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Review Article

Environmental and Microbial Factors in Inflammatory Bowel Disease Model Establishment: A Review Partly through Mendelian Randomization

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Inflammatory bowel disease (IBD) is a complex condition resulting from environmental, microbial, immunologic, and genetic factors. With the advancement of Mendelian randomization research in IBD, we have gained new insights into the relationship between these factors and IBD. Many animal models of IBD have been developed using different methods, but few studies have attempted to model IBD by combining environmental factors and microbial factors. In this review, we examine how environmental factors and microbial factors affect the development and progression of IBD, and how they interact with each other and with the intestinal microbiota. We also summarize the current methods for creating animal models of IBD and compare their advantages and disadvantages. Based on the latest findings from Mendelian randomization studies on the role of environmental factors in IBD, we discuss which environmental and microbial factors could be used to construct a more realistic and reliable IBD experimental model. We propose that animal models of IBD should consider both environmental and microbial factors to better mimic human IBD pathogenesis and to reveal the underlying mechanisms of IBD at the immune and genetic levels. We highlight the importance of environmental and microbial factors in IBD pathogenesis and offer new perspectives and suggestions for improving experimental animal modeling. Our goal is to create a model that closely resembles the clinical picture of IBD. **(Gut Liver 2024;18:370-390)**

Key Words: Inflammatory bowel disease; Environmental factor; Microbiota; Gastrointestinal microbiome; Mendelian randomization analysis

INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a global health problem with increasing incidence in recent years.[1,2] Current researches suggest that IBD results from a complex interaction between environmental factors (such as diet, lifestyle habits, air pollution, additives, drugs, etc.), microbial factors (such as changes in intestinal microbiota composition, function and metabolites), immune system and gene expression, leading to intestinal inflammation.[3-10] However, the environmental factors and microbial factors are still uncertain to be the cause or result of IBD.

Animal models are widely used and useful tools for studying the pathogenesis of IBD. Researchers can manipulate environmental factors by controlling variables, microbial factors by fecal transplantation, immune mechanisms by cell transfection, and genetic factors by gene engineering in animal models. However, the animal models induced by dextran sulfate sodium (DSS) or trinitrobenzenesulfonic acid (TNBS) only cause colonic epithelial barrier disruption and inflammatory injury that resembles colitis more than IBD, remaining a gap between the models with real human IBD environment.

Mendelian randomization is a method that uses natural experiments or instrumental variables based on genetic vari-

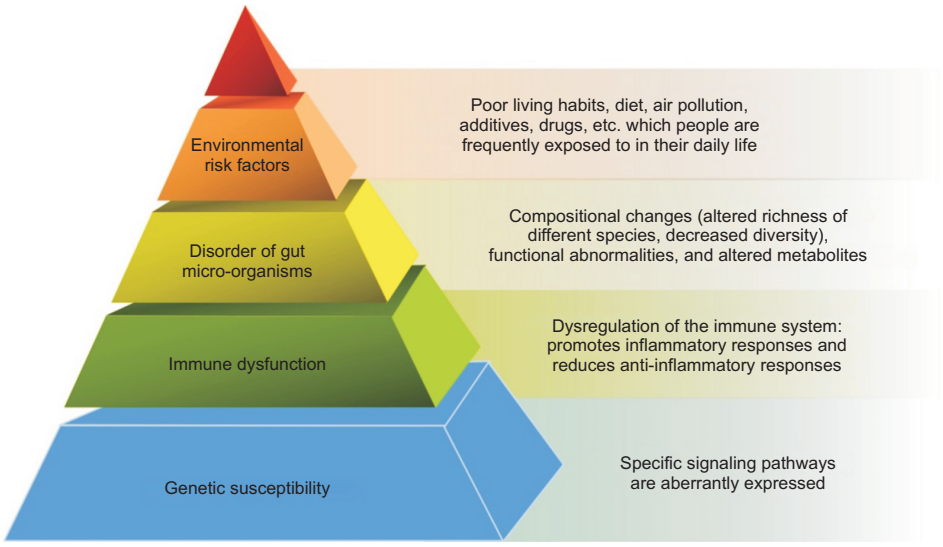

Fig. 1. Factors, including environment, intestinal microecology, genes, and immunity, involved in inflammatory bowel disease pathogenesis.

ation, which can be of a great deal of help to determine the relationship between those risk factors and IBD, providing a reference for us to select environmental and microbial factors that can be incorporated into establishing animal models.[11] It can help answer causal questions about how changing exposures can affect different outcomes. The method relies on the assumption that genetic variants only influence the outcome through the exposure, and avoids the bias of reverse causality and largely eliminates confounding.[12,13]

Aspired by Mendelian randomization, we propose that in order to create the more realistic and reliable animal models of IBD, we also consider both environmental and microbial factors, based on the existing evidence of their roles in IBD pathogenesis. A possible way to devise a model that mimics the actual human situation of IBD is to introduce several environmental and microecological risk factors in both the control and experimental groups.

In this review, we will summarize the current knowledge on the roles and interactions of environmental and microbial factors in IBD pathogenesis. The current methods for creating animal models of IBD will be evaluated for their strengths and weaknesses. Our goal is to enhance our understanding of IBD by using animal models that closely resemble the actual condition of IBD (Fig. 1).

ENVIRONMENTAL RISK FACTORS IN IBD

1. Diet

1) Diet structure

Many studies have shown that a high-fat diet increases the inflammation of the intestinal epithelium.[14-16] Among the different types of fatty acids, most unsaturated fatty acids have a protective effect against IBD, but n-6 polyunsaturated fatty acid, a kind of unsaturated fatty acid, increases the risk of UC.[17,18] Studies showed that high-fat diet can increase the level of saturated fatty acids, which can alter the host bile acid composition and disturb the intestinal microbiota, leading to inflammatory injury in IBD-susceptible mouse models.[19] Moreover, high-fat diet and antibiotics can have a synergistic effect on disrupting the intestinal microbiota and causing inflammation of the intestinal epithelium in mouse models.[20]

Another dietary component that is related to IBD is protein. Excessive intake of protein, especially animal protein (red meat), is a risk factor for IBD.[21] This may be explained by the fact that meat contains high amounts of iron, sulfur-containing amino acids and fat, which can induce inflammation of the intestinal epithelium in animal models.[19,22-24] However, reducing animal protein intake in the diet does not seem to have a significant impact on the course and severity of CD.[25]

High-sugar diet, especially high fructose diet (HFrD), can also induce pro-inflammatory changes similar to IBD in mice.[26] HFrD can increase the contact between intestinal microbes and colonic mucosa by reducing colonic mucosal thickness and altering colonic mucus quality. HFrD can also affect the composition and metabolism of intestinal microbes, such as reducing beneficial symbiotic bacteria and microbes that express bile salt hydrolase, and increasing lumen-bound bile acids.[27] In addition, fructose can directly impair the integrity of colonic cells by reducing the expression of tight junction proteins and increasing cell permeability.[28]

Dietary fiber can modulate the intestinal environment by providing substrates for microbial growth, allowing microbial species to utilize these substrates and expand their populations.[29] Therefore, low dietary fiber intake may

result in the loss of specific bacterial species in the digestive system,[30] which may contribute to the development of chronic inflammatory diseases such as IBD. In mice colonized with human microbiota, low intake of microbiota accessible carbohydrates, a type of dietary fiber, led to a significant reduction in microbial diversity within three generations, and this effect was not reversed by restoring normal microbiota accessible carbohydrate intake.[31]

2) Microelements

Zn^{2+} deficiency impairs the integrity of the intestinal epithelial cell barrier and increases its osmotic pressure, leading to intestinal epithelial injury in IBD.[32,33] A population-based study found that patients with CD and UC had significantly lower levels of iron, selenium and manganese than healthy controls.[34] In mice, Mn deficiency aggravated colitis induced by DSS, and Mn-deficient mice showed damage to intestinal tight junctions and increased intestinal permeability. Supplementing Mn improved the tolerance to colitis.[35] Selenium deficiency was observed in 30.9% of IBD patients.[36] Selenium and selenoproteins are important for counteracting oxidative stress, dampening inflammatory signaling pathways and enhancing anti-inflammatory M2 macrophage numbers. Low selenium levels may worsen the inflammation.[37] Aluminum can induce intestinal inflammation through various mechanisms, such as triggering inflammatory response to bacteria, affecting epithelial cell renewal and claudin expression, disrupting the intestinal barrier and promoting granuloma formation. Oral aluminum exacerbated intestinal inflammation in mice induced by 2,4,6-TNBS or DSS, as well as chronic colitis in interleukin 10-deficient (IL-$10^{-/-}$) mice.[38] Vitamin D deficiency reduces the number and function of regulatory T cells, which increases the susceptibility to CD and aggravates its symptoms.[39,40]

3) Emulsifiers

Processed foods often contain synthetic emulsifiers that help mix oil and water. However, these substances can harm the gut by changing its bacterial composition.[41] For example, when researchers added polysorbate 80 to a model of human gut bacteria, they found that the diversity of bacteria decreased by half and the expression of genes related to bacterial adhesion and invasion increased.[42] These genes can trigger inflammation in the gut lining.

Another substance that can affect gut bacteria is carrageenan, which provides sulfur for bacteria that produce hydrogen sulfide (H_2S), such as *Bilophila wadsworthia*. H_2S can damage DNA and cause inflammation in the colon.[14]

Maltodextrin is another substance that can influence gut bacteria. It is a modified starch molecule that can increase the formation of *Escherichia coli* biofilms in mice.[43] Biofilms are sticky layers of bacteria that can attach to surfaces and resist antibiotics. People with CD, a type of IBD, have higher levels and activity of a gene that breaks down maltose, a sugar related to maltodextrin, than healthy people.[44]

Some food additives and drugs contain nanoparticles, which are very small particles. In animal models, orally administered nanoparticles have been shown to accumulate in the intestinal epithelium, activate NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome and increase intestinal inflammation.[45]

2. Air pollution

In mice models, short-term exposure to certain particles in the air will lead to increased intestinal osmotic pressure and hyperactive innate immune response; long-term exposure to these particles will significantly increase the expression of inflammatory cytokines and change the composition of intestinal microbiota.[46]

There are different opinions on how various types of particles in the air affect IBD. Exposure to O_x in childhood will increase the risk of IBD;[47] epidemiological evidence shows that CD incidence is high in areas with high NO_2 concentration in the air, and UC incidence is high in areas with high SO_2 concentration in the air, suggesting that NO_2 and SO_2 are involved in the inflammatory response process of CD and UC respectively.[48] Exposure to air pollutants ($PM_{2.5}$, O_3, and CO) may increase the risk of IBD, and the most sensitive season is mainly in warm seasons, and the impact on CD is more obvious than on UC.[49] PM_{10} is a highly complex mixture of elements and organic carbon, metals, sulfates, nitrates and organic pollutants. Short-term treatment with PM_{10} changed the expression of immune genes in wild-type mice, enhanced the secretion of pro-inflammatory cytokines in the small intestine, increased intestinal permeability, and induced hyporesponsiveness of splenocytes. Long-term treatment of wild-type and IL-$10^{-/-}$ mice increased the expression of pro-inflammatory cytokines in the colon and changed the concentration and composition of short-chain fatty acids (SCFAs). The disease of IL-$10^{-/-}$ mice increased, manifested as enhanced histological damage.[46]

The promotion or reduction of mucosal inflammation by H_2S depends on its concentration. When H_2S comes from endogenous metabolism, H_2S can maintain the integrity of the mucus layer, but when intestinal microorganisms produce excessive H_2S, H_2S can inhibit colon epithelial cell proliferation by reducing mitochondrial ATP production, because it inhibits complex IV in mitochondrial respiratory chain, and interferes with various signaling pathways in colon cells.[50]

Many studies have shown that reactive oxygen species may play a pathogenic role in IBD.[51] Exogenous sources, also known as exposures, include ultraviolet radiation, chemotherapy drugs, environmental toxins, ionizing radiation, inflammatory cytokines, etc.[52] And endogenous sources include mitochondria, lipoxygenase, NADPH oxidases, peroxisome, cytochrome p450, which have high oxygen consumption.[53] Moderate production of reactive oxygen species is beneficial for humans.[54] Excessive production of reactive oxygen species induces oxidative damage and disrupts homeostasis.[55]

3. Smoking

It has been reported that nicotine may modulate the pathogenesis of IBD by stimulating DNA damage in intestinal cells, inducing microflora dysbiosis and increasing susceptibility to infection, enhancing the likelihood of epigenetic changes and modulating the intestinal immune response.[56] For instance, noncoding microRNAs pathways may account for part of the epigenetic mechanism, where the upregulation of miR-124 is considered to have an important anti-inflammatory role.[57,58] Smoking can alter the intestinal microecology to some degree. It is a risk factor for CD patients, but a protective factor for UC patients. Smoking cessation may result in an increased incidence of UC.[3] A possible mechanism that could account for this phenomenon is that nicotine worsens *Mycobacterium avium* subspecies paratuberculosis infection in macrophages of CD patients, causing a shift to M1 polarization and excessive production of pro-inflammatory cytokines. On the other hand, in UC, nicotine activates cholinergic pathways via α7nAChR, causing a shift in macrophage polarization to M2 and an increase in IL-10, and conferring anti-inflammatory effects such as a reduction in the levels of pro-inflammatory cytokines such as IL-6, IL-12, and tumor necrosis factor-α.[59]

4. Drugs

Some drugs can harm the gut by affecting its function or microbiota. For example, non-selective cyclooxygenase inhibitors in nonsteroidal anti-inflammatory drugs can reduce protective prostaglandins such as prostaglandin E and increase intestinal osmotic pressure and mucosal damage. Both short-term and long-term use of nonsteroidal anti-inflammatory drugs increases the risk of IBD.[3]

Proton pump inhibitors, which reduce stomach acid production, also increase the risk of IBD and its subtypes when used for a long time.[60]

Antibiotics can change the diversity, richness, and evenness of intestinal microbiota in healthy people, making them more susceptible to inflammatory damage.[3,61] A meta-analysis showed that most antibiotics were associated with IBD, especially metronidazole and fluoroquinolones.[62] Triclosan and triclocarban, which are antibacterial chemicals in many products, can worsen colitis in mice by altering intestinal microbiota.[63]

Oral contraceptive pills contain estrogen, which has anti-inflammatory and immune-mediated effects.[64] However, oral contraceptive pills also increase the risk of IBD, especially CD and UC.[65]

5. Surgery

1) Tonsillectomy

Some surgical procedures can affect the risk of IBD by removing or altering parts of the body that are involved in immune function or microbiota. For example, tonsillectomy, which removes the tonsils, may also influence the development of IBD by affecting immune function or microbiota. A meta-analysis showed that tonsillectomy was positively correlated with CD development, but no evidence was found that tonsillectomy had a protective effect on UC development.[66]

This can be explained by some known findings. First, $CD4^+FOXP3^+$ Treg cells constitute an important T cell compartment in palatine and lingual tonsils,[67] $CD4^+CD25^+$ Treg cells play a key role in maintaining tolerance by inhibiting activation and proliferation of autoreactive T cells.[68] Powrie *et al.*[69] demonstrated that transfer of $CD4^+CD25^+$ Treg cells could prevent the development of IBD and even reverse established gastrointestinal inflammation in IBD animal models. Therefore, tonsillectomy involving depletion of tonsillar Treg cells may play a role in CD development. Second, tonsillectomy may alter gut microbiota in CD development by affecting bacterial invasion or colonization in the gut. It is also possible that tonsillectomy affects IBD development by acting as a surrogate marker for hygiene conditions or antibiotic use.[66]

2) Appendicectomy

Appendectomy has a complex and inconsistent relationship with IBD. A meta-analysis showed that appendectomy increased the risk of CD by 61%, but this risk declined to baseline levels after 5 years or more,[70] it can even become a protective factor of IBD after this long time.[71] However, some studies suggested that appendectomy for appendicitis had a protective effect against UC, especially in patients under 20 years old.[72-74] A study in Asian populations showed that appendectomy increased the incidence and risk of both CD and UC, regardless of appendicitis.[75] The appendix may act as a reservoir of gut bacteria that regulate immune response to host microbiota.[76] It may also serve as a biofilm inoculant for commensal microbiota in the prox-

imal colon and terminal ileum. The appendix can balance pro-inflammatory and anti-inflammatory responses in the gut and maintain homeostasis.[77] More research is needed to determine whether changes in appendix or surrounding gut microbiota or related immune responses are involved in mediating these risks.

A mouse model of appendectomy after appendicitis was proposed by Cheluvappa[78] as a unique experimental model to study the effects of appendectomy on colitis. This model showed that appendectomy protected mice from experimental colitis in a way that depended on their age, bacteria, and antigen exposure. Appendectomy reduced T helper cell 17 (Th17) cell activity, autophagy, interferon activity and endothelin-vascular activity in the colon, which are all involved in inflammation and immune response. These changes reduced colitis pathology in the mice.[79-81]

GUT MICROORGANISMS IN IBD

1. Composition and function of normal intestinal microorganisms

The disruption of the gut microbiome is associated with various diseases, such as IBD, colorectal cancer and metabolic disorders. The most abundant bacteria in the human gut microbiome are *Firmicutes* and *Bacteroidales*, while the less abundant ones vary greatly among different individuals. The ratio of *Firmicutes* and *Bacteroidales* is abnormal in many diseases. The main functions of the gut microbiome include digestion and absorption of nutrients, regulation of host metabolism, immune system development and function, prevention of pathogens, and gut-brain axis modulation.[7,82]

Fungi account for about 0.1% of the total microbes and can survive on any mucosal surface of the human body. There are about 160 species of fungi in the human body, among which *Candida* species dominate, such as *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis*. Fungi can be affected by environmental factors, mainly diet, especially carbohydrate (sugar) intake, and long-term antibiotic use can induce fungal infection. Fungal abnormalities can induce IBD. The body has a specific anti-fungal cell wall immune response such as Toll-like receptors, complement system, and so on. These immune functions are abnormally activated and cause damage to the intestinal epithelium, leading to the occurrence and development of IBD.[83-85]

Viruses (mostly phages) infect bacteria in the gut and integrate their genetic material into the bacterial genome or exist as extrachromosomal plasmids, thus participating in the formation and regulation of the gut microbiome. Due to the strong evolutionary ability of viruses, there is a large difference in the gut viral community among individuals, but the difference in the viral community at different times within the same individual is small, indicating that it has a certain stability. The human gut viral community may also be regulated by environmental factors such as diet, similar to other gut microbial communities.[83,86,87]

There are quite complex interactions between the microbial communities of bacteria, archaea, parasites, viruses and fungi in the gastrointestinal tract and health or disease states. The relationship network contained therein still needs researchers to further explore and understand.

2. The disorders of intestinal bacteria in IBD

The gut microbiome of IBD patients has abnormal changes, and the resilience of the gut microbiome of IBD patients is significantly reduced.[88] The changes can be divided into three aspects: changes in the composition of gut microbes (including microbial abundance and diversity), changes in the function of gut microbes and changes in the metabolites of gut microbes.

Common changes in microbial abundance associated with IBD include an increase in facultative anaerobes such as *E. coli* and a decrease in obligate anaerobic SCFA producers. Other changes may not have individual differences and are not common in all IBD patients.

Increased abundance of pathogens includes *Enterobacteriaceae, Pasteurellaceae, Veillonellaceae, Proteobacteria, Fusobacteriaceae, Bifidobacterium bifidum,* and *Lactobacillus lactis* in both UC and CD, whereas *Bacteroides fragilis* in CD patients.

Decreased abundance of probiotics include *Firmicutes, Clostridiales* (especially clusters IV and XIV disappear almost completely), *Erysipelotrichales*, butyrate-producing bacteria, *Lachnospiraceae* and *Ruminococcaceae* in CD, *Bacteroidales* in UC. In CD patients, beneficial bacteria *Faecalibacterium prausnitzii* and *Roseburia intestinalis* are significantly reduced.[5,89-91]

Blautia, Ruminococcus are key bacterial groups in CD and UC.[5] *Blautia* is a symbiotic obligate anaerobe that plays an important role in maintaining intestinal ecological balance and preventing inflammation by upregulating regulatory T cells in the gut and producing SCFAs.[92]

Ruminococcus can degrade and convert complex polysaccharides into SCFAs and other nutrients for their hosts. In CD, *Ruminococcus* synthesizes and secretes a complex polysaccharide glucorhamnan with a rhamnose backbone and glucose side chains, which can effectively induce dendritic cells to secrete inflammatory cytokine tumor necrosis factor-α.[93] *Faecalibacterium* is a genus of anaerobic bacteria that belongs to the family Ruminococcaceae, which is

one of the important producers of butyrate, which has anti-inflammatory effects, maintains the activity of bacterial enzymes, and protects the digestive system from intestinal pathogens.[94]

Compared with healthy individuals, the gut microbial diversity of IBD patients is lower. The imbalance of gut microbes causes damage to intestinal epithelial cells, increases intestinal permeability and disrupts mucosal integrity. However, it is not clear whether the decrease in gut microbial diversity is a cause or a consequence of IBD.[95,96]

The functional disorder of the gut microbiome in IBD patients will cause a series of reactive changes in the body, mainly including the reduction of SCFAs and amino acids synthesized by microbes, increased oxygen consumption, increased sulfate transport, and malnutrition.

Among them, SCFAs are an important energy source for intestinal epithelial cells and can activate signaling cascades that control immune function through cell surface G protein-coupled receptors such as GPR41, GPR43 and GPR109A. They can protect the integrity of the gastrointestinal mucosa. After the function of the microbiome changes, the SCFAs synthesized by the microbes decrease, leading to impaired intestinal mucosal protection mechanism and abnormal immune function. These changes are strongly correlated with IBD.[97,98]

The functional disorder of the gut microbiome is also related to the expression of some specific genes, which increases the susceptibility to IBD. Many genes associated with IBD are related to immune function, especially the interaction between the immune system and microbes,[99,100] such as nucleotide oligomerization domain 2 (NOD2), autophagy-related 16-like 1 (ATG16L1), caspase recruitment domain-containing protein 9 (CARD9), and C-type lectin domain family 7 member A (CLEC7A).[5]

Among them, NOD2 expressed in intestinal epithelial cells is involved in expressing a defense factor against intracellular bacteria that can promote immune response to common pathogenic microbes. In NOD2-deficient mouse models, their microbiome changes and their ability to maintain stability decreases, and their susceptibility to colitis increases.[101]

The metabolites of feces, serum or mucosa in IBD patients are different from those in healthy populations.[102,103] Ileal CD is associated with increased tryptophan, bile acids and unsaturated fatty acids.[104,105] The levels of taurine and cadaverine in UC patients are increased, and the changes in levels of carnitine, ribose and choline are related to inflammation levels.[106]

3. The role of intestinal microbiome in IBD

In summary, gut microbes play an indispensable role in the occurrence and development of IBD. After the original balance of the gut microbiome is disrupted, it will lead to impaired intestinal mucosal protection mechanism, changes in the expression of specific genes (regulating inflammation-related genes), immune dysfunction (inhibition of inflammatory system function weakened, inflammatory response enhanced). These changes will increase the level

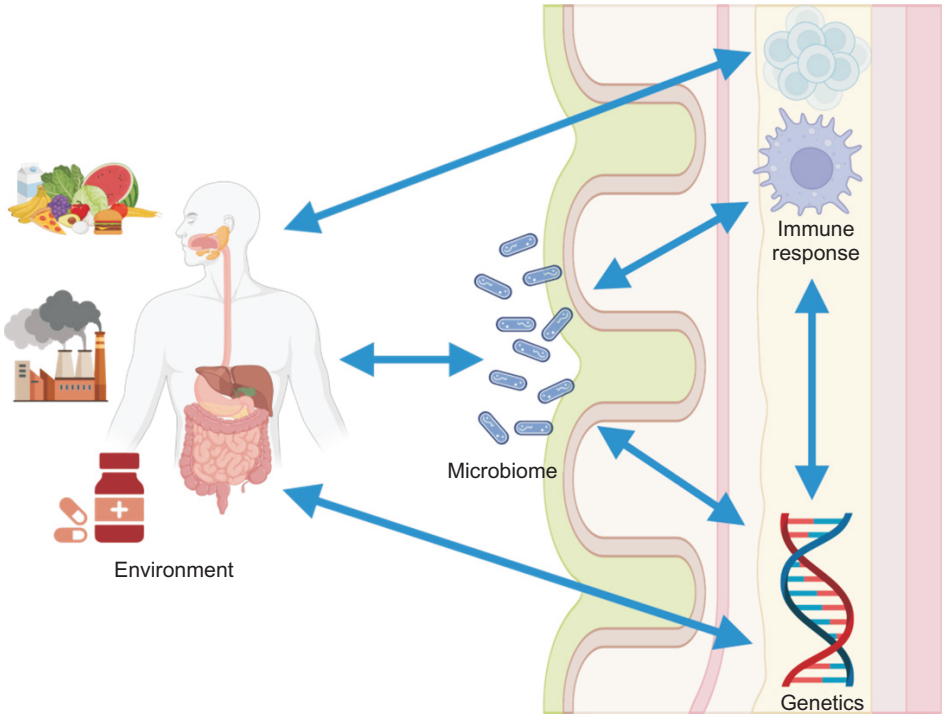

Fig. 2. The relationship between inflammatory bowel disease (IBD) and environment, genes, immune response, and intestinal microecology. Microbial factors interact with the environment, immune system and genes to participate in the pathogenesis of IBD. Therefore, when establishing the IBD animal model, we can change the intestinal flora by certain means, so that the intestinal microecology of the animal model is in a state of disorder, to better simulate the IBD of humans.

of inflammation in the intestine, aggravate inflammatory damage to the intestinal epithelium, and further lead to disease occurrence and development. In the future, the applicability and efficacy of microbial community changes for IBD treatment still need further research, and using our designed environmental factors combined with microbial factors modeling method may facilitate this direction of research (Fig. 2).

THE INTERACTION BETWEEN ENVIRONMENTAL FACTORS AND INTESTINAL MICROORGANISMS

1. Environmental factors can trigger the changes in gut microorganisms

Understanding the complex interactions among environmental factors, gut microbiota and IBD can help us prevent and treat IBD based on adjusting environmental factors. Meanwhile, we can explore and optimize the methods of modeling IBD mice based on the synergistic effects of environmental factors on microbiota. Table 1 summarizes the effects of some environmental factors with strong evidence on gut microbiota.

An increase in dietary fructose content can lead to changes in various bacterial populations, including the expansion of mucin-degrading *Akkermansia muciniphila*. *A. muciniphila* is a known mucin-degrading bacterium that is associated with colitis.[107,108] *In vitro* experiments found that fructose can directly inhibit the growth of *Lactobacillus johnsonii* and *Bifidobacterium pseudolongum*, which is parallel to the decrease in bile salt hydrolase expression, and fecal-bound bile acids levels increased significantly after HFrD feeding. Bile acids have been shown to have adverse effects on barrier function both *in vivo* and *in vitro*.[109] Experiments showed that HFrD aggravated colitis in IL-10$^{-/-}$ mic.[27]

In mice fed with a high casein diet, the relative abundance of *Bacteroidetes* increased and *Firmicutes* decreased. The study found that casein reduction was associated with increased gene expression involved in cell adhesion, such as AOCS3, LAMC1, KRT17, and DES, as well as genes related to actin cytoskeleton and intermediate filament organization. High casein diet mice had reduced expression of cell adhesion genes, increased microbiota-dependent intestinal permeability, which was related to increased microbial density, reduced microbial diversity and reduced intestinal barrier function.[110]

In an analysis of pre-IBD patients and healthy controls, it was found that a high-fat diet combined with antibiotic use increased the risk of pre-IBD by 8.6 times, suggesting

Table 1. Environmental Factors Triggering Changes to the Gut Microbiota

Environmental factors		Gut microbiota (increased bacteria)	Gut microbiota (decreased bacteria)	Host effects	Cohort/animals	Study subject
Diet	High fructose diet	*Akkermansia muciniphila*	*Lactobacillus johnsonii*, *Bifidobacterium pseudolongum*	Decreases colonic mucosal thickness and alters colonic mucus quality; alters intestinal microbial populations and metabolism; decreases tight junction protein expression and increases cell permeability.	Dextran sulfate sodium (DSS)-induced colitis	Mice
	High casein diet	*Bacteroidetes*	*Firmicutes*	Decreases in casein were associated with increased expression of genes involved in cell adhesion, such as AOCS3, LAMC1, KRT17, and DES, as well as increased genes related to the actin cytoskeleton and intermediate filament organization. reduced expression of cell adhesion genes in mice on high casein diet and increased microbiota-dependent intestinal permeability were associated with increased microbial density, as well as reduced microbial diversity and intestinal reduction in barrier function.	DSS-induced colitis	Germ-free mice, specific pathogen free mice
	Low microbiota accessible carbohydrates diet	*Clostridiales*	*Bacteroidales*	Microbiota-accessible carbohydrates abundant in dietary fiber are the main source of carbon and energy for the distal gut microbiota.	Normal	Humanized mice

Table 1. Continued

Environmental factors		Gut microbiota (increased bacteria)	Gut microbiota (decreased bacteria)	Host effects	Cohort/animals	Study subject
	High-fat diet + history of antibiotic therapy	*Escherichia coli*		High-fat diet and streptomycin treatment led to a long-term reduction in mitochondrial bioenergy in mouse colonic epithelial cells, which reduced oxygen consumption in epithelial cells thereby increasing oxygenation of epithelial cells and diffusion of oxygen into the intestinal lumen, and an increase in oxygen bioavailability leading to an increase in Enterobacteria.	Normal	C57BL/6J mice
	LFD (low-fat diet, high dietary fiber)	*Bacteroidetes*	*Actinobacteria*	LFD increases the number of mycobacteria containing the primary producers of acetate, and the microbiota metabolizes tryptophan into indole metabolites that act as ligands for the aryl hydrocarbon receptor and exert protective and anti-inflammatory effects via interleukin-22.	Ulcerative colitis	Human
	Animal-based diet	*Alistipes*, *Bilophila**, *Bacteroides*	*Roseburia*, *Eubacterium rectale*, *Ruminococcus bromii*	Animal-based diets similar to high-fat diets promote the growth of *Bilophila wadsworthia*, a sulfite-reducing bacterium whose production of H_2S is thought to cause an inflammatory response in intestinal tissues and is associated with inflammatory bowel disease.	Normal	Human
	Maltodextrin	*Salmonella**		Inhibition of host antimicrobial responses to enhance intracellular survival and mucosal colonization.	Normal	C57BL/6J mice
	Polychlorinated biphenyls (PCBs)	*Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, *Firmicutes*	*Proteobacteria*	Alterations in the gut microbiota are likely to be a novel mechanism leading to immunological alterations in response to PCB exposure, and in addition, alterations in the gut microbiota can affect PCB-induced disruption of the intestinal barrier and transfer of lipopolysaccharides to the blood stream.	Normal	C57BL/6J mice
Gas particles	PM_{10}		*Bifidobacteria*	The decrease in *Bifidobacteria* may be due to an enhanced inflammatory luminal environment in mice, such as an increase in interferon-γ and tumor necrosis factor α.	IL-$10^{-/-}$ mice model	Mice
	Smoking	*Bacteroides-Prevotella**, *Bifidobacteria*	*Faecalibacterium prausnitzii*	Smoking can alter smooth muscle tone and affect endothelial function through nitric oxide production, or affect the integrity of the intestinal mucosal barrier; mediate oxidative stress; and affect microbial populations, with *Bacteroides* exerting pro-inflammatory effects and both *Bifidobacteria* and *F. prausnitzii* enhancing immunomodulatory pathways.	Crohn's disease	Human
Drugs	Ciprofloxacin (Cp)		*Bacteroides dorei*, *Akkermansia muciniphila*, *Roseburia*	The observed response to Cp may include direct effects due to intrinsic or acquired resistance of the strain to Cp, as well as indirect effects mediated through numerous ecological interactions between microbial populations.	Normal	Human
	Clindamycin		*Bacteroides*	High levels of specific erm genes (resistance genes) can still be detected in stool samples long after the selection pressure for antibiotics has subsided.	Normal	Human
Surgery	Appendectomy	*Escherichia-Shigella**, *Veillonella**, *Klebsiella**, *Megasphaera**, *Flavonifractor**, *Ruminococcus gnavus**, *Streptococcus**	*Roseburia*, *Barnesiella*, *Butyricoccus*, *Odoribacter*, *Butyricimonas*	Changes in short-chain fatty acids producing microorganisms after appendectomy may contribute to the development of specific diseases.	Healthy appendectomy patient, healthy patient	Human

*Microbiotas that are clearly more harmful to inflammatory bowel disease.

that individuals who consume a high-fat diet and have a history of antibiotic use are more likely to develop pre-IBD. Using animal models, it was shown that mice exposed to pre-IBD risk factors, namely having a history of antibiotic treatment and being fed a high-fat diet, had increased levels of fecal calprotectin and an expansion of *E. coli* in their gut microbiota. Moreover, the mice in the high-fat diet group excreted significantly more fecal *E. coli* than the mice in the low-fat diet group.[20]

In a study of UC patients, compared with baseline, LFD (low-fat diet, high dietary fiber) resulted in a significant increase in *Bacteroides* and a significant decrease in *Actinobacteria*, indicating that dysbiosis was improved after LFD. LFD was given to patients in the baseline group and iSAD (improve standard American diet, although high dietary fiber was added, it was still a high-fat diet) group respectively. After LFD, *F. prausnitzii* increased significantly compared with iSAD. Compared with baseline, *Prevotella* increased significantly.[111] LFD increased clostridial clusters containing major acetate producers, and the composition of microbiota was also significantly correlated with acetate. Microbiota metabolized tryptophan to indole metabolites, which acted as ligands for aryl hydrocarbon receptor and exerted protective and anti-inflammatory effects through IL-22.[102] This indicates that low-fat diet can reduce inflammatory markers, correct intestinal microecological disorder to some extent, and improve symptoms of IBD patients.

Short-term consumption of a diet composed entirely of animal or plant products can change the structure of microbial communities and offset individual differences in microbial gene expression. Animal-based diets increased the abundance of bile-tolerant microorganisms (*Bilophila, Bacteroides,* and *Alistipes*), and reduced the levels of *Firmicutes* that metabolize dietary plant polysaccharides (*Roseburia, Eubacterium rectale,* and *Ruminococcus bromii*).[112] In contrast, plant-based diets led to an increase in *Firmicutes* numbers. The most increased bacterium in animal-based diets was *B. wadsworthia*, which is a sulfate-reducing bacterium whose H_2S production is thought to induce intestinal tissue inflammatory response and is associated with IBD.[19]

Maltodextrin exposure impaired intestinal epithelial cells and macrophages' ability to clear *Salmonella*. Maltodextrin exposure enhanced intracellular survival and mucosal colonization by inhibiting host antimicrobial responses, leading to the formation of new protective niches for *Salmonella.*[43] These results suggest that consumption of processed foods containing polysaccharides may lead to an increased risk of intestinal infection, which may be an environmental trigger for the development of chronic inflammatory diseases such as IBD.[113]

Oral polychlorinated biphenyls (PCBs) exposure significantly altered the abundance of gut microbiota in mice, mainly by reducing the level of *Proteobacteria.*[114] The changes in gut microbiota are likely to be a novel mechanism leading to immunological changes that are a response to exposure to PCBs. In addition, the changes in gut microbiota can affect the disruption of intestinal barrier and the translocation of lipopolysaccharide to the bloodstream caused by PCBs.[115]

Although the gut microbiota stabilized after treatment with ciprofloxacin, it still changed compared to the undisturbed original form. This was due to the fact that *Bacteroides dorei*, *A. muciniphila,* and several *Roseburia* species could not recover.[116] Another study showed that using clindamycin for 7 days resulted in a loss of diversity of *Bacteroidetes*. Even after 2 years of treatment, this situation did not recover.[117] It is not clear whether the observed effects on gut microbiota are the result of direct action of antibiotics or secondary effects (such as changes in physicochemical parameters or immune response modulation in the gut). It is also possible that the resilience of microbiota affects the response to antibiotic treatment. However, the exact mechanism of how antibiotic-induced stress perturbations of gut microbial communities translate into complex disease etiology remains to be studied.[88]

Feeding mice with PM_{10}-containing mouse feed reduced the number of *Bifidobacteria* in their feces, which have a known role in maintaining host intestinal barrier and regulating mucosal immune system.[118] PM_{10} exposure also increased serum lipopolysaccharide levels, leading to an increased proportion of intestinal microbial translocation. The disruption of intestinal barrier may be due to PM_{10} directly stimulating the immunoreactivity of basal macrophages. Therefore, early exposure to PM_{10} pollutants in the air can lead to premature occurrence of intestinal mucosal inflammation in genetically susceptible hosts and reduced bacterial clearance rate.[119]

The mechanism of smoking-related changes in microbiota is not clear. A study of active CD patients showed that smokers had a higher abundance of *Bacteroides-Prevotella* than non-smokers.[120] On the other hand, smoking cessation led to an increase in *Firmicutes* (*Clostridium coccoides*, *E. rectale*, and *Clostridium leptum*), *Actinobacteria* (*Propionibacteriaceae* and *Bifidobacteria*), and a decrease in *Bacteroidetes* (*Prevotella* and *Bacteroides*).[121] Some studies have shown that *Bacteroides* strains induce CD4 T cell-mediated colitis in animal models.[122] And in humans, IL-12-positive intestinal dendritic cells are more often coexisting with CD patients with higher *Bacteroides.*[123]

The appendix plays an important role in regulating gut

microbiota and mucosal immunity. The intestinal bacterial composition of samples from appendectomy (HwA) was less diverse than that from non-appendectomy (HwoA) samples. The abundance of *Escherichia-Shigella, Veillonella, Klebsiella, Megasphaera, Flavonifractor, Ruminococcus gnavus,* and *Streptococcus* in HwA subgroup was significantly higher than that in HwoA group, and there was a trend of recovery towards HwoA with time after surgery. The abundance of *Roseburia, Barnesiella, Butyricicoccus, Odoribacter,* and *Butyricimonas* species was lower, most of which were SCFA-producing microorganisms. Over time after surgery, *Roseburia, Butyricicoccus, Odoribacter* and *Butyricimonas* became more abundant. The abundance-related network in appendectomy samples showed more complex fungal-fungal and fungal-bacterial community interactions, and the effect on fecal fungal community was more obvious and lasting than that on bacteria.[124]

2. Gut microorganisms can alter the environmental microbes

A systematic review of environmental hygiene and IBD risk factors found that people who grew up in hygienic environments had a higher risk of IBD, such as Western horse-raising families. The bacteria in horse manure increased the microbial diversity in the air, and children may enter the gut through breathing or fecal-oral route, thus playing a protective role and not prone to IBD.[125,126]

Being in some relatively unclean sanitary conditions, such as keeping pets, contacting farming environment, sharing bedrooms, having siblings, etc., can reduce the risk of IBD. This may also be related to the increased microbial diversity in the air leading to better establishment of intestinal microecology.[125] For example, prenatal or postnatal exposure to agricultural environment may be related to bacterial colonization, such as *Acinetobacter lwoffii* and *Lactococcus lactis*, which in turn can affect Th1-mediated immune response.[127] Exposure to household dust produced by domestic dogs can increase the abundance of *L. johnsonii*, leading to reduced production of Th2-mediated cytokines, reduced activated T cells and reduced immune damage.[128]

APPLICATION OF ENVIRONMENTAL AND MICROBIAL FACTORS IN THE ESTABLISHMENT OF IBD ANIMAL MODELS

1. Mainstream molding methods

Currently, animal models for studying IBD are mainly divided into five categories: chemically induced models, cell transfer models, spontaneous models, congenital (spontaneous gene mutation) models and genetically engineered models. Since no model can fully reflect human IBD, and each type of model has its own advantages and disadvantages, a suitable modeling method is usually chosen according to the research purpose. Below we discuss some of the mainstream modeling methods in research.

1) Chemically induced models

DSS-induced colitis is currently the most widely used mouse colitis experimental model, established by administering molecular weight 40-50 kDa DSS in drinking water.[129] However, when evaluating the therapeutic effect of some candidate drugs on IBD using the DSS model, the following issues need to be considered:[130] inconsistent DSS doses (1% to 5%) and treatment times (3 to 7 days) in different protocols affect the severity of inflammation, and genetic background is a key factor determining mouse susceptibility to DSS colitis,[131] which may interact with each other when used for modeling.

TNBS-induced colitis is obtained by intrarectal injection of a mixture of ethanol and 2,4,6- TNBS, similar to chronic CD.[132] New therapies targeting IL-12 and IL-23 have been translated from TNBS mouse experiments into successful human trials, demonstrating the clinical importance of this model.[133] This model has also been shown to help understand the mechanisms by which gene mutations may cause IBD, a good example being the exploration of NOD2-deficient mice.[134] At the same time, this model is suitable for exploring possible mechanisms by which host intestinal microbiota interactions affect human diseases.[135,136] However, TNBS batches, TNBS doses, mouse strains and microbial status of animal facilities can all affect intestinal inflammation activity and thus lead to differences in response.[137]

Oxazolone colitis is caused by intrarectal injection of a mixture of oxazolone (4-ethoxymethylene-2-phenyl-2-oxazoline-5-one) and ethanol, and distal colonic inflammation is very similar to UC and can be used as a model for human UC.[138] This model reveals the role of type 2 immune responses.[139] However, clinical trials of new therapies targeting IL-13 derived from the oxazolone colitis model were not significant.[140,141] The construction of acute disease models has problems such as rapid weight loss and high mortality in mice. In addition, administration of lower doses of oxazolone results in a biphasic inflammatory response characterized by rapid death in most mice and selective survival in a few mice with mild, transient disease.[138]

2) Cell transfer models

Transfer of mouse $CD45RB^{high}CD4^{+}$T cells from healthy

donors to severe combined immunodeficiency mice induces transmembrane colitis by adoption.[130] One advantage of this model is that it allows examination of early immune events associated with intestinal inflammation, including regulatory T cell responses.[138] The role of IL-17 and IL-23 in IBD has also been well demonstrated in the CD45RB model.[142,143] However, human severe combined immunodeficiency patients have a low incidence of IBD,[144] and this model does not accurately simulate the factors that affect CD development and progression in research.

3) Spontaneous models

IL-10-deficient mice develop spontaneous small intestinal colitis characterized by progressive cellular infiltration in the cecum, colon, rectum and small intestine, with high incidence of transmural lesions and colorectal adenocarcinoma observed in 6-month-old mice. Many very early-onset IBD children have IL-10 and/or IL-10R mutations, so they are important for studying pediatric IBD.[145,146] Intestinal microbiota plays a major role in this colitis. In fact, many microbiological studies, such as probiotics, have been using IL-10 deficient mice for research.[130] But IBD patients are not always deficient in IL-10 and may not benefit from IL-10 treatment.[147]

2. Usage of environment factors in animal models

Based on Mendelian randomization studies on the relationship between environmental factors and CD and UC, it was found that smoking was positively associated with CD, but not with UC risk.[148] However, several studies failed to demonstrate a significant association between smoking characteristics (such as initiation/cessation and intensity) and UC or CD risk.[149,150] In a study on a wide range of modifiable factors for IBD, it was pointed out that smoking was associated with increased UC risk, while vegetable fruit intake, appendectomy, high vitamin D levels and other factors were protective factors for UC. Antibiotic exposure, appendectomy were associated with increased CD risk, while vegetable fruit intake, blood zinc levels, high vitamin D levels and other factors were protective factors for CD.[151] Another study on the potential causal relationship between food and IBD risk found that high intake of poultry and cereals had a significant causal relationship with CD, while high oily fish intake levels were found to have a statistically significant association with UC risk.[152]

We summarized the environmental factors that can be used to improve IBD experimental animal models (Table 2), mainly including dietary patterns, gas particles and other aspects, which have been shown to have a more definite relationship with IBD onset in clinical observations and have been studied more in depth, and the main effects on the host are also clearer. Among them, those factors that remain relevant to the risk of IBD after screening by Mendelian randomization studies are listed as recommended factors.

3. Usage of microbial factors in animal models

Thanks to the development of deep sequencing technology for the variable regions of bacterial 16S ribosomal RNA genes, we have gained a deeper understanding of the complex intestinal bacterial ecology.

The fecal extracts of IBD patients or intestinal microbiota transplanted directly into animal models can induce inflammatory responses similar to IBD in animal models. Infusion of fecal extracts from UC patients into mouse colon can cause colonic inflammation, characterized by

Table 2. Environmental Factors That Can Be Used to Improve Experimental Models of IBD

Environmental factor	Effect
Low dietary fiber diet*	Loss of specific bacterial species and significant reduction in microbial diversity
Deficiency of zinc*	Reduced intestinal barrier function, elevated osmotic pressure, and induced intestinal epithelial damage
Deficiency of vitamin D*	Reduces the number and function of regulatory T cells
Smoke exposure*	Affects the integrity of the intestinal mucosal barrier, mediates oxidative stress, and has pro-inflammatory effects
High-fat diet	Disturbed intestinal microecology induces inflammatory damage in a mouse model of genetically susceptible IBD
High sugar diet	Reduction of protective commensal and bile salt hydrolase expressing microorganisms, increase of lumen bound bile acids, disruption of barrier function, pro-inflammatory effects and aggravation of colitis in IL-$10^{-/-}$ mice
High casein diet	Increased intestinal permeability, increased microbial density, decreased diversity, and reduced intestinal barrier function
Oral aluminum	Mediates intestinal inflammation and facilitates the formation of granulomas
PM_{10}	Enhances secretion of pro-inflammatory cytokines in the small intestine, increases intestinal permeability, and induces splenocyte hyporesponsiveness
High concentration of H_2S	Inhibits the proliferation of colonic epithelial cells
Excessive ROS	Induces oxidative damage and disrupts endostasis

IBD, inflammatory bowel disease; IL, interleukin; $PM_{10,}$ particulate matter 10 μm or less in diameter; H_2S, hydrogen sulfide; ROS, reactive oxygen species.
*Factors recommended by Mendelian randomization.

pathological changes such as intestinal epithelial cell damage, inflammatory cell infiltration and increased mucus production.[153] Transplantation of intestinal microbiota from CD patients into mice can also result in inflammatory responses similar to CD in mouse intestine.[154] When germ-free mice were transplanted with fecal microbiota from IBD patients, they activated host Th17 responses, manifested by increased numbers of Th17 cells in the intestine, decreased numbers of RORγt^{+} Treg cells, leading to exacerbation of IBD severity and worsening of disease status.[155] In addition, using antibiotic-altered microbiota transplanted into germ-free Nod2$^{-/-}$ mouse model, it led to more severe DSS-induced colitis.[101]

These studies all illustrate that changing the abundance of certain specific bacterial species by microbial transplantation are likely to bring practicable modeling effects, and we can further improve the animal model of IBD based on the study of pathogenic mechanisms of microbial factors.

However, the intestinal microbiota of IBD patients vary widely and are often not universal. It is unclear whether the changes in each bacterial group are causes or consequences of IBD. Using microbial factors to model rashly may result in poor model performance due to reverse causality errors. Therefore, the results of Mendelian randomization studies are very meaningful. Mendelian randomization studies indicate the increase of *Ruminococcus* and *Enterobacteriaceae* are associated with a higher risk of IBD.[156,157] Combined with the findings of other studies, *R. gnavus* and two strains under *Enterobacteriaceae* can be recommended for inclusion in animal model construction.

We have compiled a list of "microbial factors recommended for use in experimental animal models" (Table 3), paying extra attention to the effects of direct inoculation of bacteria into the intestine. That is, it can cause enhanced inflammatory response, increased disease susceptibility, altered osmotic pressure, and other IBD risk factors in the model.[5,158-160] Among them, those factors that remain relevant to the risk of IBD after screening by Mendelian randomization studies are listed as recommended factors.

Table 3. Microbial Factors That Can Be Used to Improve Experimental Models of Inflammatory Bowel Disease

Microbial factors	Effects
Clostridium difficile (genera)	Increased epithelial permeability and luminal fluid accumulation Causes intestinal epithelial cell death Induces IL-8, TNF-α, IL-1 and IL-6 production
Adherent-invasive *Escherichia coli* (AIEC) (strain)*	AIEC strain LF82 induces intestinal inflammation in transgenic mice expressing human CEACAMS and in conventional mice treated with streptomycin Persistence of AIEC in macrophages induces increased production of TNF-α and IL-6 AIEC strains induce IL-8 production in epithelial cells
Ruminococcus gnavus (genera)*	In Crohn's disease, complex polysaccharides with a rhamnose backbone and glucose side chains are synthesized and secreted to effectively induce dendritic cells to secrete the inflammatory cytokine TNF-α
Bacteroides family–*Prevotella* (genera)	Induces CD4 T cell-mediated colitis Induces production of more IL-12-positive intestinal dendritic cells
Enterotoxigenic *Bacteroides fragilis* (strain)*	Causes deficiency of E-cadherin in intestinal epithelial cells Induces IL-8 production by intestinal epithelial cells Induces colitis in a mouse model and promotes intestinal inflammation through Th17 response
Fusobacterium varium (strain)	High levels of n-butyric acid are produced in the culture supernatant of the bacillus, which kills Vero cells *Escherichia coli* culture supernatant induced colonic ulcer, crypt abscess, inflammatory cell infiltration and apoptosis in mice *E. coli* induces adaptive immune responses and *E. coli* antibodies were detected in ulcerative colitis patients
Fusobacterium nucleatum (strain)	Causes intestinal epithelial cell death through autophagy activation Affects the expression and distribution of zonula occludens-1 Exacerbates colitis by distorting pro-inflammatory M1
Campylobacter concisus (genera)	Causes death of intestinal epithelial cells *C. concisus* increased TLR4, MD-2, and COX-2 in intestinal epithelial cells Induces IL-8 production by intestinal epithelial cells and macrophages *C. concisus* Zot protein caused intestinal epithelial cell death and enhanced macrophage response to commensal *E. coli*

IL, interleukin; TNF, tumor necrotizing factor; CEACAMS, carcinoembryonic antigen-related cell adhesion molecules; Th17, T helper cell 17; M1, classically activated macrophage; TLR4, Toll-likereceptor4; MD-2, myeloid differentiation protein-2; COX-2, cyclooxygenase-2.
*Factors recommended by Mendelian randomization.

HYPOTHESIS AND INNOVATION ON THE ENVIRONMENTAL AND MICROBIAL FACTORS

1. E+M factors can better establish IBD experimental animal models

In recent years, with the advancement of gene detection and analysis technology, we have found more and more IBD susceptibility gene loci, and have made great progress in understanding the contribution of genes to IBD.[161] Meanwhile, both adaptive and innate immune responses are thought to play an important role in the pathogenesis of IBD.[162] However, currently only about 25% of IBD heritability can be explained by genetic studies,[163] a phenomenon known as "the mystery of missing heritability of common traits" or "genetic vacuum." Therefore, one possibility is proposed that interactions between genes and their products can explain most of the apparent vacuum and account for a considerable number of causes of IBD.[164] This suggests that in the pathogenesis of IBD, microbial and environmental factors may interact with genetic factors.

Recent studies have found that only genetic susceptibility is sufficient to drive ileitis in mice with caspase-8 specific deficiency in intestinal epithelial cells (Casp8$^{\Delta IEC}$ mice), but germ-free Casp8$^{\Delta IEC}$ mice did not show any signs of colitis or colonic *E. coli* cell death on histopathology, indicating that it was disease-associated microbial communities that determined the occurrence of colonic inflammation.[165] In addition, a study on the effect of high sugar diet on IL-10$^{-/-}$ mouse colitis onset mentioned that no sugar-induced exacerbation of colitis was observed in germ-free mice, but if microbial communities from sugar-treated mice were transplanted into germ-free mice, susceptibility to colitis increased,[166] fully illustrating that the effect of environmental factors on disease severity is microbe-dependent. Therefore, we do not recommend using environmental factors or microbial factors alone for modeling.

In summary, we innovatively propose a hypothesis that "combination of environmental and microbial factors can induce IBD better in establishing animal model." And we suggest that when constructing animal models for studying the pathogenic mechanisms of IBD, such as immune and genetic aspects, as well as for conducting drug experiments for IBD, both environmental and microbial factors should be taken into account in order to better simulate the human IBD environment and make the research results more realistic and reliable. Currently, the limitations of mainstream IBD mouse models include not reflecting the genetic and environmental diversity of human populations, nor explaining inherent variables such as drug exposure, smoking, and diet seen in human studies.[167] Whether dysbiosis precedes IBD development and initiates inflammatory processes, or merely reflects changes in immune and metabolic environment of inflamed mucosa, remains to be answered. To address these issues, thinking about how to construct an animal experimental model that reflects environmental and microbial factors as important factors in IBD pathogenesis becomes crucial. Here, we explore the feasibility of using environmental factors (E)+microbial factors (M) alone for modeling.

2. The feasibility of using E+M factors alone for building animal models

Diet has been shown to have a significant impact on the composition of the gut microbiota, altering its function and metabolism at the genomic level.[168] There is increasing epidemiological evidence of the role of diet in the pathogenesis of IBD. Therefore, when modeling, it is possible to consider using diet-induced (E)+simulation of gut microbial pathogenic factors (M) in IBD patients to induce experimental animal IBD.

High-fat diet fed mice are a common animal model for detecting the effects of fat intake *in vivo*. A research of high-fat diet animal models shows it can increase the level of saturated fatty acid, which impairs the intestinal epithelial cells and intestinal immune system while the animal models' gut microbes are unaffected.[169] We presumed it is resulted from the difference between human and animal models.

Thereby, based on this model, we can include microbial factors in animal models to simulate human's IBD pathogenic mechanism. We can consider using the same enterotoxigenic *B. fragilis* bacterial solution as mentioned in the previous text, which has a clear causal relationship with IBD in Mendelian randomization studies, to create intestinal microbial pathogenic factors by enema on the basis of the high-fat diet model, further causing damage to intestinal epithelial cells barriers, inducing MMP-7 and the activation of transcription factors such as nuclear factor kappa-B and activator protein 1, and promoting intestinal inflammation through Th17 response.[160,166]

Such animal models can be used in exploring the related mechanisms from immune and genetic aspects in depth, and answer the specific role of environmental and microbial factors in the pathogenesis of IBD.

However, at present, only environmental factors and microbial factors are used to induce IBD experimental models with instability and uncertainty of effect, and more research is needed to determine the optimal dose, induction time, mode, etc. to make this type of model easy to repeat experiments and popularize.

Environmental factors include gas particles, drugs, surgery, etc. in addition to diet. Although there is controversy over whether smoking is associated with IBD based on Mendelian randomization studies, smoking is a relatively recognized risk factor for CD in clinical practice. Compared with never smokers, smokers have almost twice the risk of developing CD.[167] Direct interactions between IBD susceptibility genes, such as NOD2 and ATG16L1, and smoking have been found.[168] Therefore, after giving experimental mice smoke exposure to simulate human smoking environmental factors for research, it has the potential to make the experimental model closer to the disease mechanism of smoking CD patients in a short time. It is worth noting that currently observed smokers *Prevotella* in *Bacteroidetes* increased abundance.[123] Therefore, although there is no Mendelian randomization evidence to support the interaction between *Prevotella* and IBD, the existing research results still make us think that it is worth recommending to add *Prevotella* as a microbial factor that can be combined with smoking model. If assisted *Prevotella* bacterial fluid enema mice or may play a synergistic pro-inflammatory effect to ensure that mice intestinal microbiota toward smoking caused by adverse direction development, increasing the probability of successful modeling. If IBD model can be induced by smoke exposure (E)+*Prevotella* (M) alone, it will provide strong support on the view that smoking is a driving factor for IBD occurrence, and intestinal microbial changes play a key role in it (Fig. 3).

3. The combination of E+M factors and existing models

Another approach is to apply environmental factors and microbial factors to the improvement of mainstream models, such as supplementing the DSS-induced mouse model with dietary induction (E)+simulated IBD patient intestinal microecological disorder (M) or smoking (E)+*Bacteroides-Prevotella* (M) mentioned above. The purpose of doing so is to increase the complexity of IBD onset in experimental animal models and reduce the difference between mouse-induced IBD under experimental conditions and human IBD in actual situations. Due to strictly controlled experimental conditions, mice derived from mainstream modeling methods are genetically homogeneous, consume monotonous food, and live in a clear environment, which is different from the environment humans are exposed to. Supplementing E+M factors not only has a more comprehensive consideration when exploring the pathogenesis, but also is beneficial for evaluating the therapeutic effect of drugs. It can reduce the probability of events such as the occurrence of too harsh conditions for the applicable population of developed drugs or the discovery of ineffective treatment for IBD in clinical studies. For example, the etiology of colitis induced by oxazolone alone does not fully reflect the etiology of UC, resulting in no obvious therapeutic effect in phase IIa clinical trials using IL-13 antibody (anrukinzumab)[140] or tralokinumab (another IL-13 neutralizing antibody)[141] for UC patients. Considering the repeatability and reliability of experimental results, we also need to strictly control the supplemented E+M factors, such as strictly controlling the composition, concentration and duration of smoking, the concentration of enema bacterial fluid, etc. In the future, researchers should also try to use those factors with more definite causal relationship in Mendelian randomization studies in Tables 2 and 3 for combination, in order to find the most suitable combination of environmental factors and microbial factors for improving different modeling methods. We also look forward to the follow-up Mendelian randomization studies to verify the other risk factors in IBD.

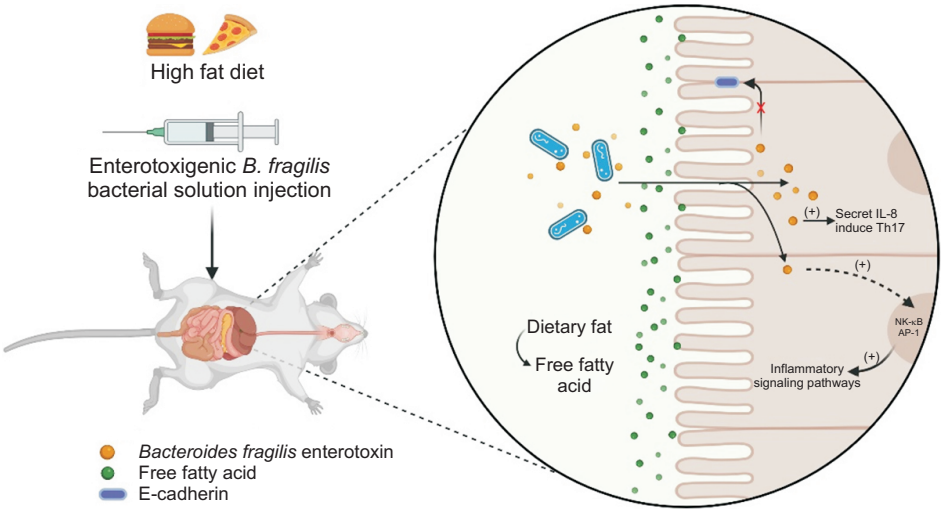

Fig. 3. Schematic diagram of the E+M molding mechanism. High-fat diet combined with enterotoxigenic *Bacteroides fragilis* bacterial solution to construct an animal intestinal inflammation model. E, environmental factor; M, microbial factor; IL-8, interleukin 8; Th17, T helper cell 17; NF-κB, nuclear factor kappa; AP-1, activator protein 1.

SUGGESTIONS FOR FURTHER RESEARCH

Despite the fact that Mendelian randomization provided strong support for our theory, our suggestion of using environmental factors and microbial factors alone to establish animal models may still need to be better validated by building more experimental animal models of IBD in practice. But it is necessary and meaningful to consider both environmental factors and microbial factors when building animal models for the pathogenic mechanism of IBD. Our review highlights the importance of environmental factors and microbial factors, and provides new insights for experimental animal modeling. Pursuing models that are closer to the real situation of IBD is considered as the future direction of research and effort.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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