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REVIEW

The gut−liver axis in drug-induced liver injury (DILI): Interplay between gut microbiota, intestinal barrier, and immune system

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Immune system

Abstract Drug-induced liver injury (DILI) is an adverse hepatic reaction with a complex etiopathogenesis, involving direct cellular damage by the drug, immune-mediated mechanisms, and host and environmental factors. The gut microbiota has recently been recognized as a key player in human physiopathology. In DILI, animal models and patients have shown significant alterations in gut microbiota composition. Evidence indicates that patients with DILI often present intestinal barrier dysfunction, characterized by increased permeability and the translocation of pathogen-associated molecular patterns (PAMPs) to the liver. This process may contribute to the onset or aggravation of liver injury by triggering harmful immune responses. Furthermore, dysbiosis can alter bacterial metabolite production, thereby affecting intestinal and hepatic homeostasis. The gut microbiota can also modify the efficacy and toxicity of drugs on an individual level, thereby increasing the risk of DILI. This review provides an overview of the current evidence on the mechanisms by which the gut microbiota contributes to inflammation, immune recruitment, and exacerbation of liver damage in DILI. A more in-depth understanding of how

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the gut microbiota alters intestinal and hepatic homeostasis is essential for advancing our knowledge of DILI pathogenesis, integrating novel biomarkers into clinical practice, and developing microbiota-based interventions.

1. Introduction

Drug-induced liver injury (DILI) is an adverse reaction caused by the use of drugs, herbs, and dietary supplements that leads to liver dysfunction. It is currently a leading cause of acute liver failure (ALF) and liver transplantation, and a significant reason for drug withdrawal, with substantial clinical and economic implications worldwide[1]. DILI can be classified into three types according to the mechanism of action of the drug responsible for liver toxicity[1]. Intrinsic DILI is a predictable, dose-dependent damage with a short latency period that can be easily reproduced in animal models. *N*-acetyl-*p*-aminophenol or acetaminophen (APAP) is the most common cause of this type of hepatotoxicity, accounting for up to 50% of the causes of ALF in Europe[2]. Idiosyncratic DILI (iDILI) is unpredictable, with a longer latency period, and typically not dose-dependent, although a threshold dose is required to initiate the cascade of events leading to liver damage. This type of hepatotoxicity is the most common in clinical practice, and the drugs most commonly associated with this type of DILI are antibiotics, cardiovascular drugs, central nervous system agents, and non-steroidal anti-inflammatory drugs[3]. A third type of DILI, indirect DILI, has been described, caused by drugs that may aggravate pre-existing liver conditions or drugs that alter the immune system, such as immune checkpoint inhibitors used in the treatment of certain types of cancer[4].

Population-based prospective studies in Europe estimate an annual incidence of DILI of around 14−19 cases per 100,000 inhabitants[3]. Even so, iDILI is thought to be underreported, and its diagnosis is one of the biggest challenges for physicians because there are no specific tests or diagnostic and prognostic biomarkers, and its symptoms often mimic those of other liver diseases, such as autoimmune hepatitis. This problem is aggravated by the large number of approved drugs with hepatotoxic potential, the rising incidence of herbs and dietary supplements hepatotoxicity, and the increasing use of immunotherapeutic agents, especially immune checkpoint inhibitors[4,5].

The proposed pathological mechanisms underlying DILI involve the generation of drug-reactive metabolites, oxidative stress, mitochondrial dysfunction, inhibition of canalicular transporters, and immune-mediated hepatotoxicity, with the adaptive immune response as a critical element in iDILI. These processes are further influenced by drug-related factors, host-specific responses, individual genetic variability, and environmental factors[6,7]. The dysfunction of the gut−liver axis is increasingly recognized as a key contributor to the pathophysiology of multiple liver diseases, including alcoholic liver disease, metabolic dysfunction-associated steatotic liver disease (MASLD), autoimmune liver diseases, and hepatocellular carcinoma[8−10]. Recently, the gut−liver axis has also been associated with DILI pathogenesis. Studies in animal models and in patients with DILI have revealed significant changes in the composition of the gut microbiota. There is also evidence of intestinal barrier dysfunction and increased intestinal permeability. Although the molecular events behind these changes are not fully understood, it has been suggested that dysbiosis and intestinal barrier dysfunction may act as pathogenic drivers in DILI, potentially inducing or exacerbating liver injury by modulating the immune response through various mechanisms[11,12]. Furthermore, gut microbiota can modify a drug's hepatotoxic potential or the liver's detoxification capacity. These findings have provided a rationale for exploring potential new therapeutic strategies in DILI, aimed at modulating the gut microbiota with prebiotics, probiotics, postbiotics, fecal microbiota transplantation (FMT), and agents targeting microbial-host signaling pathways[13,14].

This review aims to provide an integrated overview of the current state of knowledge regarding the role of the microbiota-gut liver axis in the pathogenesis of DILI. First, we introduce the key components of the gut−liver axis and the general mechanistic aspects of DILI. We then summarize recent evidence on how the gut microbiota may contribute to DILI by disrupting specific elements and signaling pathways involved in the bidirectional gut−liver crosstalk. Finally, we discuss therapeutic strategies to modulate the alterations in the gut−liver axis observed in DILI.

2. Components of the gut−liver axis connection

The close relationship between the gut, its microbiota, and other extraintestinal organs is increasingly accepted[15]. In particular, the term gut−liver axis refers to the bidirectional anatomical and functional connection between the gut, along with its microbiota, and the liver. The liver is the largest internal organ and a biochemical factory that plays a vital role in metabolism, detoxification, and the production of bile acids (BAs)[16]. Through the portal vein, the liver constantly receives products derived from the gut microbiota and diet, as well as pathogens and toxic compounds that will be metabolized or detoxified. In turn, the gut receives *via* the bile duct different bioactive compounds generated in the liver that regulate the composition and distribution of gut microbiota[17], such as primary BAs.

The human gut microbiota is a vast and diverse ecosystem of more than 100 trillion microorganisms, including bacteria, fungi, viruses, archaea, and protozoa, that establish symbiotic relationships among themselves and with the human host[9]. The gut microbiota is first established at birth[18]. Although the adult human microbiota generally maintains a basic stability in its biochemical functions, its abundance and composition may change throughout life due to factors such as age, diet, lifestyle, drugs, environment, and host genetics[19]. In the normal human gut, bacteria comprise the vast majority of the microbiota, predominantly residing in the distal segment of the gastrointestinal tract. In a healthy adult gut microbiota, the most dominant bacterial phyla are Bacillota (formerly known as Firmicutes), which includes several important genera such as *Lactobacillus, Clostridium,* or *Eubacterium*; and

Bacteroidota (also referred to as Bacteroidetes), which includes genera such as *Bacteroides* and *Prevotella*[20]. These two phyla represent more than 90% of the bacterial community, followed by the subdominant phyla Actinobacteria (Actinomycetota), with *Bifidobacterium* as one of the most important genera, and Pseudomonata (also referred to as Proteobacteria)[19]. A healthy gut microbiota performs essential functions, including preventing pathogen colonization, contributing to the metabolism of nutrients and xenobiotics, regulating intestinal barrier integrity, and supporting the development and maintenance of immune homeostasis[21]. These effects are mediated both directly by the microbes themselves and indirectly through their metabolites, such as short-chain fatty acids (SCFAs), secondary BAs, and tryptophan-derived compounds.

A critical element of the gut−liver axis is a healthy, functional intestinal barrier that permits the selective transport and absorption of nutrients while serving as the first line of defense against exogenous antigens, microbiota-derived products, and pathogens. The intestinal barrier consists of several physicochemical layers[21]. These include the chemical and antibacterial mucosal layer, which is the most direct point of contact with the gut microbiota and luminal antigens[22]; the intestinal epithelium, which is a monolayer of intestinal epithelial cells (IECs) that are physically bound to each other by critical protein complexes and interspersed with specialized cells[23]; and the gut vascular barrier, which prevents the translocation of bacteria and their metabolites into the portal venous system when the mucosal and epithelial barriers are compromised[24]. The intestinal barrier also includes an inner functional layer, which constitutes the first immunological line of defense within the gut−liver axis[25]. The intestinal immune system is organized in a specialized structure known as gut-associated lymphoid tissue. It contains inducer compartments, where cells of the adaptive immune system undergo initial priming and differentiation, and effector sites, with different immune cells distributed throughout the lamina propria and overlying the intestinal epithelium[26,27].

In a bidirectional mechanism, the intestinal immune system controls certain functions of the gut microbiota itself and maintains intestinal homeostasis[21]. Imbalance in the composition and function of the normal gut microbiota, known as gut dysbiosis, can induce pro-inflammatory responses and immune dysregulation in the intestinal microenvironment, affecting the balance of helper T cells (Th)1, Th17, Th2, and regulatory T cells (Treg) responses[28]. Gut dysbiosis and intestinal immune system dysfunction have been linked to gastrointestinal diseases such as inflammatory bowel disease and celiac disease, as well as extraintestinal diseases, including rheumatic arthritis, metabolic syndrome, neurodegenerative disorders, or cancer[29].

Finally, the liver is particularly enriched in hepatocytes and non-parenchymal cells, including liver sinusoidal endothelial cells (LSECs), and innate immune cells, such as Kupffer cells (KCs) or innate lymphocytes. In response to injury or inflammation, it can also recruit more immune cells from the circulation[30], acting like a second immunological firewall in the gut−liver axis. Under normal conditions, the liver is constantly exposed to dietary and gut microbiota antigens that escape immune surveillance by the gastrointestinal mucosa. For this, the liver has a unique immune regulatory mechanism that maintains a balance between immune response activation and immunotolerance[31,32]. When the intestinal barrier is compromised, there is an increased translocation of PAMPs, such as lipopolysaccharide (LPS) or flagellin, and bacterial—derived metabolites from the gut into the systemic circulation. Through the hepatic portal circulation or the mesenteric lymph nodes of the intestinal lamina propria, PAMPs and bacterial metabolites can reach the liver, potentially inducing or aggravating the liver injury by causing a harmful immune response[10].

3. The gut−liver axis and DILI

3.1. General pathological mechanisms in DILI

The exact mechanisms underlying DILI remain poorly understood. Most insights into the mechanisms of hepatocyte death in DILI are based on preclinical models, with a focus on intrinsic DILI, particularly the APAP-induced liver injury animal model[33]. However, despite intense efforts, current preclinical models are unable to replicate the complexity and multifactorial interactions characteristic of iDILI in humans[34], which accounts for the majority of cases of hepatotoxicity in clinical practice[3].

Most drugs are recognized by the human body as xenobiotics and must undergo biotransformation. This process takes place mainly in the liver and involves a series of chemical reactions, including oxidation, reduction, or hydrolysis reactions (phase I), followed by conjugation reactions (phase II), which facilitate their excretion *via* the kidneys or the bile as more soluble compounds. In some cases, this process produces chemically reactive metabolites, which, together with the inability of certain individuals to detoxify them properly, can increase cellular stress in hepatocytes. This represents the initial step in liver damage[35,36]. The parent drug, along with reactive metabolites resulting from drug metabolism by liver enzymes such as human cytochrome (CYP) 450, located in the inner mitochondrial membrane and smooth endoplasmic reticulum of hepatocytes, can cause damage to hepatocytes through several concurrent mechanisms. These include the generation of reactive oxygen species (ROS), covalent binding to mitochondria and the endoplasmic reticulum, leading to cellular stress and mitochondrial dysfunction[37] and alteration of BAs homeostasis through impairment of the canalicular bile salt export pump (Fig. 1). All these processes trigger hepatocyte cell death, including apoptosis and necrosis[6]. However, this alone cannot explain the liver damage observed in DILI patients.

There is now clear evidence that the immune response plays a role in DILI pathogenesis. The innate immune response is particularly involved in the initial response to drug-induced stress[38,39]. Injured hepatocytes release damage-associated molecular patterns (DAMPs), such as high-mobility group box protein 1, S100 proteins, cytokeratin-18, and mitochondria-derived DAMPs, which can activate liver resident immune cells, including KCs, natural killer (NK), and NKT cells. In susceptible individuals, the adaptive immune system is subsequently activated, with T-cell-mediated liver injury providing the basis for iDILI development[40−42]. The most widely accepted hypothesis for iDILI is that drugs or their metabolites act as haptens, covalently binding to endogenous proteins to generate drug-protein adducts[43]. In liver-draining lymph nodes, these adducts can be processed by antigen-presenting cells (APCs) and presented as neoantigens by major histocompatibility complex (MHC)-I and MHC-II molecules to naïve $CD8^{++}$ cytotoxic T (Tc) cells and $CD4^{++}$ helper T (Th) cells, respectively[40]. The naïve T cell priming or sensitization and subsequent conversion to effector T cells require co-stimulatory signaling by mature APCs, previously activated by DAMPs and PAMPs, through CD80/CD86 receptors binding to

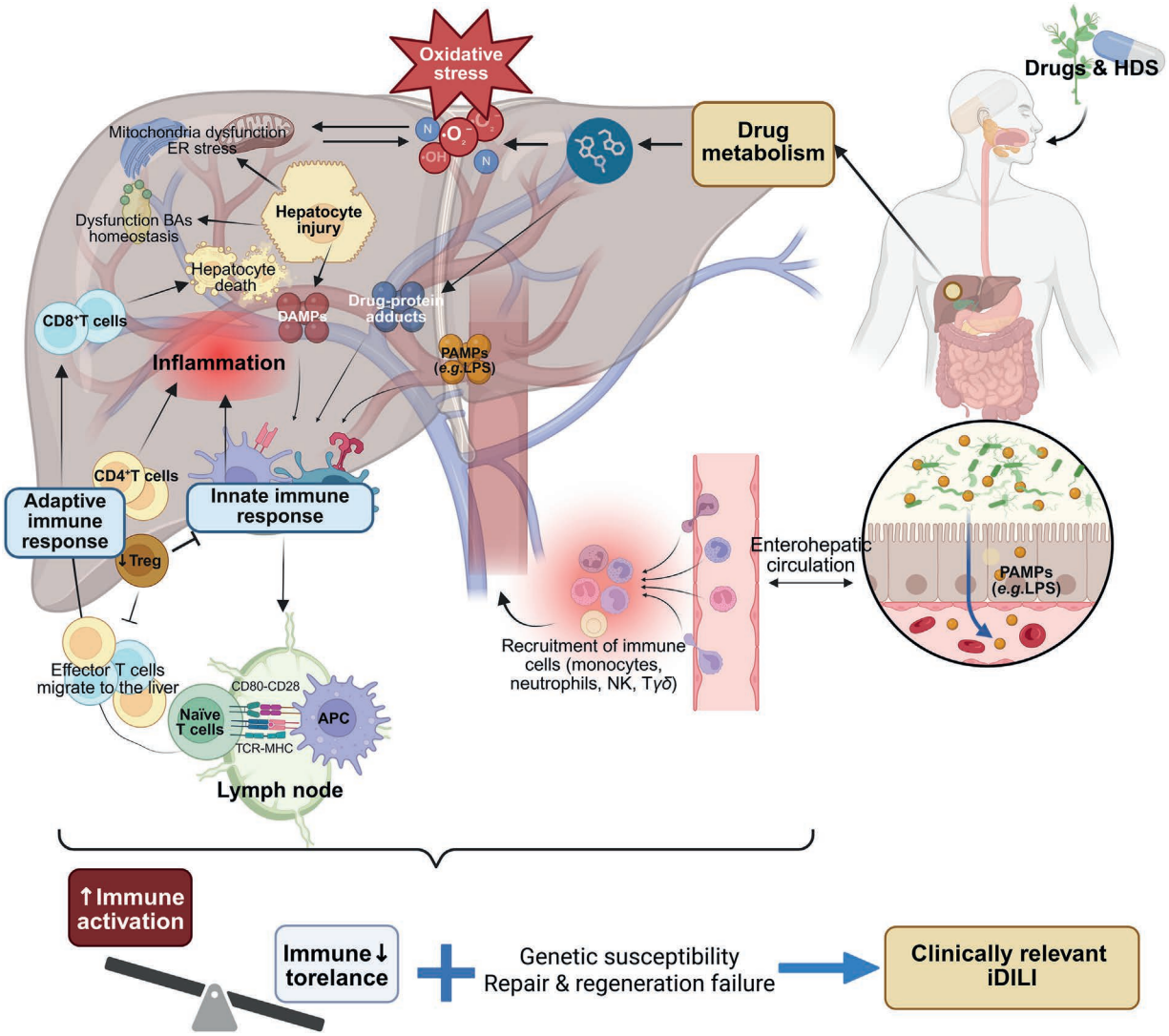

Figure 1 General physiopathological mechanisms proposed in idiosyncratic drug-induced liver injury (iDILI). iDILI results from a complex interaction between the drug and host factors that determine the type of liver damage and the clinical course. The drug- and/or reactive metabolite-induced hepatocyte damage triggers a multifaceted host response. Cellular damage, including oxidative stress, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and/or dysfunction of bile acids (BAs) homeostasis, can ultimately lead to cell death, cell membrane swelling and rupture, and the release of intracellular contents, including damage-associated molecular patterns (DAMPs). Furthermore, gut dysbiosis can exacerbate liver damage by disrupting the intestinal barrier, increasing the translocation of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), and intensifying the innate immune response in the liver along with immune recruitment. Additionally, certain drugs can generate neoantigens that, when presented by specific major histocompatibility complex (MHC) molecules, trigger an adaptive immune response mediated by cytotoxic $CD8^+$ T cells and helper $CD4^+$ T cells. This response exacerbates inflammation and liver injury, providing the basis for immunopathological mechanisms in iDILI development. Although the liver possesses reparative and immune tolerance mechanisms that typically prevent serious injury, a breakdown of this tolerance—favored by host factors such as age, genetics, pre-existing diseases, and concurrent medication—can lead to clinically significant liver injury. APC, antigen-presenting cell; HDS, herbal and dietary supplements; NK, natural killer cell; TCR, T-cell receptor; Tγδ, gamma-delta T cells; Treg, regulatory T-cells.

CD28 on T cells, as well as a suitable cytokine microenvironment. These effector T cells will migrate to the liver, where they can recognize drug-modified antigenic peptides in the context of MHC molecules expressed on the surface of damaged hepatocytes, exert a specific T-cell immune response, and generate memory T cells. While effector Th cells orchestrate immune response by producing cytokines and upregulating membrane co-stimulatory molecules, effector Tc cells mediate cytotoxic reactions by releasing TNF-α, TNF-α-related apoptosis-inducing ligand on their surface with their respective receptor on hepatocytes, and releasing granules containing perforins and granzymes[42]. Other hypotheses are that drugs might bind directly to the T cell receptor or MHC molecule independently of a peptide (the pharmacological interaction model) or, in rare cases, the drugs or their reactive metabolites may alter the MHC binding groove and, consequently, the range of peptides that can be presented[44]. In this context, immune tolerance mechanisms may be crucial for maintaining liver homeostasis, including Treg responses, inhibitor immune checkpoint receptors such as CTLA-4 and PD-1/PD-L1[45], myeloid-derived suppressor cells, and tolerogenic APCs[46]. In fact, it has been suggested that a

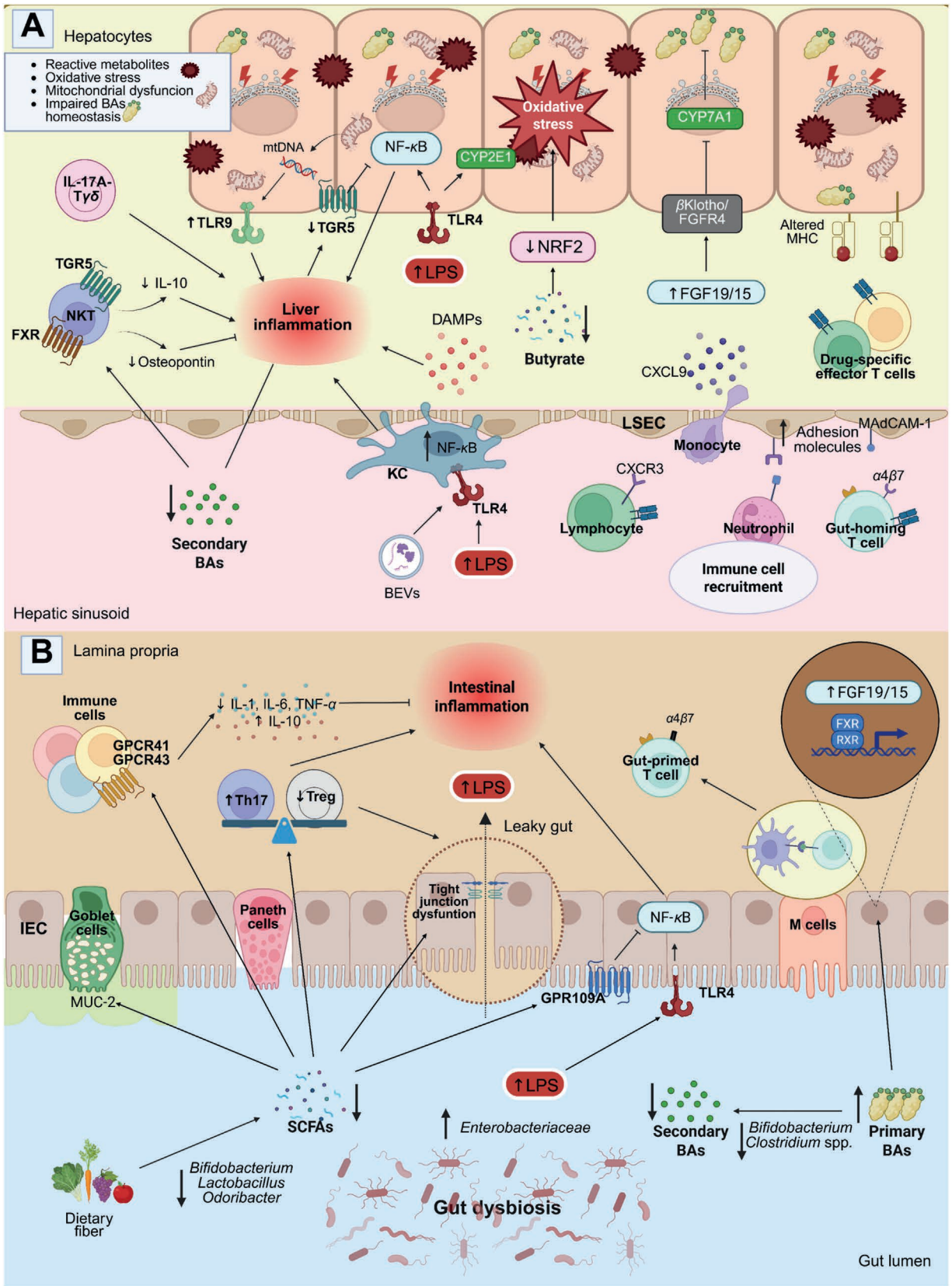

Figure 2 Proposed mechanisms of gut-microbiota contribution to drug-induced liver injury (DILI). (A) Molecular mechanisms produced by gut microbiota in the liver. Excess primary bile acids (BAs) in DILI may lead to liver damage by releasing mitochondrial DNA (mtDNA), which activates toll-like receptor (TLR) 9 and stimulates the production of pro-inflammatory cytokines. Upon reaching the intestine, primary and conjugated BAs can be transformed into secondary BAs by gut bacteria such as *Clostridium* spp. (*e.g.*, *C. scindens* and *S. hylemonae*), as well as a

disruption or impairment of immunotolerance mechanisms is involved in iDILI (hypothesis of defective adaptation)[47]. Therefore, the balance between immune activation, immunotolerance, and repair and regeneration mechanisms is crucial for the progression or resolution of liver damage in idiosyncratic reactions[40,48].

3.2. Contribution of the gut microbiota in DILI

Recent evidence suggests that the gut microbiota is implicated in the pathophysiology of DILI. The principal hypothesis is that gut microbiota has a bidirectional connection in DILI, meaning it can be either the cause or the result of the final phenotype. Drugs can alter the composition and function of the gut microbiota. This gut dysbiosis could lead to altered drug metabolism, dysregulation of the intestinal barrier, and, consequently, worsened liver inflammation and oxidative stress. This may be due to the presence of certain bacterial genera or the production of specific bacterial products or metabolites, such as LPS, SCFAs, or secondary BAs. In a feedback loop, liver injury may perpetuate gut dysbiosis and promote BAs imbalance. Additionally, changes in the gut microbiota may increase susceptibility to the hepatotoxicity of certain drugs. For this reason, therapies that harness the beneficial effects of certain bacterial profiles in DILI are also being tested[49]. The elements of the gut−liver axis that appear altered in DILI and their potential contributions to the pathological mechanisms will be discussed in detail below (Fig. 2).

3.2.1. Changes of the gut microbiota

Multiple studies in animal models and patients with DILI induced by different drugs have observed changes in gut microbiota (Table 1[50-60]).

A representative parameter of dysbiosis repeatedly detected in both animal models and DILI patients is the alteration of the Bacillota/Bacteroidota (formerly Firmicutes/Bacteroidetes) ratio[8,57,60]. This imbalance may already influence drug toxicity, given that Bacteroidota has a higher diversity of cytochrome P450 enzymes and produces a higher variety of secondary metabolites than Bacillota[61,62]. The general scenario in DILI is that changes in the gut microbiota are characterized by an alteration of bacterial richness and diversity, alongside a relative overgrowth of bacterial groups with pathogenic potential and a relative reduction in commensal bacteria that are considered beneficial[50,54,57,58,63,64].

3.2.1.1. DILI by APAP. APAP is a widely used analgesic and antipyretic pharmacological drug. While it is usually safe at therapeutic doses, an overdose can lead to hepatotoxicity, which is a remarkable cause of ALF in Western countries[65]. Studies on the gut microbiota in APAP-induced liver injury reveal significant changes in richness and diversity in animal models, with bacterial compositions that differ from those of control mice[51,52,66]. Evidence suggests that an imbalance in the gut microbiota exacerbates APAP-induced liver injury or increases susceptibility to DILI, either directly or *via* its metabolites. In the study by Xia et al.[51], an increase in the genera *Oscillibacter*, *Mucispirillum*, and *Colidextribacter* in mice with APAP-induced liver injury was positively correlated with analytic parameters of liver injury, inflammatory cytokines, and cell infiltration. In addition, *Lactobacillus*, *Candidatus_Saccharimonas*, and SCFAs were negatively correlated with parameters of liver damage and proinflammatory factors. In fact, *Lactobacillus* species are well known for their anti-inflammatory and hepatoprotective properties[67,68]. Another study in mice with APAP-induced liver injury found that treatment with APAP reduced anti-inflammatory and SCFAs-producing bacteria, such as

genus like *Bifidobacterium* involved in prior deconjugation. However, in DILI, a decrease in secondary BAs-producing bacteria, including these *Clostridium* species and *Bifidobacterium,* has been detected. This reduction may diminish the beneficial effects associated with secondary BAs, such as reduced anti-inflammatory protection through their action on the hepatocyte Takeda G protein-coupled receptor 5 (TGR5). Depletion of secondary BAs can suppress the polarization of natural killer (NK) T cells toward a regulatory subtype by binding to TGR5, thereby reducing IL-10 secretion. Although primary BAs can mediate anti-inflammatory responses by reducing osteopontin production by NKT cells *via* the nuclear receptor farnesoid X receptor (FXR), this is likely insufficient given the already predominant immunologically altered environment and the global imbalance in BAs. A decrease in short-chain fatty acids (SCFAs)-producing bacteria in DILI, including *Bifidobacterium, Lactobacillus*, or *Odoribacter*, leads to a reduction in SCFAs. In particular, butyrate depletion may reduce the antioxidant effect of nuclear factor E2-related factor 2 (NRF2). Increased levels of lipopolysaccharide (LPS) derived from gut dysbiosis reach the liver *via* the portal vein and are recognized by Kupffer cells (KCs), which are activated, thereby potentiating and aggravating liver inflammation. Bacterial extracellular vesicles (BEVs) can also promote LPS/TLR4 signaling on KCs. LPS can also increase *CYP2E1* expression, thereby increasing reactive oxygen species (ROS) production and hepatocyte death. The proinflammatory environment and chemoattractant signals (*e.g.*, CXCL9) promote the overexpression of adhesion molecules in liver sinusoidal endothelial cells (LSECs), which ultimately mediate leukocyte adhesion and transendothelial migration into the injured liver. These immune cells contribute to both the damage and the regenerative and reparative processes. Gut-derived lymphocytes could migrate to the liver and contribute to immune dysregulation, inflammation, and liver immune attack in DILI. Furthermore, IL-17A-producing gamma-delta ($\gamma\delta$) T cells have been associated with neutrophil infiltration and inflammation in acute liver injury. (B) Molecular mechanisms produced by gut microbiota in the intestine. The decrease in SCFAs-producing bacteria in DILI reduces the levels and beneficial effects of SCFAs at the intestine, which include 1) down-regulation of nuclear factor kappa B (NF-κB) and intestinal inflammation associated with the TLR4-LPS activation pathway *via* the GPR109A receptor in intestinal epithelial cells (IECs), 2) protection of intestinal and mucosal barrier integrity and function by strengthening tight junction (TJ) proteins and stimulating mucin 2 (MUC2) production by goblet cells, and 3) downregulation of pro-inflammatory cytokines and upregulation of anti-inflammatory cytokines *via* G-protein coupled receptor (GPCR) 43 and GPCR41 expressed on immune cells. Dysfunction of TJ proteins promotes increased intestinal permeability and the translocation of microbiota-derived products, such as LPS, which is elevated in the serum of DILI patients, likely due to increased Enterobacteriaceae. LPS signaling *via* TLR4 expressed in IECs also potentiates the inflammatory state in the intestine. Accumulation of BAs in the liver is involved in the pathophysiology of DILI. Elevated levels of BAs in plasma have been linked to the severity of liver damage. In a negative feedback mechanism, increased primary BAs enhance the production of fibroblast growth factor (FGF)19 in the intestine, which travels to the liver and, through its action on the FXR-FGFR4/β-Klotho axis, blocks the CYP7A1 enzyme, and with it, the hepatic synthesis of BAs. Dysbiosis, LPS translocation, and reduced SCFAs levels can induce an imbalance in Th17/Treg responses, promoting intestinal and hepatic inflammation and disrupting this critical hepatic immunological balance.

Lactobacillus and *Odoribacter*, and significantly increased genera such as *Enterococcus*, *Bacteroides,* and *Oscillibacter*[52].

3.2.1.2. DILI by methotrexate (MTX). MTX is an antimetabolite drug used to treat various cancers and autoimmune diseases. Its main adverse effect is MTX hepatotoxicity. In a study by Wang et al.[53], administering MTX to mice caused hepatotoxicity and significant dysbiosis, as evidenced by a significant increase in the Bacillota/Bacteroidota ratio. The observed gut microbiota profile showed increased levels of proinflammatory bacteria (*Enterococcus*, *Collinsella*, *Streptococcus*, and *Aerococcus)* and decreased levels of anti-inflammatory bacteria (*Lactobacillus* and *Bifidobacterium*). Furthermore, this alteration in the gut microbiota appeared to be associated with changes in different metabolic pathways in the liver.

3.2.1.3. DILI by antithyroid drugs. The first-line treatment for Graves' disease is antithyroid drugs, such as methimazole and propylthiouracil, but a significant side effect of these medications is iDILI. Current findings indicate that animal models and patients with Graves' disease treated with antithyroid drugs exhibit reduced gut microbiota diversity and alterations in gut microbiota composition. However, it is unclear whether these alterations are a cause or a consequence of the disease[69−71]. The most relevant study on DILI induced by antithyroid drugs was conducted by Sun et al[54]. These authors observed alterations in the gut microbiota structure in both Sprague−Dawley rats and patients treated with antithyroid drugs. Although there were certain differences, they suggested that the endotoxemia hypothesis may mediate hepatotoxicity by this medication. Notably, there was a decrease in *Bacteroides* and an increase in *Blautia* in the treated patients compared to controls, which correlated negatively and positively with markers of liver injury, respectively. This suggests a role for these bacteria in liver damage. In fact, *Bacteroides* depletion has previously been related to increased oxidative stress and reduced liver function[72]. Rats that developed DILI showed an enrichment in LPS-producing genera, including Prevotellaceae_*UCG-003* and *Oscillibacter*. This finding was consistent with the increased LPS detected in serum and feces, as well as evidence of intestinal barrier dysfunction. These genera were also positively associated with liver parameters and proinflammatory cytokines, whereas decreases in *Lactobacillus* and *Faecalibacterium* were negatively correlated with indicators of liver damage. The increase in serum and fecal LPS was confirmed in humans and correlated with increased levels of the *Escherichia-Shigella* genera.

3.2.1.4. DILI by anti-tuberculosis drugs (ATD). Tuberculosis is a mycobacterial infection that represents a significant global health threat. Whilst the disease is currently preventable and there is a highly effective long-term treatment available, the first-line ATD treatment, particularly isoniazid, rifampicin, and pyrazinamide, can cause idiosyncratic adverse liver reactions[73]. Evidence shows that prolonged treatment with ATD significantly reduces gut microbiota diversity and causes persistent gut dysbiosis, even after therapy has been completed[74−76]. This occurs independently of the bacterial infection and may affect the host's immune response. Evaluation of ATD therapy in humans has revealed that, before starting the treatment (T1), patients who subsequently developed ATD-DILI exhibited a higher relative abundance of the *Bacteroides, Veillonella*, *Clavibacter*, *Corynebacterium*, *Anaerococcus*, *Gardnerella*, *Peptostreptococcus*, and *Lautropia* genera than those who did not. The *Bacteroides* genus is part of the commensal gut microbiota, but it can become an opportunistic pathogen under conditions of increased intestinal permeability, thereby exacerbating liver injury. Pei et al.[55] also observed that, once ATD-DILI had developed (T2), the anti-inflammatory genera *Akkermansia* and *Prevotella_9* were decreased in ATD-DILI patients compared to non-ATD-DILI patients. This was negatively correlated with liver parameters and proinflammatory cytokines. Conversely, *Enterococcus*, *Faecalibacterium*, and *Ruminococcus_gnavus_group* genera were increased and positively correlated with these parameters. This suggests that the development of ATD-DILI may be associated with an imbalance in the levels of anti-inflammatory and pro-inflammatory bacteria. Interestingly, continuous exposure to isoniazid has also been linked to gut dysbiosis and the clinical adaptation phenomenon or liver tolerance in mice with isoniazid-induced liver injury[56]. This phenomenon was specifically associated with an increase in the *Bifidobacterium* genus, which modulates Th17/Treg responses and hepatoprotection.

3.2.1.5. DILI by antibacterials. Although iDILI can be caused by different types of drugs, antibacterials are the main cause according to prospective DILI registries[3,77]. The administration of antibiotics has been associated with positive and negative influences on DILI development. The transient or permanent effects of different classes of antibiotics on the composition and function of the microbiota, depending on the individual, may explain the significant differences in the onset and course of different types of iDILI[19,59,78]. Dysbacteriosis was observed in an animal model that received ceftriaxone, and this change was associated with intestinal barrier damage and activation of the liver's immune system, promoting chronic liver inflammation[79]. Schneider et al.[66] observed that *Nlrp6*$^{-/-}$ mice, which are used as a dysbiotic mouse model, showed greater inflammation and liver damage following the injection of a sublethal dose of either APAP or LPS compared to wild-type mice. In both mice, administration of broad-spectrum antibiotics normalized liver injury, demonstrating the role of the gut microbiota in liver injury and a severe phenotype. In humans, iDILI patients on conventional drugs have been associated with a gut microbiota profile mainly related to a pro-inflammatory state. Rodriguez-Diaz et al.[57] observed that patients with iDILI caused by different pharmacological drugs, particularly antibacterial drugs and herbs, presented a lower abundance of *Acetobacteroides*, *Blautia*, *Caloramator*, *Coprococcus*, *Flavobacterium*, *Lachnospira*, *Natronincola*, *Oscillospira*, *Pseudobutyrivibrio*, *Shuttleworthia*, *Themicanus*, and *Turicibacter* genera. *Blautia*, *Coprococcus*, and *Oscillospira* have previously been found to be reduced in the fecal microbiota of patients with autoimmune hepatitis[80]. Indeed, reduced abundance of *Blautia* has previously been associated with increased progression of liver damage[58]. *Oscillospira* may be associated with reduced secondary BAs, which are lower in patients with severe DILI[81]. However, Haoshuang Fu et al.[82] used two-step Mendelian randomization and mediation analysis to explore the causal effects of the gut microbiota on DILI through specific plasmatic metabolites. Four taxa were causally associated with an increased risk of DILI *via* five mediating metabolites, which were *Blautia*, *Fusicatenibacter*, *Prevotella 7*, and *Oscillospira*. Román-Sagüillo et al.[59] evaluated

Table 1 Studies characterizing the gut microbiota in DILI. Taxa are listed as reported in the original studies; phylum headings do not necessarily match the families/genera listed below. "Norank" or "unclassified" indicates unclassified taxa at that rank, shown at the lowest confidently assigned level.

Comparative groups	Hepatotoxic drug	Changes of gut microbiota		Methodology	Ref.
		DILI−increased taxa	DILI−decreased taxa		
Lister hooded rats DILI *vs.* healthy control	Tacrine	Family: Enterobacteriaceae Genus: *Bacteroides*	Genus: *Lactobacillus*	16S rRNA sequencing	Yip et al. 2018[50]
C57BL/6J DILI mice *vs.* healthy mice	APAP	Phyla: Deferribacteres, Cyanobacteria, and Desulfobacterota Genera: *Oscillibacter*, *Mucispirillum*, *Colidextribacter*, and *Bacteroides*	Phylum: Actinobacteria Genera: *Dubosiella, Bifidobacterium, Lactobacillus, Allobaculum,* Prevotellaceae_*UCG-001, and Candidatus_Saccharimonas*	16S rRNA sequencing V3−V4	Xia et al. 2022[51]
Kunming DILI mice *vs.* healthy mice	APAP	Phyla: Deferribacterota Genera*: Enterococcus, Bacteroides, norank_f_norank_o_ Clostridia_UCG-014, Erysipelatoclostridium, Blautia, Colidextribacter, Gordonibacter, Eubacterium_fissicatena_group, norank_f_Eubacterium_coprostanoligenes_group, Eubacterium _nodatum_group, Family_XIII_AD3011_group, Eubacterium_brachy_ group,* and *Oscillibacter*	Phylum: Firmicutes Genera: *Lactobacillus and Odoribacter*	16S rRNA sequencing V3−V4	Xu et al. 2022[52]
Kunming DILI mice *vs.* healthy mice	MXT	Phyla: Firmicutes, Actinobacteria and Proteobacteria Genera: *Staphylococcus, Enterococcus, Collinsella, Streptococcus,* and *Aerococcus*	Phyla: Bacteroidota and Fusobacteriota Genera: *Lactobacillus, Ruminococcus, norank_f_Muribaculaceae, unclassified_f_Lachnospiraceae, norank_f_Lachnospiraceae, Eubacterium_xylanophilum_group, Phascolarctobacterium, Bifidobacterium,* and *Faecalibaculum*	16S rRNA sequencing V3−V4	Wang et al. 2022[53]
Sprague Dawley rats DILI *vs.* control	Antithyroid drugs	Phyla: Bacteroidetes, Proteobacteria, and Spirochaetae Genera: *Clostridium_sensu_stricto_1,* Prevotellaceae_*UCG−003,* and *Oscillibacter*	Phylum: Firmicutes Genera: *Lactobacillus, Romboutsia,* and *Faecalibacterium*	16S rRNA sequencing	Sun et al. 2020[54]
Treat Graves' disease patients *vs.* initial Graves' disease patients	Antithyroid drugs	Phyla: Firmicutes, and Actinobacteria Genera: *Eubacterium_rectale*, *Romboutsia,* and *Dorea*	Phylum: Bacteroidetes Genera: *Faecalibacterium, Clostridium_sensu_stricto_1,* and *Bacteroides*	16S rRNA sequencing	Sun et al. 2020[54]
ATD−DILI patients *vs.* non−ATD −DILI patients	ATD	Genera: *Enterococcus, Faecalibacterium, unclassified_f_Burkholderiacea, Cardiobacterium, Ruminococcus gnavus,* and *Tyzzerella_4*	Genera: *Prevotella_9, Akkermansia, Erysipelotrichaceae_UCG−003, Rubrobacter, and norank_f_Desulfovibrionaceae*	16S rRNA sequencing V3−V4	Pei S. et al. 2025[55]
DILI BALB/c mice *vs.* control mice	Isoniazid	Genera: *Muribaculaceae, Bifidobacterium, GCA_900066576, Blautia, Morganella, Oscillibacter, Collidexaribacter, Turicibacter, Mycoplasma, Corynebacterium, and Paludicola*	Order: Campylobacterales Species: *Anaerotruncus_colihominis, Bacteroides_yulgatus,* and *Helicobacter_typhlonius*	16S rRNA sequencing V3−V4	Liu et al. 2023[56]
DILI patients *vs.* healthy controls	Conventional drugs and dietary supplements	Phylum: Bacteroidetes	Phylum: Firmicutes Genera: *Acetobacteroides, Blautia, Caloramator, Coprococcus, Flavobacterium, Lachnospira, Natronincola, Oscillospira, Pseudobutyrivibrio,*	16S rRNA sequencing V3−V4	Rodriguez et al. 2022[57]

			Shuttleworthia, Themicanus, and *Turicibacter*		
DILI patients *vs.* health patients	Conventional drugs (antibiotics were excluded)	Phyla: Bacteroidota, Firmicutes, Fusobacteriota, and Acidobacteriota Genera: *Streptococcus*, *Faecalibacterium*, *Bacteroides,* and *Klebsiella* Key genera: *Enterococcus* and *Veillonella*	Phyla: Proteobacteria, Verrucomicrobiota, Desulfobacterota Genera: *Bifidobacterium*, *Blautia,* Ralstonia, *Subdoligranulum,* and *Dialister*	16S rDNA sequencing	He et al. 2023[58]
DILI patients *vs.* control	Conventional drugs	Family: *Sutterellaceae* Genera: *Alloprevotella, Anaerotruncus*	Family: *Barnesiellaceae*, Clostridiaceae Genera: *Eubacterium, Parasutterella* *Barnesiella, Clostridia UCG−014, Clostridium sensu stricto 1, Erysipelotrichaceae UCG−003, Monoglobus,* and *Romboutsia*	16S rRNA sequencing V3−V4	Román−Sagüillo et al. 2024[59]
DILI patients *vs.* healthy controls	Conventional drugs (27.8%) and herbs (66.7%)	Phyla: Proteobacteria and Actinobacteria	Phyla: Firmicutes and Bacteroidetes Class: Clostridia Order: Clostridiales Genera: *Bacteroides* and *Bifidobacterium*	16S rRNA sequencing	Zhao et al. 2022[60]

Abbreviations: DILI, drug-induced liver injury; APAP, acetaminophen; MXT, methotrexate; PM, *Polygonum multiflorum*; ATD, anti-tuberculosis drugs; rRNA, ribosomal RNA; rDNA, ribosomal DNA.

the specific gut microbiota profile in amoxicillin-clavulanic-induced liver injury in humans. They suggested that the disrupted gut microbiota profile observed may predispose patients with iDILI to inflammation, intestinal permeability, and worsening liver injury. Specifically, they detected an increase in the relative abundance of the family *Sutterellaceae* and the genera *Alloprevotella* and *Anaerotruncus*, along with a decrease in the genera *Eubacterium* and *Parasutterella*. They also highlighted a reduction in the relative abundance of the *Barnesiellaceae* and Clostridiaceae families, with significant reductions in *Barnesiella*, *Clostridia UCG-014,* and *Monoglobus* genera compared to a non-iDILI acute hepatitis control group.

Currently, it is difficult to determine whether gut dysbiosis is a direct cause of DILI or a consequence of liver damage and intestinal barrier dysfunction resulting from drug intake, or probably both. Evaluating this becomes particularly complex in the case of antibiotics, given that they themselves eliminate certain species of gut microbiota. In addition, most studies on gut microbiota and DILI have been conducted in animal models, making it challenging to extrapolate results to humans due to complex interactions with the human microbiota. On the other hand, as DILI is a low-prevalence condition, it is difficult to obtain a significant cohort of patients and to collect fecal samples at the time of and before liver injury[83]. Understanding the precise role of the gut microbiota in DILI could lead to new targets for early diagnosis, prevention, and treatment by monitoring its composition and function.

3.2.2. Effect of gut microbiota on drug pharmacokinetics and toxicodynamics

The interaction between drugs and the gut microbiota is a two-way process: drugs can cause dysbiosis, and the gut microbiota can generate different responses to drugs in individuals. The gut microbiota can directly modulate the expression, secretion, or action of enzymes involved in drug absorption, metabolism, activation, and inactivation—a phenomenon known as pharmacomicrobiomics. This can affect the viability, efficacy, and toxicity of the drug in an individual, potentially increasing its toxic potential and thereby raising the risk of hepatotoxicity[84,85]. In a model of tacrine hepatotoxicity, changes in the gut microbiota (*Lactobacillus*, *Bacteroides*, and family Enterobacteriaceae) were shown to increase liver damage in rats through increased activity of the intestinal beta-glucuronidase enzyme[50]. The gut microbiota is also able to induce cytotoxicity in HepG2 cells by converting geniposide to genipin, one of the major bioactive components of the traditional Chinese medicine *Gardeniae Fructus*[86,87]. On the other hand, the microbiota can modulate the expression of metabolic enzymes, compete for the metabolism of certain drugs, and increase the sensitivity of the liver to chemical injury through the metabolites they produce[88]. For example, phenylpropionic acid produced by gut microbiota can attenuate APAP-induced liver damage in mice by reducing the hepatic *Cyp2e1* levels, the principal enzyme that converts APAP into its toxic metabolite[89]. The microbiota-derived metabolite 1-phenyl-1,2-propanediol (PPD) has been shown to reduce glutathione (GSH) levels, the main detoxification mechanism for the toxic metabolite of APAP, in a rat model of APAP-induced liver injury. A reduction in GSH reduces the liver's ability to protect itself, and could contribute to mitochondrial ROS production, hepatocyte necrosis, and ALF[11,90]. Gong et al.[91] observed that *Citrobacter freundii* and *E. coli*, both PPD-producing bacteria, were increased in mice with APAP-induced liver injury, enhancing the production of PPD and

exacerbating APAP liver toxicity. In addition, the metabolite PPD was involved, at least in part, in daily changes in hepatotoxicity induced by APAP. It is known that liver injury induced by APAP has daily variation, being more severe when APAP is administered at night because of the lowest levels of GSH at this time. On the contrary, PPD levels were higher at night, thereby increasing susceptibility to liver damage in mice. P-Cresol is a protein derived from tyrosine produced by gut microbiota. Changes in the gut microbiota leading to increased p-cresol levels may increase the toxicity of drugs such as APAP due to p-cresol competition for the glutathione-dependent detoxification pathway[92].

When evaluating changes in the gut microbiota, it is essential to consider not only the beneficial or harmful effects of the altered microbiota but also its direct and indirect effects on drug pharmacokinetics and toxicodynamics. Although a significant proportion of the evidence in this field has been studied in animal models of APAP-induced liver injury, the presence of certain gut microbiota could have the potential to exacerbate the hepatotoxic effect of particular drugs and increase the risk of DILI.

Highlights

- Animal models and patients with DILI exhibit gut dysbiosis, which is characterized by changes in the richness, diversity, and composition of the gut microbiota.
- Several studies have revealed an altered Bacillota/Bacteroidota ratio, with a gut microbiota profile enriched in pro-inflammatory genera, and a reduction in anti-inflammatory and hepatoprotective genera, such as *Bifidobacterium* and *Lactobacillus.*
- The gut microbiota can directly, or *via* bacterial metabolites, modulate the pharmacokinetics and toxicodynamics of certain drugs. This can potentially increase their toxicity and reduce the liver's ability to protect itself, consequently increasing the risk of hepatotoxicity.
- The most likely hypothesis is that gut dysbiosis acts as both a causal and resulting factor in DILI. In a vicious feedback cycle, drug-induced or pre-existing gut dysbiosis may intensify liver inflammation; in turn, hepatotoxicity triggers gut dysbiosis.

3.2.3. Alteration of bacterial metabolite production

The gut dysbiosis observed in DILI may alter bacterial metabolite production, with implications for the development and progression of liver damage. Two major bacterial metabolites playing important roles in maintaining intestinal epithelial integrity, immune homeostasis, and regulation of oxidative stress and inflammation have been identified in DILI: SCFAs and secondary BAs[93,94].

3.2.3.1. The role of SCFAs in DILI. SCFAs are one of the main metabolites produced by the gut microbiota as a consequence of the fermentation of non-digestible complex carbohydrates from the diet[95]. The most common SCFAs are acetate, propionate, and butyrate, and to a lesser extent, formate, valerate, and caproate. The main bacteria responsible for the production of SCFAs are *Bacteroides*, *Bifidobacterium*, *Faecalibacterium,* and *Lactobacillus*[96]. Among their functions, SCFAs act as a source of energy for enterocytes and modulate physiological processes of the intestinal barrier, metabolism, and immune response[97]. These functions are exerted through the action on G protein-coupled receptors (GPCRs) that are differentially expressed on IECs, immune cells, and endocrine cells[98]. The action of SCFAs on GPCRs, particularly GPR109A, can inhibit nuclear factor kappa-B (NF-κB) activation in IECs and immune cells. Through GPCR43 and GPCR41 receptors (also known as FFAR2 and FFAR3, respectively) expressed on immune cells, SCFAs can decrease the production of pro-inflammatory cytokines and upregulate anti-inflammatory cytokines, as well as increase the activity of colonic regulatory T cells[93,99].

Current evidence of SCFAs effects in DILI comes from animal models. In general, a reduction in fecal levels of SCFAs[51,64], and especially butyrate, the main component of SCFAs, has been observed in animal models of DILI. This means that its beneficial effects on the intestinal barrier are reduced, as well as its anti-inflammatory and antioxidant effects on the liver. A reduction in SCFAs in APAP-induced liver injury has been linked to a decrease in the number of bacteria that produce SCFAs, such as *Lactobacillus*, *Bifidobacterium,* or *Odoribacter*[51,52,100](Fig. 2B). In the study by Xia et al.[51], administration of *Akkermansia muciniphila* (*A. muciniphila*) ameliorated APAP-induced liver injury, in part by directly increasing SCFAs production and remodeling the microbiota community in favor of SCFAs-producing bacteria. On the other hand, in the study by Li et al.[100], ampicillin administration exacerbated APAP-induced liver injury, partly due to butyrate depletion in the feces of mice caused by antibiotic-induced dysbiosis. However, butyrate levels were restored by supplementation with the probiotic *Lactobacillus* spp., which ameliorated liver necrosis and function by activating nuclear factor E2-related factor 2 (Nrf2) signaling, a master regulator of redox homeostasis. This antioxidant effect of butyrate, mediated by its action on Nrf2 and mitochondrial function, was also demonstrated in HepG2 cells[101,102]. On the other hand, butyrate can downregulate NF-κB and inflammation by activating the TLR4−LPS pathway *via* its interaction with the GPR109A receptor on IECs, thereby conferring protection against intestinal inflammation[103,104]. Indeed, intragastric administration of the probiotic *Bifidobacterium longum* has been shown to ameliorate LPS-induced acute liver injury in mice by actively remodeling the intestinal microbiota and producing acetate and butyrate, which directly modulate the TLR4/NF-κB pathway and inflammation[105]. Moreover, butyrate also helps maintain the integrity and function of the intestinal and mucus barriers. Butyrate influences tight junction (TJ) protein assembly, increasing the expression of claudin-1 and ZO-1, decreasing LPS translocation, and promoting MUC2 production by goblet cells[106−108]. Butyrate deficiency has previously been associated with TJ lesions and impaired intestinal permeability in animal models of ulcerative colitis[109].

3.2.3.2. The role of BAs in DILI. Primary BAs are molecules synthesized in hepatocytes from cholesterol by two main pathways: the classical and the alternative[110]. In humans, the main primary BAs are cholic acid (CA) and chenodeoxycholic acid (CDCA), whereas in mice, the predominant primary BAs are CA and muricholic acid[111]. Many primary BAs are conjugated with glycine or taurine, which will be stored as bile salts until they are secreted into the duodenum to help food digestion[108]. Approximately 95% of BAs returned to the liver *via* the enterohepatic circulation[108,112]. The remaining primary BAs can be excreted or deconjugated by bacterial bile salt hydrolases and undergo further modification by the commensal gut microbiota, resulting in more hydrophobic secondary BAs[113]. Deoxycholic acid (DCA) and lithocholic acid (LCA) are the major secondary BAs in humans, whereas DCA, hyodeoxycholic acid, and murideoxycholic acid

are the predominant secondary BAs in mice[114]. The main bacteria mediating the conversion of primary to secondary BAs are members of the class Clostridia, particularly *Clostridium scindens* and *C. hylemonae*, as well as related species possessing 7α-dehydroxylation activity. *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* contribute indirectly by deconjugating BAs *via* bile salt hydrolases, facilitating subsequent transformation by *Clostridium* spp[115]. BAs regulate their own liver metabolism, as well as lipid and glucose metabolism, intestinal barrier integrity, and inflammatory response, mainly by binding to the nuclear receptor farnesoid X receptor (FXR)[116] and the plasma membrane bile acid receptor Takeda G protein-coupled receptor 5 (TGR5), also known as G protein-coupled bile acid receptor 1 (GPBAR1)[117,118]. These receptors are expressed in many cell types, including IECs, hepatocytes, and immune cells. Abnormal metabolism of BAs would lead to alteration of the FXR and TGR5 signaling pathways, and this has been implicated in inflammatory and gastrointestinal disorders, as well as hepatic liver disease[119,120].

Several studies have shown that DILI is associated with a dysfunction of BAs metabolism and transport. Specifically, an increased primary/secondary BAs ratio has been observed in the serum of DILI patients compared to healthy controls, with an accumulation of primary BAs and conjugates (*e.g.*, glycochenodeoxycholic acid (GCDCA), glycocholic acid (GCA), taurochenodeoxycholic acid (TCDCA), and taurocholic acid (TCA)), and decreased secondary BAs (*e.g.*, DCA and LCA)[59,81,121−124], contributing to an imbalance of the BA pool towards more hydrophobic and potentially hepatotoxic forms. Specific BAs profiles have been proposed as potential biomarkers for certain types of DILI[121,125−127] and certain primary and conjugated BAs have been positively correlated with prognosis and severity[60,121,128,129]. Monte et al.[130] have recently proposed that BAs species and/or ratios can improve the prognostic value of the Model for End-Stage Liver Disease score in DILI, enabling the early identification of patients at risk of severe disease progression.

The accumulation of toxic BAs in the liver has been proposed as a mechanism of liver damage in DILI, contributing to increased oxidative stress, mitochondrial dysfunction, and immune activation. This accumulation is associated with the effect of certain drugs, which can directly affect BAs synthesis and secretion or export to systemic circulation[131−133]. However, the gut microbiota can contribute to impaired BAs homeostasis. The study by Zhao et al.[60] found lower levels of *Bifidobacterium*, *Clostridia*, and *Clostridiales* in DILI patients compared to healthy controls. These bacteria convert primary to secondary BAs and deconjugate BAs, respectively. This reduction in bacteria could explain the decrease in secondary BAs and the increase in conjugated primary BA levels. In addition, serum fibroblast growth factor 19 (FGF19) levels were elevated in DILI patients and correlated with disease severity. Liver transcriptome analysis showed that the hepatic expression of *CYP7A1* and genes related to BAs synthesis and BAs uptake and excretion (*NTCP*, *OATP1B1*, *OATP1B3*, and *BSEP*) were decreased. Binding of BAs to the FXR receptor (CDCA is the most potent agonist) in enterocytes is known to promote the synthesis and release of FGF19 in humans (FGF15 in rodents), which enters the liver *via* the portal vein and binds and activates the β-Klotho/FGFR4 receptor complex[134]. This binding activates hepatic FXR and, consequently, inhibits *CYP7A1* expression, the rate-limiting enzyme in the classical pathway of hepatic BAs, providing a negative feedback mechanism to prevent excessive synthesis of BAs[60]. CYP8B1 has also been found to be compromised in liver injury, and the proinflammatory environment in DILI patients may contribute to the inhibition of *CYP8B1* gene transcription[135,136]. This inhibition mechanism is an attempt by the liver to prevent further accumulation of BAs and thus further liver damage in DILI (Fig. 2). Conversely, ibuprofen has been associated with gut dysbiosis and increased fecal excretion of BAs in mice, reducing hepatic BAs biosynthesis by potentially deactivating the intestinal FXR−FGF15 signaling pathway and inducing *Cyp7a1*[137]. This mechanism suggests that the ultimate goal in DILI is to restore BAs homeostasis to minimize or prevent liver injury. The activation of the FXR-FGF15/FGF19-FGFR4/β-Klotho axis by BAs also plays a key role in maintaining intestinal epithelial integrity and exerts anti-inflammatory effects[138]. Supplementation with DCA or LCA has been associated with promoting intestinal stem cell renewal and proliferation and inhibiting NLRP3 inflammasome activity in macrophages[139].

In a mouse model of triclosan-induced liver damage, xenobiotic treatment was shown to cause accumulation of total BAs and a reduction of secondary BAs DCA and LCA in the systemic circulation, as well as decreased TGR5 and increased TLR9 expressions in liver tissue[64]. Something similar may occur in DILI, as elevated levels of serum mitochondrial DNA (mtDNA) have been detected in APAP-induced ALF patients and were related to the clinical outcome[140]. Excess of BAs found in DILI may lead to liver damage through the release of mtDNA, which activates TLR9 and stimulates the production of pro-inflammatory cytokines[141] (Fig. 2A). TGR5 activation has also been linked to the activation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α). PGC1α is a master regulator of mitochondrial biogenesis, oxidative stress, and energy metabolism[142,143]. In animal models of MASH, dual activation of TGR5/FXR has been shown to reverse steatosis, inflammation, and liver fibrosis by restoring mitochondrial function and reducing oxidative stress. Reduced TGR5 in DILI may indicate decreased PGC1α. This effect may partially contribute to oxidative stress and mitochondrial dysfunction in DILI due to the inability to generate additional mitochondrial mass in order to cope with stressful situations or increased cellular energy requirements. On the other hand, TGR5 is mainly activated by unconjugated secondary BAs, such as LCA and DCA[144], whose activation has hepatoprotective and anti-inflammatory effects[145−147]. In particular, it has been observed that secondary BAs can inhibit the production of pro-inflammatory cytokines induced by LPS through their action on TGR5[148]. TGR5 activation has also been linked to the polarization of pro-inflammatory M1 macrophages towards anti-inflammatory M2 macrophages[143]. The reduction of DCA and LCA in DILI may reduce the anti-inflammatory effect mediated by the TGR5 signaling pathway.

Some immune cells also express the TGR5 receptor and, in particular, secondary BAs activate TGR5 on NKT cells[149]. NKT cells play an important role in regulating hepatic immune homeostasis through the recognition of CD1d-restricted glycolipid antigens, the secretion of inflammatory and anti-inflammatory cytokines[150], and the elimination of senescent hepatocytes. Two subtypes of NKT cells have been described: the proinflammatory subtype I, principally activated by lipids, and the type II subtype, which plays a protective role in liver inflammation[151,152]. In a mouse model of immune-mediated hepatitis, activation of TGR5/GPBAR1 protected mice from liver damage by favoring the polarization of NKT cells towards the natural killer regulatory subtype T10 (NKT10) and the expansion of IL-10-secreting type II NKT cells. Secondary BAs are TGR5 agonists, so their reduction

in DILI could reduce this anti-inflammatory effect[153] (Fig. 2A). Another study has demonstrated that gut microbiota also aggravates hepatitis by activating NKT cells *via* DCs, which are stimulated by glycolipids from pathogenic bacteria during dysbacteriosis[154]. FXR is also expressed in liver NKT cells, and its activation may inhibit osteopontin production, a potent pro-inflammatory mediator. In a mouse model of concanavalin A-induced acute liver injury, FXR activation rescued the mice from liver injury. This protective effect was associated with negative regulation of NKT efflux to the liver and decreased markers of NKT activity, including osteopontin[155]. In DILI, the increase in primary BAs and potent FXR agonists may reduce NKT-mediated osteopontin production, thereby reducing liver inflammation as the liver attempts to control it. However, this anti-inflammatory effect is likely insufficient to control inflammation in an immunologically altered environment and in the context of overall BAs imbalance. Osteopontin has been suggested as a potential prognostic biomarker in DILI[156], although its exact role in liver injury is unclear, as it has been associated with both proinflammatory and regenerative roles[157].

Highlights

- *A re*duced level of SCFAs has been found in animal models of DILI, probably due to a reduction in SCFAs-producing bacteria such as *Bifidobacterium* and *Lactobacillus.*
- Reduction of SCFAs could contribute to worsening liver damage by reducing the organ's antioxidant and anti-inflammatory effects, affecting the intestinal barrier integrity, and allowing higher levels of LPS to reach the liver. This promotes the LPS-TLR4 signaling pathway and liver inflammation.
- The primary/secondary BAs ratio is increased in DILI patients, reflecting an imbalance in BAs homeostasis towards more hydrophobic and potentially hepatotoxic forms.
- The imbalance in BAs has been linked to an inhibition of hepatic BA efflux and a reduction in the number of bacteria involved in converting primary BAs into secondary BAs and deconjugating BAs.
- The FXR-FGF15/FGF19-FGFR4/β-Klotho axis has emerged as an important pathway for maintaining BAs homeostasis by controlling BAs biosynthesis *via CYP7A1* in DILI.
- Total liver BAs accumulation can lead to the release of mtDNA, which activates TLR9 signaling. Together with the reduction of secondary BAs and their anti-inflammatory effects mediated through TGR5, these processes can contribute to liver inflammation in DILI.
- NKT cells are a type of unconventional T cell that could play an important role in the pathogenesis of DILI by inducing TGR5 and FXR signaling pathways due to the dysfunction of BAs homeostasis and dysbacteriosis present in DILI.

3.2.4. Dysfunction of the intestinal barrier

The intestinal barrier is a highly specialized barrier consisting of a mucosal layer, an intestinal epithelium, and a local immune system whose integrity and function are essential for the proper functioning of the gut−liver axis. The liver is one of the main organs affected when the integrity of the intestinal barrier is disrupted, as 70% of blood from the intestine enters the liver *via* the portal vein[158]. Indeed, liver injury may be triggered or exacerbated by intestinal immune activation, which promotes inflammation and the recruitment of immune cells to the liver[159]. Evidence suggests that gut dysbiosis is associated with intestinal barrier dysfunction and intestinal inflammation and, consequently, increased intestinal permeability, driving the onset and progression of several liver diseases[31]. This "leaky gut" contributes to the translocation of gut microbiota-derived products into the systemic circulation and the liver[49]. Studies in animal models and patients with DILI suggest that there is physical and chemical damage to the intestinal barrier and altered intestinal immune homeostasis, triggered by gut dysbiosis and by DAMPs and cytokines secreted by the damaged liver.

3.2.4.1. Alteration of the mucus layer's elements. Dysbiosis has been associated with alterations in the mucus layer in DILI. Some studies suggest a reduction of goblet cells, *Muc2* expression, and mucus excretion in animal models of DILI[51,64,160]. Specifically, Xia et al.[160] observed that the abundance of *Muribaculaceae* was negatively correlated with mucus layer thickness and positively correlated with intestinal permeability tests in mice with methotrexate-induced liver injury. *A. muciniphila* is an anaerobic Gram-negative bacterium of the phylum *Verrucomicrobia* that colonizes the intestinal mucus layer, where it degrades mucin and produces regulatory metabolites such as SCFAs[161]. Reduced levels of *A. muciniphila* have previously been associated with mucus layer thinning, impaired barrier function, and intestinal inflammation[162,163]. In DILI, its abundance appears to vary depending on the etiology. A significant decrease of *A.muciniphila* has been reported in antibiotic-associated DILI, correlating negatively with liver function parameters and pro-inflammatory cytokines[55], and probably with the lack of its beneficial effects on the intestinal barrier. However, in APAP-induced liver injury, an expansion of *A. muciniphila* has been observed along with increased mucus thickness, possibly reflecting a compensatory mechanism to preserve barrier integrity in response to a higher liver damage induced by the sublethal dose of the drug[66].

3.2.4.2. Alteration of intestinal epithelium elements and intestinal permeability. Intestinal permeability reflects, to some extent, the integrity of the intestinal epithelium[164]. Intestinal permeability can be measured *in vivo* by administering fluorescein isothiocyanate-conjugated (FITC)-dextran[165], but also indirectly and non-invasively through the measurement of peripheral markers related to intestinal homeostasis and structures present in the intestinal barrier, such as components of the TJ proteins, the intestinal fatty acid binding proteins (I-FABP/FABP2), or the endotoxin LPS and the proteins involved in its signaling pathway, including LPS-binding protein (LBP) and CD14[166].

In a murine model of APAP-induced liver injury, APAP decreased occludin mRNA levels and reduced claudin protein levels, alongside increased serum LPS levels and gut dysbiosis, ultimately exacerbating liver inflammation and damage[51]. The intestinal damage was correlated with the direct apoptotic effect of APAP in the intestine and the reduction of *A. muciniphila*. This was in line with Chopyk et al.[167], who demonstrated that APAP overdose caused the rapid apoptosis of intestinal cells, particularly crypt base stem cells, with long-term consequences for gut barrier integrity. Niu et al.[168] observed that mice treated with APAP exhibited gut leakiness, characterized by elevated fecal albumin levels, reduced levels of occludin and claudin-4 proteins in the colon, and a disrupted colonic epithelial barrier. These effects were associated with gut dysbiosis and direct APAP-induced

damage. The main contributing factor was the overexpression of the chemokine CCL7 ligand in epithelial colonic cells. All these alterations allowed bacterial products and immune cells to translocate to the liver, resulting in more severe liver damage and inflammation compared to control mice. Mice treated with methotrexate also showed epithelial damage, intestinal inflammation, elevated serum FITC-dextran levels, and decreased expression of intercellular junction proteins, including ZO-1, claudin-1, and E-cadherin[160]. Intestinal barrier dysfunction was ameliorated by oral administration of *Lactobacillus*, a genus reduced by the MTX treatment. In rats with liver injury induced by antithyroid drugs, evidence of intestinal barrier dysfunction has been observed through the detection of elevated serum levels of FITC-dextran and transmission electron microscopy images[54]. Sun et al.[54] observed that gut dysbiosis induced by antithyroid drugs was associated with side effects, including intestinal barrier dysfunction and LPS production. Recently, our group has observed that patients with iDILI caused by conventional drugs had a decrease in serum zonulin levels during the acute phase of liver damage, reaching practically normal levels after 30 days. In addition, no significant differences in serum I-FABP levels were detected compared with healthy controls[169]. However, regarding the reduced zonulin levels in patients with iDILI, these results contradict existing evidence for other conditions associated with increased intestinal permeability, such as celiac disease, type 1 diabetes, obesity, and MASLD[170,171]. Specifically, zonulin levels were significantly higher in patients with MASLD than in controls, and there was a clear correlation between zonulin levels and the severity of liver steatosis and inflammation. The proinflammatory cytokines or chemokines secreted by the damaged liver or by intestinal inflammation induced by gut dysbiosis may increase paracellular intestinal permeability in DILI patients without affecting the expression of TJ proteins or through mechanisms other than the zonulin signaling pathway[172,173], as has already been observed in other pathological entities[174]. One plausible hypothesis is that increased activity of phosphorylated myosin light chain kinase (p-MLCK) in the colon mediates alterations in TJ proteins in response to chemotactic signals, such as CCL7. This mechanism has previously been described in APAP-induced liver injury in animal models. Additionally, zonulin regulates intestinal permeability in a reversible fashion; thus, reduced zonulin levels in DILI patients may be a human protective mechanism in an attempt to enhance the integrity of the intestinal barrier and prevent the translocation of PAMPs from entering the bloodstream, a phenomenon that has also been suggested in patients with hepatitis B[175].

3.2.4.3. Promising markers of intestinal permeability. Growth differentiation factor 15 (GDF15) is considered a cytokine induced by cellular and mitochondrial stress and pathological conditions. GDF15 is widely expressed in various cell types, including the gastrointestinal tract, the liver, and macrophages. Tissue expression levels of GDF15 are strongly correlated with circulating levels, making it a reliable indicator of tissue damage[176,177]. Our group has recently demonstrated that GDF15 downregulates TJ proteins, such as ZO-1 and claudin-1, in colonic organoids, thereby increasing intestinal barrier permeability. Indeed, plasma GDF15 levels were correlated with circulating markers of increased intestinal permeability (specifically, LBP)[178]. Research conducted in animal models has indicated an increase in GDF15 following various forms of liver damage[176]. To date, only one study has demonstrated GDF15 upregulation in an animal model of APAP-induced liver injury. However, GDF15 overexpression and exogenous administration have shown no hepatoprotective effects[179]. The role of GDF15 in DILI has not yet been studied in humans, but, as in other liver diseases, GDF15 may be elevated due to mitochondrial dysfunction in these patients. Previous accumulated evidence suggests that GDF15 exerts a protective effect on acute and chronic liver damage, acting as an anti-inflammatory, anti-hepatosteatotic, and anti-fibrotic molecule, inhibiting excessive ROS production and mitochondrial damage[180]. GDF15 has also been related to gut microbiota reprogramming and intestinal homeostasis[181]. In DILI, GDF15 may act as a protective compensatory mechanism in response to cellular stress by modulating the inflammatory environment, the gut microbiome, and intestinal barrier function.

Although evidence suggests that intestinal barrier dysfunction is present in DILI, the mechanisms underlying increased intestinal permeability, given the complex interactions among the different layers of the human gut, remain unknown. On one hand, some drugs can directly damage the intestinal barrier, such as the overdose of APAP. On the other hand, intestinal dysbiosis, which is observed both in animal models and patients with DILI, is likely to be partly involved in the dysfunction of the intestinal barrier, both directly and by promoting a pro-inflammatory environment in the intestine[28]. Conversely, the proinflammatory environment triggered during liver injury could induce or be triggered by increased intestinal permeability and/or dysbiosis, perpetuating a proinflammatory loop[182]. In any case, a disrupted intestinal barrier leads to increased intestinal permeability, resulting in the translocation of PAMPs and bacterial-derived metabolites into the liver, exacerbating liver injury by aggravating the immune response. The different results observed in animal models and in patients with DILI are probably due to the involvement of multiple factors in intestinal permeability, the different drug doses used, and the lack of standardization in the methods used to indirectly measure it.

3.2.5. Role of pathogen-associated molecular patterns (PAMPs)

3.2.5.1. Gut dysbiosis, LPS, and liver damage. LPS, one of the most studied bacterial endotoxins, is a component of the outer membrane of Gram-negative bacteria that can be released into the circulation and act as PAMPs. In humans, the gut microbiota is the largest source of LPS. In health, small amounts of LPS reach the liver *via* the portal vein, but the liver can exert an immune tolerance role and remove LPS from the body[10]. However, when intestinal barrier function is altered, increased levels of bacteria-derived compounds can translocate to the liver and promote the activation of the innate immune system, thereby triggering proinflammatory immune responses that contribute to the development and progression of various liver diseases, such as alcoholic liver disease, MASLD, MASH, liver fibrosis, and hepatocellular carcinoma[183]. Specifically, LPS is recognized by the TLR4/MD2 receptor complex on the surface of innate immune cells, a process facilitated by CD14. This binding activates an internal signaling cascade that depends on the myeloid differentiation factor 88 (MyD88) protein. This cascade culminates in the activation of NF-κB, which leads to the production of proinflammatory cytokines that contribute to or exacerbate liver inflammation[184,185]. LPS can also increase the expression of *CYP2E1*, leading to the formation of ROS that can induce hepatocyte death and promote hepatotoxicity[186]. The increased level of LPS in blood is highly indicative of intestinal overgrowth and dysbiosis, intestinal barrier dysfunction, increased intestinal

permeability, and bacterial translocation[66,187]. The severity of liver damage has also been related to the degree of endotoxemia and levels of pro-inflammatory cytokines [188,189].

3.2.5.2. The endotoxemia hypothesis in DILI. In recent years, several studies in animal models and in DILI patients have demonstrated elevated LPS levels in blood, and there is evidence of the involvement of the LPS−TLR4 signaling pathway in DILI. Animal models have shown that LPS signaling pathways are required to reproduce DILI caused by some drugs[190,191], and that TLR4 knockout mice show improved survival after APAP-induced liver injury[192,193]. For this reason, targeted therapeutic strategies have been developed, including anti-TLR4 antibodies and LBP protein inhibitor peptides, which have been shown to significantly reduce liver damage in animal models of APAP-induced liver injury, as well as the use of probiotics that act on the LPS/TLR4/NF-κB axis[194−196]. Enterobacteriaceae, a family within the Pseudomonata phylum (Proteobacteria), is one of the main LPS-producing bacteria. Enterobacteriaceae is increased in animal models and DILI patients by different drugs[50,53,54,58](Fig. 2B). The participation of LPS in the pathogenesis of DILI has been evidenced in animal models of APAP[51], antithyroid[54], and methotrexate-induced liver injury[160], which showed high levels of LPS in serum[51,54,160], increased expression of TLR4 and MyD88[51], activation of metabolic pathways related to LPS biosynthesis[54], and increased expression of proinflammatory cytokines and liver inflammation[160]. Although fewer studies have been conducted in humans, increased levels of LPS, LBP, and soluble CD14 have also been demonstrated in the plasma of patients with iDILI caused by different drugs, herbs, and dietary supplements at the time of acute damage. These levels returned to normal after about 4 weeks of follow-up, but remain significantly higher than those of healthy controls[169,197].

3.2.5.3. LPS and the role of macrophages in DILI. TLR4, a member of the PRRs, is expressed in all liver cell types, including KCs, hepatocytes, LSECs, and HSCs, but KCs are one of the main targets of LPS in the liver. KCs are the macrophages resident in the liver and, together with recruited monocyte-macrophages, constitute one of the main cells of the hepatic innate immune system. Due to their strategic location in the reticuloendothelial liver system, KCs act as the first line of defense in the liver[198]. In various pathological conditions, an excessive activation of KCs is induced by microbiota-derived LPS *via* the TLR4 axis[199]. In DILI, KCs in the liver may respond to elevated LPS, triggering TLR4−NF-κB signaling and worsening liver inflammation (Fig. 2A). Schneider et al.[66] observed that FMT from *Nlrp6*$^{-/-}$ mice, which were considered dysbiotic with intestinal barrier dysfunction, to wild-type mice exacerbated liver injury induced by APAP, and this was related to monocyte polarization towards a proinflammatory Ly6C^{hi} phenotype. The involvement of macrophages during liver damage in DILI is also supported by elevated levels of macrophage colony-stimulating factor-1 receptor (MCSF-1R) and soluble CD163 (sCD163) detected in the serum of iDILI patients, which have been positively correlated with enzymes that reflect liver function status, such as AST, ALT, and TBL[169,197]. The MCSF-1R is a transmembrane receptor that regulates macrophage proliferation, differentiation, and survival, and is involved in chemotaxis and migration of immune cells[200]. In DILI, MCSF-1R has been recently described as a promising prognostic marker[201] and the MCSF-1R signaling can induce the expression of CD163 in macrophages[202]. CD163 is a receptor scavenger expressed on the surface of activated macrophages, which can be rapidly cleaved and released into the circulation as soluble sCD163 upon activation by inflammatory signals, especially LPS, being a highly specific indicator of macrophage activation[203−205]. Further research is required into the role of sCD163 in DILI, given its potential dual role in inflammation. sCD163 levels have been positively correlated with the severity of liver damage in DILI patients, along with the degree of macrophage activation in the liver tissue itself[197,206,207]. However, sCD163 has also demonstrated immunoregulatory effects by decreasing T cell activity and proliferation *in vitro*[208], so elevated sCD163 levels may also indicate an immunomodulatory response in DILI. The participation of macrophages in DILI is controversial. Several studies have observed both pro-inflammatory and hepatoprotective and reparative functions, depending on the time at which liver damage is evaluated[209,210]. CD163 has also been described as a marker of macrophage polarization towards the M2 phenotype, with anti-inflammatory and tissue remodeling functions[209]. These M2 anti-inflammatory macrophages could derive from the switch of pre-existing pro-inflammatory M1 macrophages in the liver[204] or the recruitment of blood monocytes to the liver. In animal models of APAP-induced liver injury, KCs have also been associated with the promotion of activated IL-4-produced NKT cells, which inhibit the recruitment of neutrophils, reducing the degree of liver inflammation induced by APAP[211]. The dysfunction in BAs homeostasis may also contribute to the anti-inflammatory phenotype in DILI by the action of BAs on monocyte or macrophage receptors[212].

3.2.5.4. Flagellin and TLR5 signaling pathway in DILI. - Flagellin is a highly conserved structural protein expressed in the bacterial flagellum. Flagellin can be recognized as PAMPs by TLR5 in hepatic innate immune cells, especially hepatocytes, which are the main cellular source of TLR5[213]. Recently, TLR5 signaling has been shown to play a critical role in regulating immune responses and has been implicated in the pathogenesis of acute and chronic liver injury[214]. Xiao et al.[215] observed that high doses of flagellin induced overactivation of TLR5 signaling in mice, causing an acute inflammatory response in the liver. However, other studies suggest that TLR5 signaling may protect the liver from severe stress. In the study by Zhou et al.[216] flagellin attenuated acute liver damage in mice with APAP-induced liver injury, reducing histological and biochemical markers of injury, oxidative stress, and inflammation. This protective effect was abolished by the addition of a TLR5 antagonist. The mechanism of flagellin protection *via* TLR5 was, at least in part, explained by the promotion of Nrf2 translocation to the nucleus of hepatocytes, dependent on the activation of TLR5−JNK/p38 pathways, reducing liver toxicity by APAP. TLR5 signaling has also been shown to mitigate concanavalin A-induced hepatitis by limiting T-cell/NKT cell activation and subsequent pro-inflammatory cytokine release, and by increasing CD4^{+}CD25^{+}FoxP3^{+} Treg activity in the liver[217,218]. For this reason, an exogenous TLR5 agonist, such as CBLB502, has been proposed as a potential treatment for inflammatory liver diseases. TLR5 activation also appears to play a positive role in regulating the initial events of liver regeneration after partial hepatectomy[219]. However, it has also been shown that flagellin can induce inflammation by activating cytosolic nucleotide-binding oligomerization domain-like receptors[220]. Therefore, the roles of flagellin and TLR5 in liver injury remain controversial, and further studies are needed to

elucidate their mechanisms of action in immune-mediated liver injury and hepatotoxicity.

Highlights

- Animal models and DILI patients show evidence of intestinal barrier dysfunction, including impaired mucus and intestinal epithelial layers, as well as indications of the involvement of the endotoxemia hypothesis.
- Intestinal barrier dysfunction is likely to be caused by the direct action of the drug, drug-induced gut dysbiosis, or factors secreted by the damaged liver.
- These alterations increase intestinal permeability, allowing the translocation of PAMPs, particularly LPS secreted by gut microbiota, to the liver, which could potentially contribute to or exacerbate inflammation and liver injury.
- Flagellin and TLR5 signaling pathways may be involved in the pathogenesis of DILI, but their exact roles are unclear. Further research is required to determine whether these pathways play a proinflammatory or protective role in liver injury.

3.2.6. Recruitment of immune cells and activation of the hepatic immune response

3.2.6.1. Drivers of immune recruitment to the injured liver.- LSECs are the most abundant non-parenchymal cell type in the liver. These specialized endothelial cells are directly exposed to blood from the systemic circulation *via* the portal vein, including blood from the intestine. LSECs are located at the interface between hepatic sinusoids and the liver parenchyma, which contains hepatocytes and HSCs within the space of Disse[221]. Under normal conditions, LSECs act as APCs and regulate the entry of circulating leukocytes into the liver parenchyma. They play a key role in immune surveillance and inflammation. In cases of liver injury or inflammatory liver diseases, the recruitment of immune cells from the bloodstream to the liver increases, and the nature and distribution of the infiltrate determine the type and progression of the resulting liver damage[158,222−224]. DAMPs released during liver injury or PAMPs resulting from increased intestinal permeability can induce an inflammatory phenotype in LSECs and the KCs activation after recognition by their PRRs[225]. This involves the release of pro-inflammatory mediators through NF-κB activation and the assembly of the NLRP3 inflammasome, which contribute to inflammation and liver injury[226−228]. At the same time, chemoattractant signals are released and specific adhesion molecules are overexpressed, including intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1, and mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1). These molecules ultimately promote the adhesion and transendothelial migration of leukocytes into the injured liver by binding to integrins expressed on immune cells[222,225]. The migration of immune cells, including neutrophils, monocytes, eosinophils, and lymphocytes, to the liver is related not only to liver injury but also to the triggering of repair and regeneration mechanisms in DILI[210,229,230]. In animal models and humans with APAP-induced liver injury, increased levels of CXCL9, one of the CXCR3 ligands, have been associated with liver immune infiltration and death of hepatocytes[231] (Fig. 2A). Wang et al.[232] identified the CXCL10−CXCR3 axis as a critical pathway to recruit hepatic NKT and $CD4^{++}$ T cells in APAP-induced liver injury mice. Inhibition of CXCR3 blocked APAP-induced recruitment of immune cells. The accumulation of activated Tc cells and mononuclear phagocytes has also been directly associated with impaired liver function in patients with iDILI[233].

3.2.6.2. Th17/Treg homeostasis in liver injury. In a recent advanced immunophenotyping study led by our group, we detected increased expression of CCR6 and integrin $\alpha4\beta7$ in different subpopulations of circulating lymphocytes of iDILI patients compared to healthy controls[234]. This result, along with the evidence of immune infiltration in liver biopsies of DILI patients[235,236], supports the recruitment of lymphocytes to the liver parenchyma, as has already been observed in other autoimmune and inflammatory liver diseases[237]. CCR6 is a chemokine receptor activated by its ligand, the chemokine CCL20. CCL20 is expressed in tissues with active inflammation, favoring the recruitment of immune cells. Indeed, studies have demonstrated a direct correlation between CCR6 expression and the intrahepatic migration of Th17 lymphocytes in inflammatory liver diseases. Based on their cytokine secretion, Th cells can be divided into four main subtypes: Th1, Th2, Th17, and Treg[238,239]. An imbalance between the Th17 proinflammatory response and the immunoregulatory Treg response has been associated with acute and chronic liver diseases[240,241]. Gut microbiota is not only responsible for the intestinal balance of Th17/Treg cells but also effectively promotes liver Th17 and Treg cell-related immune responses[242,243]. Imbalance of Th17/Treg responses has been linked to intestinal dysbiosis, LPS translocation, and neutrophil migration to the liver, contributing to gut−liver axis dysregulation[244,245]. As previously mentioned, immunotolerance mechanisms, including Treg activity, may be critical in determining the prognosis and overcoming iDILI[45,246].

3.2.6.3. Gut-primed T cell hypothesis. The increased expression of integrin $\alpha4\beta7$ in different circulating lymphocyte populations[234] supports the hypothesis that gut-primed lymphocytes may home to the liver in iDILI, potentially contributing to immune dysregulation, inflammation, and hepatic injury (Fig. 2A). This is similar to what has been described for other liver diseases, such as primary sclerosing cholangitis, autoimmune hepatitis, or MASLD[12,159,247]. The heterodimeric integrin receptor $\alpha4\beta7$ regulates intestinal T cell recruitment *via* interaction with its ligand MAdCAM-1, which is constitutively expressed by endothelial cells and intestinal lamina propria[248,249]. Dysbiosis and PAMPs, leading to local intestinal inflammation and a leaky gut, have been shown to enhance the generation of gut-primed T cells, which express the integrin $\alpha4\beta7$ and CCR9. In liver injury, the hepatic endothelium can aberrantly express the gut-specific chemokine CCL25 and MAdCAM-1, which mediate the pathological recruitment of gut-primed T cells to the liver[250]. Chemokines secreted by epithelial target cells (such as injured hepatocytes) then bind to chemokine receptors, such as CCR6 or CXCR3, on effector T cells[251]. Indeed, deletion or blockade of MAdCAM-1 can reduce hepatic inflammation in acute immune-mediated hepatitis mice by decreasing the recruitment of pro-inflammatory immune cells to the liver[252,253].

Interestingly, iDILI patients show reduced levels of the soluble form of MAdCAM-1 and increased levels of the soluble form of ICAM-1[169]. MAdCAM-1 and ICAM-1 can be secreted as soluble molecules (sMAdCAM-1 and sICAM-1, respectively) into serum and urine. The reduction of sMAdCAM-1 could be indicative of endothelial dysfunction due to damaged LSECs by the drug or its

reactive metabolites[254]. Alternatively, it may serve as a regulatory mechanism to limit excessive migration of intestinal lymphocytes into the injured liver. The elevation of sICAM-1 has also been observed in other liver diseases such as primary biliary cholangitis, autoimmune hepatitis, or alcoholic liver disease[255,256]. In iDILI, this sICAM-1 elevation may indicate systemic inflammation or an ongoing reparatory process, as ICAM-1 has been related to both pro- and anti-inflammatory roles[257].

3.2.6.4. Potential role of unconventional T cells. Using mass cytometry, our group has detected significant differences in the frequency of circulating T$\gamma\delta$ cells and MAIT cells during the acute and recovery phases in iDILI patients compared to controls[234]. Both of these unconventional T cells are primarily located in barrier tissues, including the gut and the liver, and have been linked to dysregulation of the gut−liver axis and proinflammatory and immunoregulatory roles[244,258−260]. Studies have shown that alterations in the gut microbiota modulate the activity of intestinal and liver T$\gamma\delta$ cells, contributing to the development and resolution of liver diseases[261,262]. In experimental models of APAP-induced liver injury, serum high-mobility group box 1, a DAMP released from injured hepatocytes, activated TLR4^{++} macrophages and promoted the release of IL-23. This immune response generated IL-17A-producing T$\gamma\delta$ cells in the liver, which mediated neutrophil infiltration and inflammation. Depleting T$\gamma\delta$ cells significantly reduced IL-17A levels, attenuating neutrophil recruitment and liver injury[263]. Drug-induced gut dysbiosis or factors secreted by the injured liver may also contribute to the recruitment of intestinal or circulation IL-17A-producing T$\gamma\delta$ cells to the liver, regulated by CCR6 and gut commensal microbiota[264]. However, the role of these cells remains unclear, as T$\gamma\delta$ cells can also mitigate the harmful effects of autoreactive T cells and NKT cells in *in vivo* models of autoimmune diseases[265,266] or protect against liver fibrosis by inducing apoptosis of profibrogenic cells[229,267]. MAIT cells are particularly abundant in the liver (up to 50% of total T cells) and can respond rapidly to bacterial or inflammatory stimuli, exerting cytotoxic and proinflammatory functions[268]. Changes in the gut microbiota affect MAIT cell function, contributing to the pathophysiology of liver diseases[259]. In other liver conditions, MAIT cells have been found to be decreased in the blood but increased in the inflamed liver, with a hyperactivated phenotype but a functionally deficient state. The increased translocation of PAMPs from the intestine, especially in a context of gut dysbiosis, increased intestinal permeability, or inflammation, could contribute to MAIT dysfunction and, eventually, apoptotic cell death[269−272]. However, MAIT cells also contribute to immune tolerance and promote tissue repair[273]. Loss of MAIT cells has also been linked to increased intestinal permeability in animal models[274]. In other liver conditions, activated liver MAIT cells protected against inflammation by producing regulatory cytokines and inducing M2 macrophage polarization[275]. The role of MAIT cells in DILI remains unclear. It has been reported that certain drugs can modulate their activation by interacting directly with MHC class I-related molecules, such as MR1, expressed by APCs[276]. The role of T$\gamma\delta$ and MAIT cells in liver injury and their link with gut microbiota is undoubtedly an area of research with many unknowns yet to be resolved.

Highlights

- There is evidence of immune cell recruitment to the liver in both animal models and patients with DILI, which contributes to liver injury but also triggers repair and regeneration mechanisms.
- The pathological recruitment of gut-primed T cells to the liver is a plausible hypothesis that could lead to immune dysregulation, inflammation, and hepatic injury in DILI.
- Alterations in the gut microbiota can influence the activity of MAIT and T$\gamma\delta$ cells, thereby contributing to liver disease. Further research is needed to clarify the role of these unconventional T cells in DILI.

3.3. Targeting the gut−liver axis for DILI prevention and treatment strategies

The main therapeutic strategy for cases of DILI is to discontinue the suspected drug. As DILI patients exhibit gut dysbiosis, which appears to contribute to the pathogenesis of DILI, at least in part, approaches that reshape the gut microbiota or eliminate potentially pathogenic bacteria by supplementation with probiotics, postbiotics, dietary pattern modifications[277], FMT, or antibiotics have emerged as an attractive therapeutic or preventive alternative for alleviating liver injury[13,63,278].

3.3.1. Probiotics

Probiotics are living microorganisms that confer health benefits on the host when administered at adequate concentrations[279]. Two of the most commonly used probiotics today are *Lactobacillus* and *Bifidobacterium*. These genera have shown hepatoprotective and anti-inflammatory effects in different types of liver injury[63,280,281]. Probiotic *Bacillus* spores have also shown anti-inflammatory and antioxidant effects in rats with APAP-induced liver injury[282]. The antioxidant effect of probiotics, including *Lactobacillus*, *Bifidobacterium longum R0175*, and *Streptococcus salivarius,* has also been observed in animal models of APAP- and diclofenac-induced hepatotoxicity[283−285]. Pre-treating mice with the probiotic *Limosilactobacillus reuteri* DSM 17938 mitigated inflammation and liver injury induced by LPS and D-galactosamine through propionate production[286]. The use of a probiotic mixture containing *Bifidobacterium longum*, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii subspecies bulgaricus* mitigated liver injury caused by APAP overdose in mice. This was achieved by reducing leucine levels and, consequently, inhibiting the Hippo-YAP signaling pathway, which is crucial for liver regeneration[287]. The therapeutic potential of *A. muciniphila* has also been explored in several experimental models of DILI: its administration, or that of its EVs, has been shown to alleviate APAP- and CCl_4-induced liver injury by reducing systemic inflammation and oxidative stress, modulating immune responses, and maintaining gut barrier function[51,162,288,289]. In fact, probiotics also protect against liver injury by regulating intestinal homeostasis and the LPS-TLR4 signaling pathway. Specifically, *Lactobacillus casei* and *Lactobacillus rhamnosus* JYLR-005 conferred protection against liver injury induced by anti-tuberculosis drugs by reducing intestinal permeability and the translocation of LPS to the liver[75,191]. The use of *Bacteroides fragilis* 839 has shown similar results when it comes to liver injury caused by anti-tuberculosis drugs, restoring the balance of the gut microbiota, strengthening the intestinal barrier, and suppressing the inflammatory response by down-regulating the LPS/TLR4/NF-κB pathway[290].

3.3.2. Postbiotics

A novel trend in probiotics supplementation involves replacing live microorganisms with postbiotics. These can be defined as inanimate bacterial extracts and/or their components or metabolic by-products that confer health benefits to the host, including

maintaining intestinal and immune homeostasis[291]. Probiotic lysate derived from *Enterococcus lactis* IITRHR1 and *Lactobacillus acidophilus* MTCC447 has been shown to protect against APAP overdose in cultured rat hepatocytes by modulating antioxidant ability and apoptotic processes[292]. Similarly, bioactive lysates from *Lactobacillus fermentum* BGHV110 reduced the hepatotoxic effects of APAP overdose in human HepG2 cells by promoting autophagy[293]. SCFAs are considered one of the postbiotics produced by certain commensal gut microbiota or probiotics that have demonstrated beneficial effects in different types of liver injury. Butyrate supplementation has been shown to prevent valproate-induced liver injury in HepG2 cells and primary rat hepatocytes by limiting oxidative stress and mitochondrial damage. This was confirmed in rats by the normalization of liver biomarkers and a reduction in hepatic inflammation[294]. Intragastric administration of butyrate has been shown to alleviate hepatic necrosis and inflammation caused by genipin hepatotoxicity in rats by promoting Nrf2 expression and reducing LPS translocation[87]. Therefore, supplying oral SCFAs or performing a FMT with SCFAs-producing bacteria could effectively enhance antioxidant capacities, reduce inflammation, and even reinforce the intestinal and mucosal barriers in DILI. 4-Phenylbutyric acid (4-PBA) is a chemical derivative of butyric acid that is produced naturally by gut microbiota during fermentation. Pre-treatment with 4-PBA alleviated liver injury induced by APAP, rifampicin, pyrazinamide, and fasiglifam in preclinical models[295–298]. One of the most interesting effects of 4-PBA on liver injury is minimizing endoplasmic reticulum stress. Urolithin A and phenylpropionic acid are other less-studied postbiotics that have demonstrated protective effects against APAP-induced liver injury[89,299].

3.3.3. Antibiotics

As both animal models and clinical studies of DILI cases show an alteration in the gut microbiota, which can influence the development of liver damage in different ways, the use of antibiotics has emerged as a potential therapeutic option in DILI. The effect of antibiotics appears dual, with some studies showing protective effects and others showing harmful effects in the liver. Antibiotics can eliminate bacteria that cause liver inflammation and oxidative stress, or reduce the impact of gut microbiota on the immune system or liver metabolism. For example, compared to conventional mice, germ-free mice or mice treated with antibiotics experienced significantly reduced liver injury and immunotoxicity induced by immune agonist antibody therapy due to the absence of gut microbiota activity[300]. Vancomycin pretreatment also attenuates APAP-induced liver injury in animal models by increasing 2-hydroxybutyric acid production, which results from alterations in the gut microbiota composition mediated by the antibiotic. The protective effect of 2-hydroxybutyric acid was associated with a reduction in the bioavailability of APAP and an increase in GSH levels[301]. However, the opposite effect has been described, because ampicillin administration increased APAP-induced liver injury, partly due to the dysbiosis produced, including the reduction of *Lactobacillus*[100].

3.3.4. BAs homeostasis

Restoring BAs' homeostasis and leveraging their beneficial effects could be an attractive therapeutic approach for DILI. Supplementation with the probiotic *Lactobacillus rhamnosus* elevated bacterial bile salt hydrolase activity in an animal model of liver injury induced by a xenobiotic, thereby increasing the production of unconjugated secondary BAs, such as DCA. Through the FXR receptor, BAs reduced inflammasome activation and the production of inflammatory cytokines, thereby alleviating liver injury[302]. Ursodeoxycholic acid (UDCA) is a hydrophilic, tertiary BAs with a range of hepatoprotective properties, including cytoprotective, anticholestatic, antioxidant, and immunomodulatory effects. UDCA is the first-line treatment for primary biliary cholangitis, but several studies have also suggested potential benefits of UDCA in other cholestatic and non-cholestatic liver conditions[303,304]. In fact, UDCA has shown beneficial effects in preventing and treating DILI caused by different drugs. However, firm conclusions have not yet been reached, as the evidence is mainly based on case reports and small case series[305]. The effectiveness of obeticholic acid (ODA) in treating liver injury has also been reported. ODA is a semi-synthetic primary BAs and an agonist of FXR[306,307]. The effects of ODA include preventing and reducing oxidative stress, liver injury, and inflammation by improving BAs homeostasis and normalizing the composition of the gut microbiota in an animal model of DILI[308–311]. Recent studies have demonstrated the effectiveness of novel FXR agonists, such as kaempferol-7-*O*-rhamnoside and ginsenoside Rc, in alleviating APAP-induced liver injury and oxidative stress[312,313].

3.3.5. Fecal microbiota transplant (FMT)

FMT involves transferring gut microbiota from a healthy donor into the gastrointestinal tract of a sick recipient to restore the altered gut microenvironment[314]. This strategy is approved and highly effective for the treatment of recurrent or refractory *Clostridium difficile* infection[315], which has led to its use in other liver diseases to take advantage of its benefits[316]. However, evidence of FMT's beneficial effects on DILI is limited to studies in animal models. FMT using bacteria that produce butyrate has been shown to reduce liver inflammation and the extent of liver damage in mice with severe acute liver injury[317]. Xu et al.[52] observed that FMT from mice pretreated with *Broussonetia papyrifera* polysaccharide effectively alleviated liver damage in mice with APAP-induced liver injury. This effect was mediated by an increase in the genus Prevotellaceae_UCG_001, suggesting a protective role through the degradation of polysaccharides and the production of SCFAs. FMT has been demonstrated to be effective in alleviating liver injury induced by different drugs through regulating the Th17/Treg cytokine balance[56,318]. More widely accepted and accessible strategies for patients, such as oral FMT capsules, are also being tested. In mice with APAP-induced liver injury, oral fecal gavage mitigated liver injury and oxidative stress, and improved survival rates[319]. This protective effect was mediated by an increase in *Lachnospiraceae* and butyrate levels in the mice's colon.

3.3.6. Dietary patterns

Diet may be a modifiable risk factor in determining individual vulnerability to liver injury induced by drugs, due to its influence on the gut microbiota and the metabolites produced by them. Specifically, a fiber-rich diet protected against APAP-induced liver injury, while a Western-style diet exacerbated liver injury in mice. This protective effect was mediated by an increase in gut beneficial bacteria, such as *Lactobacillus acidophilus*, and their metabolite índole-3-lactic acid, which enhanced the antioxidant and detoxifying capacity of hepatocytes[277]. However, Cho et al.[320] observed that a fructose-rich diet -the major ingredient in the Western diet-could decrease the risk of APAP-induced liver injury in mice by modulating the composition of the gut microbiota and the metabolites they produced, as well as the liver antioxidant and

metabolic capacity against APAP overdose[321]. In a human observational study, a healthy dietary pattern was associated with a lower risk of antituberculosis-induced liver injury, suggesting protective effects. While direct causality cannot be established, the underlying mechanisms are thought to be related to the modulation of the gut microbiota and the gut−liver axis[322]. For this reason, it has been suggested that maintaining a healthy gut microbiota through dietary patterns could be an effective and simple way to prevent or reduce the risk of DILI.

In conclusion, reshaping the altered gut microbiota could be an attractive therapeutic alternative for alleviating liver injury. However, most studies evaluating the effects of microbiota-related interventions on liver injury have been conducted using *in vitro* and animal models, particularly in cases of APAP-induced liver injury. Indeed, further investigation is needed into the changes induced by these therapies in the gut microbiota's composition and the downstream effects on the microbiome and the human host. Clinical studies could facilitate the translation of preclinical results into novel practical interventions for DILI. Furthermore, pre-treatment with probiotics or postbiotics may be particularly interesting for preventing DILI in susceptible patients or those with chronic hepatic conditions by improving liver antioxidant, detoxifying, and anti-inflammatory capacities.

4. Future perspectives and conclusions

DILI is a multi-layered challenge that spans the entire drug lifecycle, jeopardizing patient safety and posing a significant economic burden to pharmaceutical companies in need of a deeper mechanistic understanding. Current therapeutic approaches involve withdrawing the suspected drug and, in highly selected patients, the use of corticosteroids[36,323]. However, these approaches do not fully address the pathological mechanisms underlying liver injury in DILI, and their efficacy is limited.

In recent years, significant progress has been made in understanding the pathological mechanisms underlying DILI, including the potential involvement of the gut microbiota and the gut−liver axis. Evidence suggests that the relationship between gut dysbiosis and DILI is bidirectional and complex. Many drugs that cause DILI also cause gut dysbiosis, a phenomenon particularly evident with antibiotics. Gut dysbiosis can damage the intestinal barrier, increasing intestinal permeability and allowing bacterial products and metabolites to translocate to the liver. There, they can amplify the drug-induced inflammation and oxidative stress. Conversely, the liver injury caused by the drug itself worsens and perpetuates intestinal dysbiosis, creating a vicious pro-inflammatory loop. Damage to the intestinal epithelium and increased intestinal permeability can also be promoted directly by the drug or by DAMPs and cytokines released by damaged hepatocytes and liver immune cells, further increasing the translocation of microbiota-derived products. Similarly, the accumulation of BAs associated with DILI, together with alterations in the gut microbiota that produce secondary BAs, causes an imbalance in BAs homeostasis with systemic consequences. Furthermore, a patient's gut microbiota profile can determine their risk of developing DILI, as certain bacterial genera can interfere with pharmacokinetics or affect the liver's detoxifying capacity, directly or through bacterial metabolites.

The mechanisms discussed below may differ slightly depending on whether intrinsic DILI or iDILI is being referred to. An acute high dose of a drug is representative of the underlying mechanisms of intrinsic DILI, which is usually modelled in preclinical studies. In these cases, it is probable that the alteration of the gut microbiota is a consequence of taking the drug. This intensifies or aggravates liver damage by disrupting the intestinal barrier and allowing the translocation of high levels of bacterial products to the liver. However, in cases of drugs that cause idiosyncratic reactions, gut dysbiosis likely plays a more direct, causal role. Intestinal dysbiosis could be an environmental factor that increases an individual's susceptibility to iDILI when a drug is administered, either by inducing chronic basal liver inflammation or altering drug metabolism. At the same time, the drug may amplify the hepatotoxic reaction by modifying the intestinal permeability and enhancing the immune response. On the other hand, it has been suggested that the clinical phenomenon of adaptation with the continuous administration of isoniazid, a drug related to idiosyncratic reactions, could be related to a change in gut dysbiosis induced by the antimycobacterial drug[56]. Given the rarity and unpredictable nature of iDILI, it is extremely difficult to conduct longitudinal studies in humans to determine the specific role of gut microbiota in this type of hepatotoxicity.

International efforts are underway to develop preclinical models that accurately reproduce the underlying mechanisms of iDILI in pursuit of personalized medicine[324]. In this context, new models have emerged that can provide direct, deeper insights into the mechanisms and interactions among the gut microbiota, the host, and drugs. These models include colonizing germ-free animals with unique, functionally characterized bacteria, germ-free treatment of genetically modified animals, and aseptic/humanized microbiota animal models. To reduce the use of experimental animals, advanced *in vitro* models have emerged, including microphysiological systems. These models have the potential to reveal new interactions between the liver and the gut, which could improve our understanding of the complex pathophysiological processes underlying DILI and may provide new opportunities for preclinical investigation of drug efficacy and safety[34,325]. Powerful omics-based technologies have emerged, with single-cell and spatial transcriptomics of particular interest for investigating the complex spatial compartmentalization of the liver[326]. Liver metabolic and immune zonation can result in different patterns of damage depending on where a drug is metabolized and the type of DILI. Studying how the gut−liver axis can modify these patterns and hepatic immune responses could be crucial to advancing our understanding of the etiopathogenetic mechanisms involved in DILI.

Increasing attention has been directed toward the potential role of bacterial extracellular vesicles (EVs) in the pathogenesis of liver diseases. Bacterial EVs are key mediators of intercellular communication between gut microbiota and host organs, playing an important role in the gut−liver axis[327]. One of the principal hypotheses is that disruption of the intestinal barrier in liver conditions facilitates the translocation of pathogenic factors, including bacterial EVs, into the systemic circulation, thereby exacerbating liver injury[328]. Nevertheless, to date, few studies have definitively demonstrated the translocation of bacterial EVs into the bloodstream in this context[328,329]. An exploratory study from our group has revealed significantly elevated plasma levels of bacterial EVs in patients with DILI and MASLD compared to healthy controls[330]. Specifically, fecal bacterial EVs isolated from MASLD patients with hepatic fibrosis were capable of inducing liver damage that mirrors the pathology observed in the disease. In fact, other *in vitro* studies and animal models suggested that bacterial EVs have significant effects on hepatic metabolism,

intestinal barrier integrity, and the immune system[331,332]. Although there is currently no evidence of the effect of bacterial EVs on DILI, they may contribute, at least in part, to its pathophysiology by promoting oxidative stress and inflammation.

Interestingly, an interaction between the gut microbiome, host genetics, and epigenetics has been observed in liver diseases[333,334]. Dysbiosis has been linked with DNA methylation and DNA methyltransferase activity in genes associated with metabolism or stem cell proliferation. SCFAs produced by the gut microbiota act as histone deacetylase inhibitors, thereby regulating the transcription of anti-inflammatory genes. Similarly, the pathogenesis of DILI might be influenced by interactions among genetic, epigenetic, and gut microbiome factors. However, these interactions in DILI remain unproven. Investigating how gut dysbiosis may influence genetic and epigenetic factors in DILI could shed new light on the role of the gut−liver axis in drug-induced liver injury.

It should be noted that the gut microbiome comprises not only bacteria but also fungi and viruses, which interact with the host and the immune system[335]. Alteration of fungal composition has been associated with the pathogenesis of MASLD, alcohol-associated liver disease, or cirrhosis[336]. Although there is currently no evidence of an alteration in the gut mycobiome in DILI, this could be a plausible hypothesis. Intestinal barrier dysfunction could facilitate the translocation of fungal molecules and metabolites to the liver, exacerbating liver injury. In fact, chronic alcohol consumption has been associated with fungal dysbiosis and increased translocation of β-glucans into systemic circulation, which promoted liver inflammation by binding to the C-type lectin receptor known as Dectin-1 on KCs and, consequently, hepatocyte damage[337]. However, *Candida*-derived β-glucan has also been associated with a protective role in LPS and D-galactosamine-induced fatal hepatitis[338]. The study of the human intestinal virome in liver diseases is very limited[339]. The human intestinal virome is specific to each individual and primarily consists of bacteriophages[340]. One current debate is whether alterations to the gut virome reflect the composition of the bacterial microbiota, mediate disease by influencing the gut microbiota, or directly drive immune responses. Another question is whether the virome is a consequence of the patient's disease. The study of the mycobiome and virome is still a debated topic today due to significant methodological limitations.

Different approaches to modulating the gut microbiota are currently being evaluated to alleviate liver injury in DILI, including probiotics, postbiotics, and FMT. However, these strategies are still in the early stages of development and have been mainly evaluated in animal models. Furthermore, they are untargeted therapies. Advances in genetic and biotechnological techniques could provide new opportunities to improve DILI management and promote precision and personalized medicine. These advancements could enable us to characterize an individual's gut microbiota enterotype, identify specific protective or pathological strains of gut microbiota for the liver, and design bioengineered bacterial strains or drugs that modulate specific bacterial enzymes and metabolic pathways[341].

In conclusion, while the role of the gut−liver axis in DILI remains to be fully elucidated, the existing evidence supports its significance as a key regulator of liver homeostasis and drug toxicity. A deeper understanding of how gut microbiota contributes to the physiopathology of DILI could lead to the identification of novel diagnostic and prognostic biomarkers, as well as facilitating the discovery of more effective, personalized approaches to preventing and treating DILI. Future research should integrate microbiota-derived biomarkers into clinical practice, develop targeted microbiota interventions, and embrace multi-omics strategies combined with advanced experimental models.

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Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

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