blood vessels to ensure the oxygen and nutrient supply essential for subsequent repair processes[20,21]. During angiogenesis, intestinal epithelial cells (IECs) migrate to the wound site, directed by chemokine receptors like CXCR4 and CCR6, thereby reconstructing the epithelium. Concurrently, TGF-β stimulates the differentiation of fibroblasts into myofibroblasts[22–25]. These myofibroblasts restore intestinal integrity by producing extracellular matrix (ECM) components, including collagen and fibronectin, through fibrosis, particularly in the submucosa. Between postoperative days (POD) 3 and 5, the synthesis and degradation of ECMs achieve dynamic equilibrium[26]. Tissue inhibitors of metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs) regulate the conversion of collagen from type III to type I, thereby enhancing the structural stability of the submucosal layer.

Immune homeostasis and dysregulation

Immunoregulation plays a crucial role in anastomotic healing. Successful healing represents immune homeostasis, while AL is a result of immune dysregulation (Fig. 1). Evidenced by extensive cell damage and reduced collagen production, immunological dysregulation is marked by persistent, severe inflammation and immune suppression[15,17,27,28]. In the following, we will discuss the role of immune response in the occurrence of AL, focusing on the functions of cytokines, bidirectional regulation of immune cells, and inflammation-mediated tissue damage.

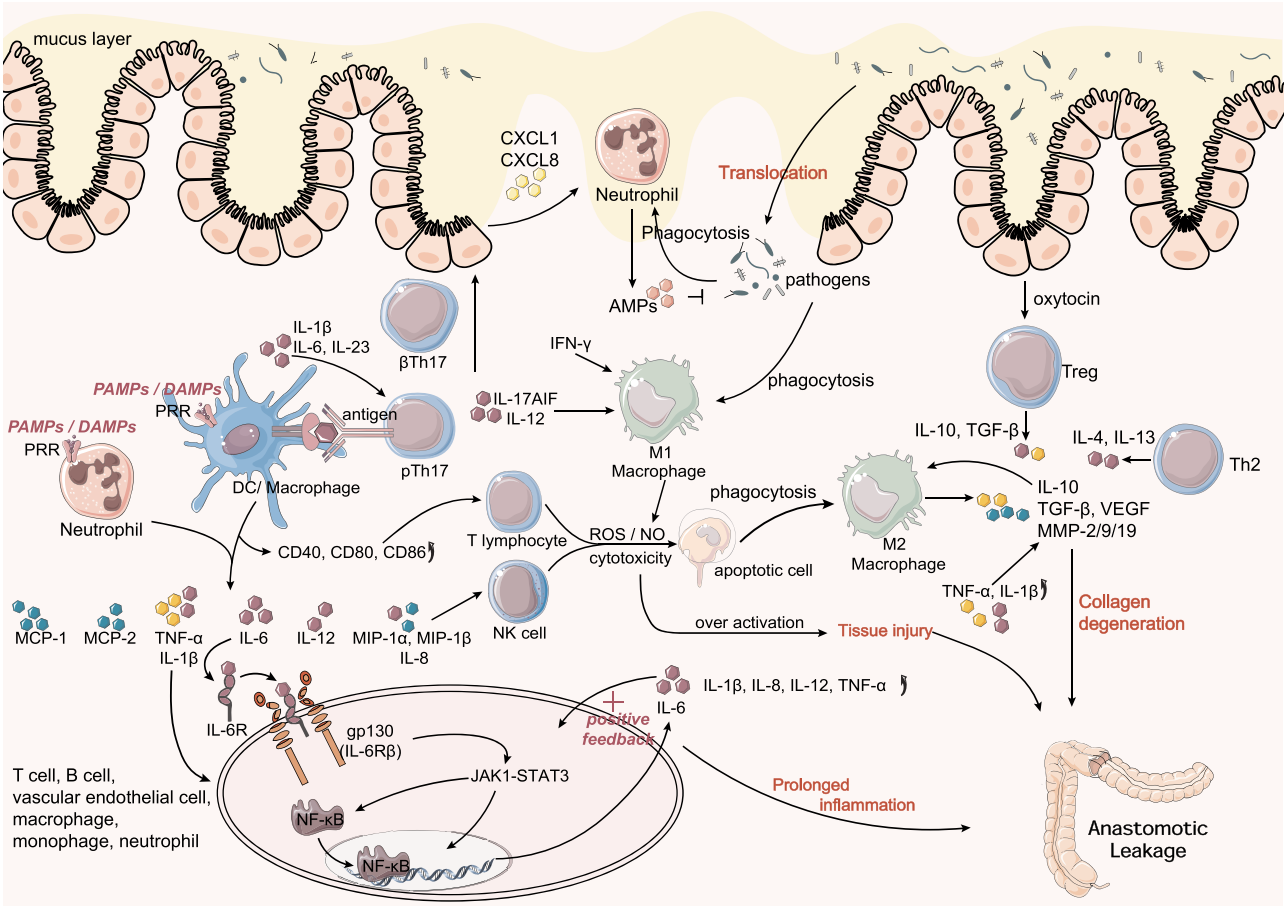


Figure 1. The mechanisms of inflammation and intestinal immunity in the formation of anastomotic leakage. Through the activation mechanisms of PAMPs or DAMPs, intestinal tissues produce inflammatory cytokines and recruit neutrophils and monocytes to the site of injury. Innate immune cells subsequently activate inflammatory pathways, leading to the production of significant amounts of cytokines, including IL-2, IL-6, and IL-12. Among these, IL-6 plays a pivotal role in amplifying the inflammatory response. The intestinal immune environment is critical in the pathogenesis of inflammation-driven anastomotic leakage. If the actions of pro-inflammatory factors are not promptly suppressed by regulatory immune cells such as Tregs, the level of inflammation may escalate uncontrollably. PAMPS, pathogen-associated molecular patterns; DAMPS, damage-associated molecular patterns; NK cell, natural killer cell; AMPs, antimicrobial peptides.

Cytokines involved in wound modulation

Acute inflammation of the anastomosis is regulated by damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), which bind to pattern recognition receptors and trigger the release of pro-inflammatory cytokines[29–33]. The positive effects of acute inflammation include reducing the intestinal microbial load and providing favorable conditions for wound healing, such as nutrients and oxygen[34–36]. However, the exacerbation and prolongation of inflammation are critical factors that disrupt anastomotic healing. Different cytokines play distinct roles in the anastomosis formation process (Table 1), with pro-inflammatory cytokines and anti-inflammatory cytokines exerting opposing effects. Inflammatory pathways in intestinal tissue primarily involve JAK1-STAT3, NF-κB, and IRF1, which release pro-inflammatory cytokines including interleukin (IL)-1, IL-2, IL-5, IL-6, IL-8, IL-12, IL-17A, interferon (IFN)-α, IFN-β, IL-15, IL-18, and IL-23[29,37,38]. Anti-inflammatory cytokines, such as transforming growth factor (TGF)-β, IL-4, IL-10, IL-11, and IL-13, play a key role in suppressing inflammation[37,38].

Overrecruitment of neutrophils leading to wound healing failure

A significant cause of wound healing failure is the excessive recruitment or activity of neutrophils, primarily due to the increased release of neutrophil extracellular traps (NETs). DAMPs are generated in the wound tissue as neutrophils release reactive oxygen species (ROS) and form NETs to eliminate microorganisms during wound healing[86]. Prolonged inflammation and elevated IL-6 levels are commonly observed in nonhealing lesions in patients, which result from excessive neutrophil recruitment. These lesions are frequently observed in individuals with peripheral vascular disease, wound infections, or type 1 or type 2 diabetes[87–89].

The role of M1 macrophages in promoting inflammation

In the initial stage of infection or inflammation, monocytes differentiate into the M1 phenotype, which is induced by lipopolysaccharide (LPS) or Th1 cytokines (e.g., IFN-γ, GM-CSF)[15]. These M1 macrophages secrete pro-inflammatory cytokines, notably transforming growth factor (TNF)-α and IL-1β, which are critical mediators of the acute inflammatory response and contribute to angiogenesis in damaged tissues[90–92]. The elevated levels of TNF-α and IL-1β subsequently upregulate the expression of MMPs, enzymes that catalyze collagen degradation in the ECM[93,94]. Importantly, IL-1β establishes a self-perpetuating inflammatory environment by promoting IL-17A production, which in turn stimulates additional IL-1β secretion, creating a positive amplification loop[42]. This cytokine cascade promotes further recruitment and activation of M1 macrophages to the site of injury, thereby exacerbating the inflammatory response and potentially delaying tissue repair.

The role of M2 macrophages in tissue reconstruction

M2 macrophages serve a critical function in anastomotic wound healing by counterbalancing M1-mediated inflammation through

Table 1

The regulatory roles of different cytokines in the anastomotic healing process

Cytokines	Function
IL-1 α [39,40]	Recruiting immune cells.
IL-1 β [41,42]	Recruiting and activating immune cells.
IL-2[43,44]	Stimulating IECs' migration.
IL-4[45,46]	Suppressing monocytes and M1 macrophages; Activating M2 macrophages; Upregulating collagen and other ECM components.
IL-6[47,48]	Promoting inflammation; Promoting the proliferation and migration of IECs; Upregulating tight junction proteins and mucins.
IL-8[49,50]	Promoting neutrophil infiltration; Promoting inflammation.
IL-10[47,51–53]	Activation of M2 macrophages; Promoting the differentiation and function of fibroblasts.
IL-11[54,55]	Stimulating the proliferation and migration of IECs.
IL-12[56,57]	Promoting Th1 cell activation and IFN- ɣ production.
IL-13[46,58,59]	Disrupting intestinal barrier function by inducing apoptosis in IECs and altering tight junction protein composition.
IL-17[60–62]	Regulating the expression and localization of tight junction proteins; Promoting inflammation.
IL-18[63,64]	Enhancing IECs' proliferation; Promoting inflammation.
IL-22[65,66]	Promoting the proliferation and repair of IECs.
IL-23[67,68]	Stimulating the production of Th17 cells.
IL-24[69–71]	Upregulating the expression of mucins; Promoting pro-fibrotic processes.
IL-27[72]	Inducing IECs' proliferation.
IL-28[72]	Maintaining the intestinal epithelial barrier function through the regulation of claudin-1.
IL-33[73,74]	Promoting the differentiation of M2 macrophages; Promoting the regeneration of IECs.
IL-34[75,76]	Stimulating intestinal fibroblasts to produce collagen.
IL-36[77,78]	Promoting the production of IL-22.
GM-CSF[79,80]	Promoting the polarization of M1 macrophages; Inhibiting the polarization of M2 macrophages.
G-CSF[81]	Enhancing the proliferation and migration of neutrophils.
TNF- α [77,82]	Promoting the proliferation of fibroblasts and IECs.
TNF- β [83,84]	Promoting epithelial cell migration and regeneration.
IFN- β [62]	Promoting inflammation; Supporting epithelial regeneration.
IFN- ɣ [85]	Promoting cell survival and the repair process.
TGF- β [83]	Enhancing the production of ECM components; Stimulating the migration of IECs.

ECM, extracellular matrix; IECs, intestinal epithelial cells.

inhibition of pro-inflammatory cytokines TNF-α and IL-1β[95]. M2 macrophages are regulated by IL-4 and IL-13, and characteristically secrete anti-inflammatory mediators such as IL-10 and TGF-β, which collectively suppress inflammatory processes while promoting tissue regeneration, ECM restructuring, angiogenesis, and homeostasis restoration. Importantly, most tissue-resident macrophages predominantly display M2-like properties and are activated through alternative pathway signaling[96]. The temporal transition from M1 to M2 predominance represents a favorable prognostic indicator in anastomotic healing, as M2 macrophages support collagen deposition and cellular proliferation. Mechanistically, excessive M1 activation can be regulated through programmed cell death, wherein IFN-γ and TLR stimulation trigger Caspase-8-dependent apoptosis and BAX/BAK-mediated mitochondrial degradation via inducible nitric oxide synthase (iNOS)[97]. Concurrently, T lymphocytes and natural killer cells, recruited via chemokine gradients, facilitate the elimination of damaged cells through ROS/NO generation and direct cytotoxicity, thereby promoting the M2-dominated reparative phase. The presence of IL-10 and apoptotic cellular debris further stimulates M2 macrophages to produce a balanced profile of MMPs, TIMPs, and anti-inflammatory mediators[98]. This process highlights the critical role of tissue injury in the expression of collagenases that degrade the matrix[99].

The bidirectional role of Th17/Treg balance in intestinal wound healing

In the intestinal microenvironment, naïve CD4+ T cells differentiate into specialized subsets, including Th17 and regulatory T (Treg) cells[100]. The differentiation of Th17 cells is meticulously governed by the local cytokine environment; notably, the simultaneous presence of IL-1β, IL-6, and IL-23 promotes the production of pathogenic Th17 (pTh17) cells, while the combination of TGF-β1 and IL-6 steers differentiation toward non-pTh17 (βTh17) phenotypes[101]. Conversely, Treg cell differentiation primarily requires TGF-β and IL-2 signaling[102]. These two T cell subsets exhibit functionally reciprocal roles: Treg cells maintain immune tolerance and suppress excessive responses, while Th17 cells predominantly mediate pro-inflammatory immune reactions[103]. Emerging evidence demonstrates that gut microbiota critically orchestrates the Th17/Treg balance through the production of metabolic derivatives, particularly short-chain fatty acids (SCFAs). Notably, specific commensal bacteria, including *Lactobacillus species* and *Akkermansia muciniphila*, have been shown to preferentially enhance Treg proliferation while concurrently inhibiting Th17 activity, thereby promoting immune homeostasis and supporting intestinal healing processes[17,104,105].

Inflammation and tissue damage caused by excessive NO/ROS

Excessive inflammatory responses are characterized by heightened production of nitric oxide (NO) and ROS, which establish a self-amplifying inflammatory cycle. Metabolically, pro-inflammatory macrophages primarily utilize glycolysis for energy generation, whereas anti-inflammatory phenotypes predominantly rely on fatty acid oxidation and oxidative phosphorylation[106–108]. Within the inflammatory microenvironment, bacterial LPS and IFN-γ significantly upregulate iNOS expression, resulting in substantial NO production. This process is further potentiated by TNF-α and IL-6, which sustain elevated iNOS expression. Mechanistically, NO impairs mitochondrial function, enhances glycolytic metabolism, and inhibits phenotypic conversion toward inflammation-resolving macrophages[109]. Concurrently, elevated ROS production is a hallmark of M1 macrophage activation[110–112]. Both mediators exert direct tissue-damaging effects; high NO concentrations generate peroxynitrite, a potent cytotoxic molecule capable of inducing cell death[113,114], while excessive ROS accumulation causes oxidative stress that compromises cellular membrane integrity, protein structure, and DNA stability, ultimately triggering apoptotic or necrotic pathways[115,116]. Furthermore, ROS overproduction stimulates MMP expression, promoting ECM degradation and exacerbating tissue injury[115,117].

Microbial-mediated AL

Taxonomic diversity, microbial gene richness, and a stable core microbiota constitute essential characteristics of healthy gut microbial communities. The colorectal tract predominantly harbors anaerobic bacteria derived from both fecal content and the specialized mucous layer[118–120]. The inner and outer mucus layers serve as critical colonization sites for beneficial commensal families, including *Lachnospiraceae, Bacteroidaceae*, and *Prevotellaceae*, which form protective barriers against potentially pathogenic bacterial species, particularly certain members of the *Firmicutes phylum* and opportunistic *Bacteroidetes*[121,122]. AL represents a complex multifactorial complication influenced by the interplay between local immune responses, bacterial metabolites, and shifts in microbiota composition (Fig. 2). While microbial translocation at the anastomosis site occurs as an expected consequence of surgical intervention, the specific microbial populations present significantly influence anastomotic healing outcomes (Table 2)[17]. Research demonstrates that disruptions to the balanced gut microbiota composition and function can substantially compromise anastomotic healing processes, potentially leading to dehiscence and subsequent leakage[17,123].

Bacterial virulence and the amplification of inflammation

Microbial translocation at the anastomosis leading to infection is a continuous process[142,143]. Initially, microorganisms colonize the wound surface and adjacent intestinal mucosa before progressively infiltrating the full thickness of intestinal tissue, ultimately leading to infection, anastomotic dehiscence, and purulent exudate[144].

Bacterial virulence can impair the anastomotic healing capacity. First, harmful microorganisms at the wound site can promote the invasion of more pathogenic or cooperative microbes by inducing immune responses or altering chemical gradients (e.g., metabolites, oxygen concentration, pH)[145,146]. *Staphylococcus aureus*, with its MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), can bind to damaged ECM components and exposed glycoproteins. MSCRAMMs have enzymatic activity that degrades tissue, aiding adhesion and invasion into deeper layers[147]. *B. fragilis* uses appendages for adhesion and releases hemolysin, while its enterotoxin disrupts epithelial tight junctions by cleaving E-cadherin[148]. Second, microbial interactions can modulate virulence and induce antibiotic resistance by altering the collective virulence and antibiotic resistance of microorganisms[149]. *Pseudomonas aeruginosa*

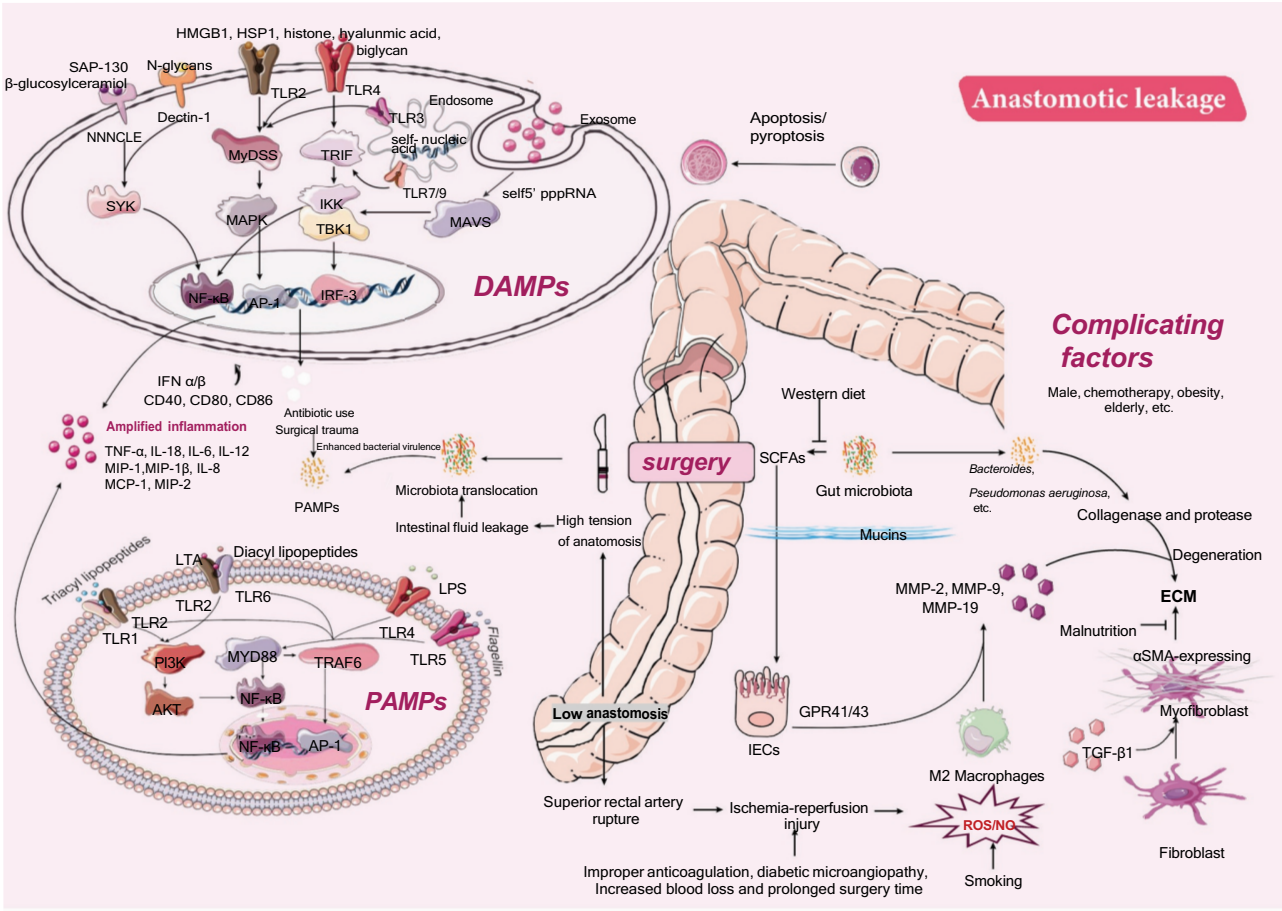


Figure 2. Perioperative management and the role of gut microbiota in promoting inflammatory responses and anastomotic healing. The initiation of inflammation is predominantly influenced by two factors: tissue damage resulting from perioperative procedures and infections arising from the translocation of gut microbiota. Surgical stress and trauma induce apoptosis and pyroptosis, leading to the release of substantial quantities of DAMPs. Conversely, PAMPs primarily originate from gut microorganisms that come into direct contact with the injured site. Both DAMPs and PAMPs activate inflammation-related signaling pathways, such as NF-κB and PI3K, which subsequently promote the expression and release of various pro-inflammatory cytokines. Furthermore, the gut microbiota plays an important role in promoting the healing and integrity of anastomoses, for example, through the production of SCFAs. However, certain microorganisms can also produce collagenases and proteases, disrupting the normal process of collagen deposition at the anastomotic site. SCFAs, short-chain fatty acids; MMPs: matrix metalloproteinases; TLR, toll-like receptor; ROS: reactive oxygen species; GPR 41/43: G protein-coupled receptor 41/43.

can enhance the pigment virulence factor, staphyloxanthin, in *Staphylococcus aureus*[150]. Antibiotic resistance is a key concern in the perioperative management of colorectal cancer patients. For instance, *Pseudomonas aeruginosa* isolates producing LasA protease make *Staphylococcus aureus* more sensitive to vancomycin and tobramycin[151].

Microbial-induced disruption of collagen remodeling

Colorectal microbiota, including *Bacteroides fragilis* and *Clostridium histolyticum*, can create a hostile environment by releasing collagenase and proteases (e.g., MMPs) that degrade collagen[5]. Type I and type IV collagen, essential structural components of the ECM, serve as primary targets for these enzymes while simultaneously being fundamental to tissue integrity and wound repair[152]. After colorectal surgery, MMP-mediated tissue breakdown occurs rapidly, while collagen production and remodeling take longer, explaining the vulnerability of anastomotic sites in the early POD[5]. Upon colonizing the anastomosis, *E. faecalis* disrupts collagen deposition and remodeling through a twofold mechanism: collagenase targets type I collagen, while coordinated MMP9 activity breaks down type IV collagen. Notably, oral phosphate supplementation has demonstrated efficacy in reducing AL by inhibiting bacterial collagenase activity at surgical sites[139].

Dysregulation of microbial metabolites

Metabolites serve as essential mediators in inflammatory regulation (Table 3). Dysbiosis, characterized by disruption of the gut microbial ecosystem, manifests through alterations in key microbial metabolites, including SCFAs, bile acids, phenolic compounds, and bacterial components such as LPS[153]. SCFAs – primarily acetate, propionate, butyrate, and valerate – are produced through microbial fermentation of complex carbohydrates and dietary fiber, exerting multifaceted physiological functions: they promote epithelial cell proliferation and differentiation, enhance regulatory T cell development, inhibit histone deacetylases to attenuate inflammatory responses, and function as the predominant energy substrate for colonocytes[154–158]. Depletion

Table 2

The role of microbiota in the occurrence of anastomotic leakage

Function	Microbiota
Secretion of SCFAs	*Alistipes*[124,125]
	Clostridium sensu stricto[125]
	Akkermansia[126,127]
Degradation of the mucin	*Lachnospiraceae*[123,128]
	Ruminococci[129]
Promotion of macrophage M1	*Escherichia coli*[130,131]
	Salmonella[132,133]
	Fusobacterium nucleatum[134]
	Streptococcus[135]
Activation of collagenase and MMPs	*Bacteroides*[125,136,137]
	Enterococcus faecalis[5]
	Pseudomonas aeruginosa[138]
	Serratia marcescens[139]
	Clostridium histolyticum[140]
Superinfection	*C. difficile*[141]

MMPs, matrix metalloproteinases; PAMPs, pathogen-associated molecular patterns; SCFAs, short-chain fatty acids.

of butyrate-producing bacterial populations can significantly compromise mitochondrial function and epithelial barrier integrity[159]. Conversely, metabolic perturbations within the gut microbiome frequently precipitate intestinal barrier dysfunction, thereby exacerbating infectious processes. The Western dietary pattern, characterized by energy-dense processed foods, refined sugars, excessive sodium, and saturated fats but deficient in essential nutrients, induces specific alterations in gut microbial composition – notably increased abundance of *Bacteroides* and *Akkermansia* – and is associated with elevated risk of chronic inflammatory disorders[160]. This dietary profile reduces SCFA synthesis and impairs primary bile acid deconjugation, suggesting that strategic modification of perioperative nutritional regimens could potentially mitigate postoperative complications[125]. However, emerging evidence suggests that perioperative dietary interventions can reduce postoperative complications. Arginine reduces the motility and virulence of *Pseudomonas aeruginosa*, while long-chain fatty acids promote its infection[161,162]. Prebiotics and fiber, such as inulin, enhance anastomotic healing by activating peroxisome proliferator-activated receptor-γ (PPAR-γ), strengthening the gut barrier, and reducing tumor implantation in murine models[163]. A colorectal cancer trial found that a modified microbiota-accessible carbohydrate (MAC) diet (30 g fiber/day) increased SCFA-producing bacteria and improved defecation. Glutamine supplementation, based on meta-analyses of 26 trials, reduces systemic inflammation and improves nitrogen balance, aiding mucosal repair[164].

Microscopic insights into clinical risk factors in AL

Clinically, AL is linked to multiple risk factors: male gender, smoking, obesity, high American Society of Anesthesiologists (ASA) scores, low rectal anastomosis, advanced cancer, emergency surgery, increased blood loss, corticosteroid use, and prolonged operation time[186–193]. While regression analyses have identified these factors, their mechanistic links to AL are often discussed macroscopically. In reality, AL arises from molecular-level changes and pathway dysregulation.

ROS/NO-mediated immune-inflammatory attack

Damaged cells release inflammatory mediators, inducing ROS/NO, leukocyte adhesion, accumulation, and complement activation, which exacerbate apoptosis and impair healing[15]. Reduced blood flow correlates with AL incidence[194–196]. Studies have shown that indocyanine green fluorescence angiography can reduce the incidence of AL[197].

Ischemia-reperfusion injury reduces energy production, triggering calcium influx and cell damage – an important pathway enhancing the effect of ROS/NO in immune cells[198]. Factors like increased blood loss, emergency surgery, and prolonged operation time can cause transient hypoperfusion, leading to ischemia-reperfusion injury (Fig. 2). In low anterior resection, ligation of the superior rectal artery immediately leads to a significant reduction in blood supply to the rectal stump and anastomotic area[199]. Ligation of the superior rectal artery in low anterior resection reduces blood supply, decreasing tissue

Table 3

Metabolites of the intestine and their regulatory effects

Metabolite	Microbiota	Function
Acetate[165]	*Bacteroidetes, Prevotella* spp., *Akkermansia muciniphila*	Modulating IEC-immune cell interactions.
Propionic acid[166,167]	*Akkermansia muciniphila, Bacteroides, Lachnospiraceae, Acidaminococcus* spp.	Binding to GRP43 on IECs, upregulating tight junction proteins occludin and ZO-1 while enhancing mucin levels.
Butyrate[159,168,169]	*Firmicutes*	Binding to GRP41 on IECs, stimulating collagen production while maintaining mitochondrial function.
L-Trp[170]	*Clostridium, Bacteroides, Lactobacillus, Bifidobacterium*	Affecting the number of colonic FOXP3+ Treg cells and local immune homeostasis.
SAA[171–174]	-	Activating the pTh17 program and enhancing IL-17 production.
Polysaccharide A[175,176]	*Bacteroides fragilis*	Triggering TLR2 on Tregs.
DCA[177,178]	*Clostridium scindens*	Downregulating CFTR expression to reduce the proliferation and migration of IECs.
UDCA[105,179]	*Clostridium baratii*	Promoting IECs migration and reducing the release of inflammatory cytokines.
Arginine[180–182]	*Bacillus megaterium*	Precursors for NO synthesis, anti-inflammatory effects, and promoting collagen synthesis.
PGE2[183–185]	-	Promoting proliferation and migration of fibroblasts and IECs, facilitating the differentiation of M2 macrophages, and inhibiting the differentiation of Treg cells.

DCA, deoxycholic acid; GRP43, G-protein coupled receptor 43; IECs, intestinal epithelial cells; L-Trp, L-Tryptophan; MMPs, matrix metalloproteinases; PAMPs, pathogen-associated molecular patterns; SCFAs, short-chain fatty acids; UDCA, ursodeoxycholic acid.

oxygen tension, which may explain higher low rectal AL rates[200]. Additionally, injury to the marginal arterial arch has a more direct impact on oxygenation in the anastomotic area and causes AL through a clearer mechanism of intestinal ischemia.

The coagulation function, vascular status, and thrombosis risk are also preoperatively critical factors affecting ischemia-reperfusion. Low-molecular-weight heparin (LMWH) is widely used as the standard drug for thrombosis prevention in the perioperative period of colorectal surgery. LMWH also has pleiotropic effects, including regulating immune function, improving endothelial function, and positively influencing vascular remodeling during tissue repair[201,202]. However, preoperative therapeutic anticoagulation is often used in patients with valvular heart disease, coronary heart disease, and coagulation disorders, who have a higher risk of poor vascular perfusion[203]. For example, aortic calcification, as a marker of systemic arteriosclerosis, requires long-term preoperative anticoagulation therapy[204]. However, warfarin anticoagulation therapy significantly increases the risk of bleeding complications in colorectal surgery[205]. For these patients, perioperative anticoagulation management becomes a more complex issue. The impact of diabetes on anastomotic healing primarily occurs through microvascular lesions. Diabetes leads to hyaline degeneration of small arteries, thickening of the basement membrane, and narrowing of the vascular lumen, which severely affects the microcirculation and tissue perfusion[206].

Disruption of collagen remodeling

Bacterial translocation is an important factor in the spread of anastomotic inflammation, and its mechanism is closely related to barrier function disruption, enhanced microbial virulence, and host immune imbalance. Among these, the occurrence of micro-leaks at the anastomosis directly leads to bacterial translocation. Excessive tension at the anastomosis can cause excessive tissue traction, mechanically damaging the integrity of the anastomosis and making intestinal fluid more likely to leak from the anastomosis. The surrounding tissue can be rapidly infected by bacteria, ultimately leading to delayed wound healing[207]. During low rectal anastomosis, the tension at the anastomosis is greater due to the limited mobility of the intestinal tract.

Surgical trauma induces virulence phenotype remodeling of the microbiota, exacerbating inflammation. Injury-released molecular signals (opioids, cytokines, ischemic byproducts) can trigger genetic mutations in *Pseudomonas aeruginosa*, promoting virulent strains with enhanced collagen-degrading activity[208,209].

The primary goal of prophylactic antibiotic use is to reduce the incidence of postoperative infections and AL[210,211]. However, inappropriate antibiotic intervention can enhance bacterial virulence. Studies show that antibiotics used in colorectal surgery, especially second- and third-generation cephalosporins, not only fail to eliminate *Enterococcus faecalis* in the gastrointestinal tract but also promote its growth and persistence[212–214]. Recent investigations have identified that *C. difficile* can predominate in anastomotic tissues and produce tissue-degrading collagenases despite antibiotic treatment. Interestingly, extended antibiotic administration suppresses commensal organisms that naturally inhibit the virulence expression of *C. difficile* and other potential pathogens[215].

Collagen formation failure

M2-type macrophages play a role in promoting collagenase production, and excessive aggregation of M2 cells may disrupt the balance of anastomotic tissue remodeling. Apoptotic cells induced by factors such as ROS/NO can promote the differentiation of M0 cells into M2 cells. Risk factors related to ROS/NO have been discussed above. Additionally, poor nutritional status impairs postoperative wound healing. Malnourished patients exhibit decreased wound healing capacity, characterized by reduced collagen synthesis, limited fibroblast proliferation, and delayed epithelialization[216]. Protein deficiency directly affects collagen synthesis – collagen, as a peptide substance, is primarily composed of glycine and proline, synthesized through intracellular and extracellular mechanisms of fibroblasts[216]. Studies on arginine supplements have shown that L-arginine can improve collagen synthesis after traumatic blood loss and enhance wound healing quality[217]. Furthermore, zinc, an essential trace element in the human body, plays a key role in wound healing: zinc deficiency can lead to reduced fibroblast proliferation and collagen synthesis, as well as protein metabolic disorders[218]. Notably, these mechanisms remain controversial in clinical data, and the relationship between nutritional factors (such as hypoalbuminemia) and AL requires further exploration – largely because AL is a complication triggered by the interaction of multiple factors[219,220].

Although glucocorticoids have powerful anti-inflammatory effects and can inhibit the production of inflammatory mediators (such as leukotrienes and prostaglandins), the intact healing of tissues is the core to prevent AL. High doses of glucocorticoids can cause nonhealing of anastomotic wounds and secondary infections by promoting protein catabolism and inhibiting protein synthesis[221].

Composite factors

The role of neoadjuvant radiotherapy as a risk factor for AL remains controversial[3,190,194,222–224]. Most patients receiving radiotherapy have middle-low rectal cancer, with higher anastomotic tension. Furthermore, neoadjuvant radiotherapy mainly uses high-energy rays to target tumors and surrounding normal tissues, leading to DNA damage and cell death[225,226]. Radiation damages the endothelial cells of small arteries in the intestinal submucosa, which may develop obliterative vasculitis after vascular injury, obstructing the supply of nutrients and oxygen to intestinal cells[227,228]. Radiotherapy can also activate the NF-κB signaling pathway, trigger inflammatory responses[229–231], and induce the production of 、 ROS, leading to oxidative stress[229,232].

The impact of obesity on postoperative AL remains a controversial topic. Studies have shown that obesity may be a risk factor for low colorectal AL[3]. The presence of adipose tissue complicates surgical field exposure, making precise anatomical operations more difficult, thereby increasing surgical trauma and anastomotic tension[233]. Adipose tissue has a relatively poor blood supply, leading to an insufficient wound blood supply, prone to liquefactive necrosis, and becoming an infection focus. Additionally, obese patients often have comorbidities such as cardiovascular, cerebrovascular diseases, or glucose-lipid metabolic disorders, which are unfavorable for wound healing.

Male gender is considered a risk factor for low rectal AL[234]. The male pelvis' deep, funnel-shaped anatomy narrows the

pelvic cavity, challenging intestinal manipulation during low anastomosis and increasing tension at the suture site. Additionally, male patients often have smoking-related risks[235]. Smoking enhances reactive ROS production while inhibiting antioxidant enzymes, inducing oxidative stress that disrupts healing[236,237]. Nicotine further impairs endothelial function by reducing NO bioavailability and increasing superoxide, compromising wound perfusion via vasoconstriction[237].

Furthermore, patients with a high ASA score or advanced tumor stage often have the combined effect of multiple unfavorable factors, such as malnutrition and chronic inflammation[186,189]. Therefore, the anastomosis in such patients is often more difficult to heal.

New tools and methodologies for diagnosing AL

AL is generally detected between POD5 and 8. The diagnostic assessment of AL primarily depends on the characteristic clinical symptoms (e.g., severe abdominal pain, high fever, increased heart rate, respiratory rate, and peritonitis), imaging findings, and laboratory examinations[238,239]. Early diagnosis of AL significantly reduces both the length of hospital stay and associated healthcare expenditures. Therefore, developing an early and accurate diagnosis of AL can benefit postoperative management and long-term patient prognosis and should be prioritized in surgical quality improvement initiatives.

Current laboratory diagnosis for AL typically relies on white blood cell count and C-reactive protein (CRP) levels (Supplementary Digital Content Table 1, available at: http://links.lww.com/JS9/E849). However, several meta-analyses on CRP and procalcitonin (PCT) have shown significant differences in their ability to predict AL accurately[240–243]. Elevation of systemic inflammation markers CRP and PCT is common in postoperative colorectal cancer patients, offering limited specificity for early diagnosis[242,244]. Additionally, using these markers as primary diagnostic indicators presents challenges in distinguishing AL from other postoperative infections.

Etiologically, biomarkers upstream of inflammation pathways are more closely linked to AL. Thus, using microbiological and immunological markers at the anastomotic site appears promising for future research. The most commonly used sample source is drainage fluid, which can not only reflect the local conditions of the anastomosis but also detect various biomarkers, including inflammatory cytokines, MMPs, and metabolites. However, current biomarkers generally show low early diagnostic accuracy (Table 4). At the same time, the accuracy of early diagnosis may gradually increase over time, and samples collected on POD3 have higher reference values than those collected on POD1[245–247]. Additionally, the selection of samples for detection is not limited to drainage fluid; studies have also used urine or feces[99,239,248–251].

While early detection is advantageous for enhancing patient outcomes, there are currently no viable biological markers that can successfully identify AL before the appearance of clinical symptoms. With the advancement of technology, the development of omics has helped illustrate the comprehensive landscape and provides clues for detecting biomarkers[214].

Summary and outlook

As a key regulator of intestinal barrier integrity, intestinal immunity occupies a central position in anastomotic healing and the pathogenesis of AL. However, due to the dynamic nature of immune–microbial interactions, their specific roles in AL may vary across different clinical scenarios. For instance, they exert protective effects during physiological healing processes but promote destructive inflammation under conditions of ischemia or infection. Therefore, it is necessary to deeply dissect the mechanisms by which intestinal immunity and microbiota regulate anastomotic healing, laying a foundation for targeted therapeutic interventions.

Currently, immune–microbial information at the anastomotic site may provide guidance for identifying high-risk patient subgroups. The potential of immune–microbial signatures as biomarkers for AL is increasingly recognized, and their analysis is expected to become an effective tool for early diagnosis. Future studies should prioritize the identification of comprehensive signatures, integrating immune cell subsets (such as regulatory T cells and macrophages), cytokine profiles (such as IL-6 and TNF-α), and microbiota, to determine optimal diagnostic combinations with high sensitivity and specificity.

Nevertheless, translating mechanistic research findings into clinical practice still faces numerous challenges, largely due to the unclear association between clinical risk factors of AL and microlevel mechanisms in the eyes of clinicians. With the deepening understanding of intestinal immunity and microbiota, future work is likely to focus on developing microbiota-targeted interventions and immune-modulatory strategies to prevent the occurrence of AL.

In summary, this is the first study to comprehensively depict the bidirectional effects of intestinal immunity and microbiota on anastomotic healing. Future research should focus on the dynamics of immune–microbial interactions in AL and explore strategies to translate these research findings into clinical practice, ultimately achieving more precise intervention and early diagnosis measures.

Table 4

Biomarkers of local inflammation in early diagnosis (before POD4) of anastomotic leakage

Reference	Biomarkers	Number of patients	Sample	POD	Cutoff	Sensitivity	Specificity	AUC
Qi XY[252], 2023	IL-6	157	Drainage fluid	3	26,430.09 pg/ml	0.86	0.55	0.73
Shi J[239], 2022	IL-1 β , IL-6, IL-10, TNF- α , MMP2, and MMP9	119	Peritoneal drainage	1	-	0.90	0.70	0.88
Yang L[250], 2013	pH	753	Pelvic drainage	3	6.798	0.99	0.95	-
Cuff SM[251], 2023	IL-1 β , IL-6, IL-8, IL-10, TNF- α and CRP	42	Peritoneal drainage	2	-	0.75	1.00	-
Komen N[214], 2014	*Enterococcus*	243	Peritoneal drainage	2	-	0.71	0.76	-
	faecalis	243	Peritoneal drainage	3	-	0.76	0.71	-

AUC, area under curve; CRP, C-reactive protein; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9n; POD, postoperative day.

Ethical approval

Not applicable.

Consent

Not applicable.

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Author contributions

K.M. contributed to the writing of the manuscript. T.G. was responsible for the revision of the article and the creation of the figures. Prof. Z.W. and Prof. Z.L. proposed study questions, designed the review structure, and reviewed supporting literature for accuracy. All authors contributed to the content of the manuscript, and reviewed, edited, and approved the final draft.

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None.

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