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REVIEW

Role of *Akkermansia muciniphila* in the development of nonalcoholic fatty liver disease: current knowledge and perspectives

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Abstract **Nonalcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome and a common cause of liver cirrhosis and cancer. *Akkermansia muciniphila* (*A. muciniphila*) is a next-generation probiotic that has been reported to improve metabolic disorders. Emerging evidence indicates the therapeutic potential of *A. muciniphila* for NAFLD, especially in the inflammatory stage, nonalcoholic steatohepatitis. Here, the current knowledge on the role of *A. muciniphila* in the progression of NAFLD was summarized. *A. muciniphila* abundancy is decreased in animals and humans with NAFLD. The recovery of *A. muciniphila* presented benefits in preventing hepatic fat accumulation and inflammation in NAFLD. The details of how microbes regulate hepatic immunity and lipid accumulation in NAFLD were further discussed. The modulation mechanisms by which *A. muciniphila* acts to improve hepatic inflammation are mainly attributed to the alleviation of inflammatory cytokines and LPS signals and the downregulation of microbiota-related innate immune cells (such as macrophages). This review provides insights into the roles of *A. muciniphila* in NAFLD, thereby providing a blueprint to facilitate clinical therapeutic applications.**

Keywords *Akkermansia muciniphila*; NAFLD; NASH; steatosis; inflammation

Introduction

Liver disease is a major worldwide health problem and heavy economic burden; liver cirrhosis and cancer account for 3.5% of global mortality [1]. Nonalcoholic fatty liver disease (NAFLD) is a potentially serious liver disease that affects approximately 25% of the global population [2]. It is one of the major risk factors for cirrhosis, hepatocellular carcinoma, and liver-related mortality [3]. NAFLD comprises a continuum of liver conditions varying from steatosis alone (NAFL) to nonalcoholic steatohepatitis (NASH). Currently, the "two-hit" theory, which consists of a first "hit" from steatosis (NAFL) and a second "hit" from other factors (e.g., oxidant stress), is not sufficient to explain NASH pathogenesis [4]. Multiple factors contribute to the development of NAFLD. In recent years, increasing evidence has indicated that the gut microbiota and its derived molecules act as vital players in the pathogenesis of NAFLD, especially the pathogenesis of NASH [5–8]. Hence, several approaches targeting the crosstalk between the gut microbiota and liver (gut–liver axis) have attempted to improve liver diseases [9].

In recent decades, *Akkermansia muciniphila* (*A. muciniphila*) has been regarded as a promising beneficial probiotic [10–13]. It is a strictly anaerobic, Gram-negative, mucin-degrading bacterium [14] that accounts for approximately 1%–3% of total bacteria in the intestinal tract of healthy adults [15]. *A. muciniphila* exhibited beneficial effects on host health and disease [16]. Several studies highlighted the therapeutic efficacy of *A. muciniphila* on metabolic disorders [17–19] and immune diseases [20]. In addition, *A. muciniphila* presented potential for the prevention of liver diseases [21–23], especially NAFLD [21]. However, key questions regarding its contribution to NAFLD need to be elaborated. Are there cause-and-effect evidence? What

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are the key components of *A. muciniphila*? How does the interaction occur between hepatic immune cells/signals and *A. muciniphila*? What are the roles of the intestinal barrier-protecting effect of *A. muciniphila* in NAFLD? In the present study, the current knowledge highlighting the role of *A. muciniphila* in NAFLD was systemically summarized, and the details of how the microbiota interferes with immunity and metabolic processes of the liver were discussed. An enhanced understanding of the *A. muciniphila*g–NAFLD interactions could facilitate the development of novel preventative or therapeutic strategies.

A. muciniphila and gut–liver axis

A. muciniphila attracted much attention for its ability to protect intestinal barrier function. Despite the mechanisms of this process are not completely understood, several theories support the role of *A. muciniphila* in maintaining such function.

First, *A. muciniphila* protects the physical and microbial barrier of the intestine. For example, *A. muciniphila* colonizes the mucus layer of the intestine, and it is involved in the turnover of mucin (degradation and synthesis) [24]. *A. muciniphila* and its-derived extracellular vesicles (AmEVs) could also promote the expression of tight junction proteins, such as Tjp1 and Occludin [25,26]. In addition, *A. muciniphila* could stimulate the proliferation and migration of enterocytes adjacent to colonic wounds [27] and accelerate the epithelial development mediated by intestinal stem cells (ISCs) [28]. Furthermore, *A. muciniphila* could promote the gene expression of antimicrobial peptides (including *Lyz1*, *DefA*, and *Reg3g*) in the gut [25] and further regulate the microbial community [25].

Second, sound evidence supported an important role of *A. muciniphila* in maintaining the intestinal immune homeostasis, including the regulation of IgA, IL-17 signals, and macrophages. For example, *A. muciniphila* was reported to promote the expression of immunoglobulin A (IgA) levels [19]. Interestingly, HFD-fed IL-17RA$^{-/-}$ mice displayed reduced abundance of *A. muciniphila* [29]. IL-17 could induce secretion of antimicrobial peptides and regulate intestinal IgA production, and it exhibited protective and pathogenic roles in the gut homeostasis [30]. Importantly, IL-17 plays a key role in the main comorbidity of NAFLD, that is, early atherosclerosis, as evident in a report on obese patients with NAFLD [31]. Thus, the findings implied that IL-17 signals could favor the enrichment of *A. muciniphila* and provided a clue for the association between *A. muciniphila* and intestinal innate immunity response. Further, researchers found that *A. muciniphila* regulated macrophages and the expression of NLRP3 of macrophages to alleviate acute intestinal inflammation [32]. Besides the innate immune cells, *A. muciniphila* coordinated the intestinal adaptive immune (T cell) responses that prevent colitis [33]. *A. muciniphila* supplementation also ameliorated TLR4-deficient-induced colitis by upregulating RORγt^{+} Treg cell-mediated immune responses in mice [34].

Lastly, the effects of *A. muciniphila*-mediated metabolites, such as short-chain fatty acids (SCFAs) and tryptophan derivatives, on the intestinal barrier have raised concern. A study showed that *A. muciniphila* promoted SCFA secretion (including acetic, propionic, and butyric acids), and antagonists of SCFA receptors could diminish epithelial development [28]. The influence of *A. muciniphila* on SCFAs was also confirmed in intestinal organoids [35]. One study reported that *A. muciniphila* regulated tryptophan metabolism [36], which activated the aryl hydrocarbon receptor (AhR) in the gut and further exerted barrier-protective effects [37]. In addition, *A. muciniphila* promoted bile acid (BA) salt uptake [38], reflected by the upregulation of *ASBT*, *IBABP*, and *OSTβ* expression in the ileum of mice, which may be beneficial to the remodeling of the gut microbiota structure [38]. Therefore, *A. muciniphila* enhances intestinal barrier function by regulating mucosal, microbial, epithelial, metabolic, and immunological components (Fig. 1).

Owing to the unique anatomy of the enterohepatic circulation, the gut–liver axis refers to the bidirectional relationship between the gut (especially the microbiota) and the liver [39]. On the one hand, the portal vein carries gut-derived products (such as lipopolysaccharide, secondary bile acids, and SCFAs) directly into the liver. On the other hand, the liver shapes intestinal homeostasis via the feed-back route. *A. muciniphila* promoted beneficial effects on hepatic immunity and metabolism on the basis of the gut–liver axis.

Once the balance of the gut–liver axis is disrupted, the gut-derived molecules in the blood induce a sustained immune response that promotes liver disorders [40]. Given its efficacy in the enhancement of the intestinal barrier, *A. muciniphila* could decrease the microbiota-derived products in circulation and further regulate the immune response in the liver. In mouse models, *A. muciniphila* markedly reduced the level of lipopolysaccharides in the serum [19,22,25] and portal plasma [41]. Accordingly, *A. muciniphila* downregulated proinflammatory factors, such as IL-6 [21], IL-1β [22], and TNF-α [23]; immune cell infiltration, such as neutrophils [22]; and fibrosis-related genes, such as *TGF-β*, *α-SMA*, *TIMP1*, *Col1a1*, and *PDGF* [23], in the liver. These studies highlighted the role of *A. muciniphila* in the interaction between microbial lipopolysaccharides and liver immunity. Recent studies found that *A. muciniphila*

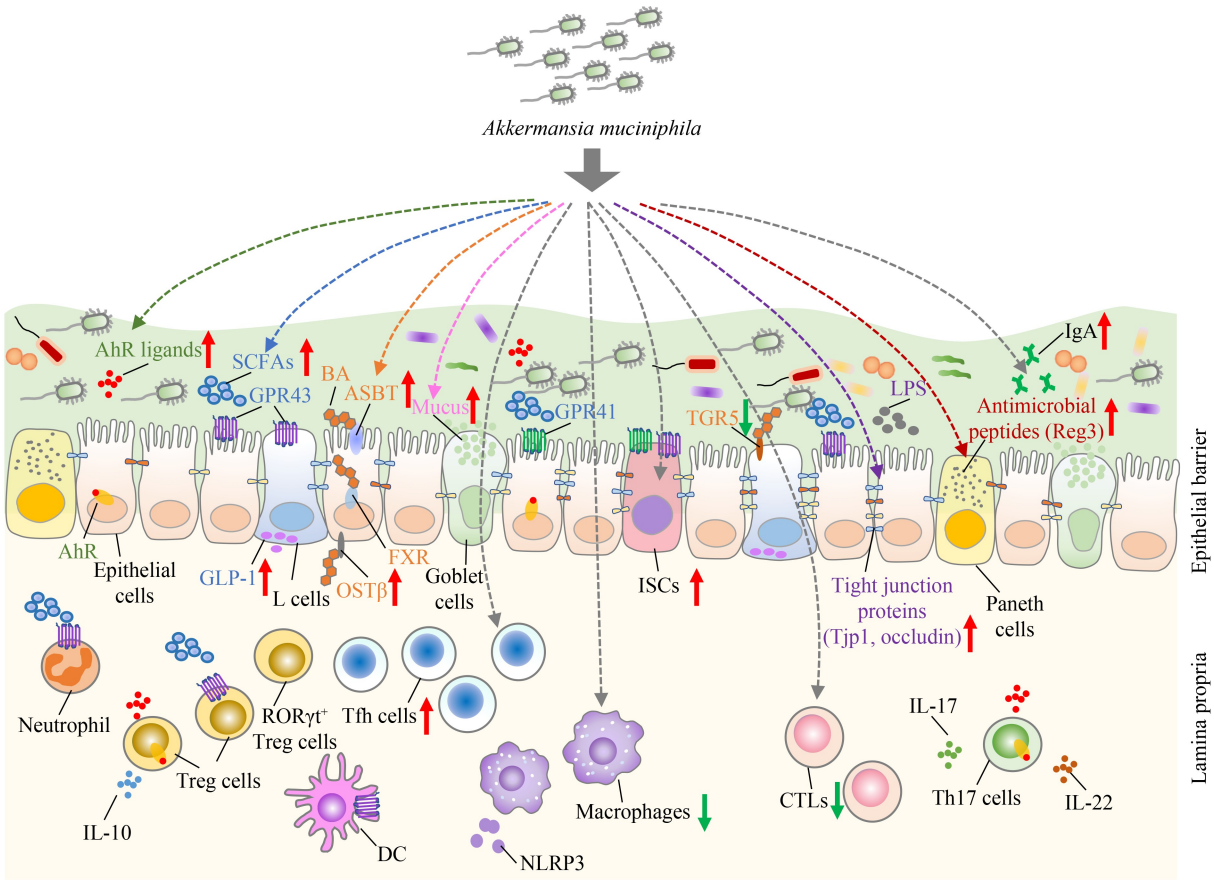

Fig. 1 *A. muciniphila* protects intestinal barrier function. *A. muciniphila* enhances intestinal barrier function by regulating mucosal (turnover of mucin), microbial (production of antibacterial peptide), epithelial (tight junction protein), metabolic (e.g., SCFAs, tryptophan metabolites, and BAs), and immunological (e.g., adaptive immune T cells) components. Abbreviations: AhR, aryl hydrocarbon receptor; BAs, bile acids; CTLs, cytotoxic T lymphocytes; DC, dendritic cell; FXR, farnesoid X receptor; GPR43/41, G protein-coupled receptors 41/43; IgA, immunoglobulin A; ISCs, intestinal stem cells; LPS, lipopolysaccharide; SCFAs, short-chain fatty acids.

could alleviate hepatic macrophage and neutrophil infiltration [22]. Wu *et al.* showed that *A. muciniphila* enriched the anti-apoptosis factor *Bcl-2* and suppressed the cytotoxic factors *Fas* and *DR5* [25]. In general, existing evidence affirmed the potential effects of *A. muciniphila* in regulating the hepatic immune response.

Interestingly, *A. muciniphila* was also found to be involved in liver metabolism pathways, such as glucose, lipid, and BA metabolism. Yoon *et al.* recently showed that *A. muciniphila* promoted glucagon-like peptide-1 (GLP-1) secretion in the intestine [42], which was previously reported to reduce hepatic glucose output [43]. A decrease in *A. muciniphila* abundance was notably accompanied by an increase in hepatic triglyceride levels [44]. By contrast, an increase in *A. muciniphila* abundance was accompanied by a decrease in total cholesterol and triacylglycerol levels [45]. Kim *et al.* further reported that *A. muciniphila* downregulated the expression of the fat synthesis gene *SREBP* in the liver [21]. Rao *et al.* revealed that *A. muciniphila* increased the expression of genes involved in cholesterol transport (*NPC1L1*) in the ileum and those involved in cholesterol transport (*LDLR*) in the liver. Thus, it acts to facilitate cholesterol transport [38]. Moreover, Rao *et al.* found that *A. muciniphila* promoted the expression of genes related to BA metabolism, including BA synthesis and efflux (*CYP7A*, *CYP8B1*, *CYP27A1*, *TGR5*, *BSEP*, *MPR2*, *MPR3*, and *FXR*) and BA salt uptake (*NTCP* and *OATP*), in the liver [38]. Plovier *et al.* suggested that pasteurized *A. muciniphila* could reduce trimetlylamine oxide production in the liver [41]. Overall, the beneficial effects of *A. muciniphila* on the liver may depend, in part, on its regulation of metabolism and immunity, both of which affect the gut.

Decreased *Akkermansia muciniphila* abundance in mice and patients with NAFLD

The role of *A. muciniphila* in metabolic diseases, such as obesity and T2DM, is a hot topic in recent studies [13]. The association between *A. muciniphila* and NAFLD, the

hepatic manifestation of metabolic syndrome, has aroused increasing concern. Differential changes in *A. muciniphila* in animals with short-term high-fat/sugar diet-induced obesity (that had not yet advanced into NAFLD) remain inconsistent [46,47]. However, previous studies reported that the administration of a high-fat diet (HFD) for 9 [48], 10 [21], 12 [49,50], or 13 [51] weeks induced fatty liver (confirmed by liver histology), and a lower abundance of *A. muciniphila* was observed in these mice. Another NAFLD mouse model induced by saccharin/sucralose diets for 11 weeks showed a decrease in *A. muciniphila* abundance [44]. Schneeberger *et al.* revealed a progressive decrease in *A. muciniphila* abundance in mice fed with HFD for 16 weeks [52]. In addition, a methionine and choline-deficient (MCD) diet for 4 [53] or 8 (unpublished data) weeks induced NASH, accompanied by a decrease in the abundance of *A. muciniphila*. Overall, a decrease in *A. muciniphila* abundance was widely observed in animal models of NAFLD induced by different diets with different intervention times (Table 1).

Studies have shown decreased *A. muciniphila* abundance in patients with metabolic syndrome, including those who are overweight and with obesity, untreated type 2 diabetes mellitus (T2DM), and hypertension [54–58]. However, the results of current clinical studies about the alterations in *A. muciniphila* abundance in patients with NAFLD or NASH are conflicting. A clinical study of obesity in females with liver steatosis found an increasing trend in the abundance of the genus *Akkermansia* as the steatosis level increased [59]. Conversely, a reduction in *Akkermansia* was observed in obese patients with NAFLD (diagnosed by ultrasonography without liver pathology), although the difference was not statistically significant [60]. Patients with T2DM with moderate/severe NAFLD also showed a decreasing trend in *A. muciniphila* abundance compared with patients with T2DM with no or mild NAFLD [61]. Another study revealed a significant decrease in *Akkermansia* in patients with NAFLD with elevated liver enzymes (diagnosed by abdominal ultrasound or computed tomography) compared with healthy controls [62]. A total of 46 patients with biopsy-proven NASH had significantly decreased *A. muciniphila* abundance levels compared with 38 healthy controls [63]. In addition, children with NAFLD demonstrated similar changes. For example, a study in pediatric patients with NAFLD showed a decreasing trend in the abundance of *A. muciniphila* when compared with healthy controls [64]. Pan *et al.* analyzed the alterations of the gut microbiota in 75 children, including 25 patients with NAFL (diagnosed by ultrasonography), 25 patients with NASH (unknown diagnostic criterion), and 25 patients who are obese without NAFLD. They showed that the abundance of *Akkermansia* in individuals with NAFL significantly decreased compared with that of control individuals [65]. Children with NASH had a lower abundance of *Akkermansia* than those with NAFL [66]. Moreover, patients with NAFLD and cirrhosis [67] showed a lower abundance of *Akkermansia* than healthy control individuals.

In addition, several studies have provided evidence for a negative correlation between *A. muciniphila* abundance and human NAFLD [58,68]. For example, patients with obesity with a low abundance of *A. muciniphila* presented with higher levels of liver enzymes (ALT and GGT) [58]. *A. muciniphila* was positively correlated with the concentration of circulating adiponectin (a potential anti-inflammatory effect [69]) levels in humans [68]. Interestingly, a study of 241 predominantly Latino children (with NASH and NAFL and control individuals) indicated that children with more severe liver pathology had enrichment of bacterial genes related to lipopolysaccharide biosynthesis, which is partly driven by *A. muciniphila*. However, no specific evidence was found for decreases in *A. muciniphila* abundance in children with NASH [70]. In general, current studies largely supported the reduction in *A. muciniphila* abundance in clinical patients with NAFLD (Table 1). However, direct evidence for the alteration of *A. muciniphila* in patients with NAFLD with histopathological diagnosis is still being further analyzed.

Therapeutic role of *A. muciniphila* in NAFLD

At present, many agents that aimed to cure NAFLD/NASH have been reported in recent papers, including antidiabetic, anti-obesity, antioxidant, and cytoprotective agents [71]. Interestingly, the abundance of *A. muciniphila* was increased in the gut after NAFLD therapies, such as probiotics, dietary fiber, prebiotics, and traditional Chinese medicine (Table 1) [72–88]. Moreira *et al.* reported that liraglutide could reverse HFD-induced NAFLD by reducing lipid droplets and inflammatory cell infiltration in the liver, and enrich the abundance of *A. muciniphila* [72]. Du *et al.* found that dietary betaine prevented hepatic steatosis and increased ALT/AST in mice with HFD treatment for 23 weeks, accompanied by an increase in *A. muciniphila* [73]. A clinical study of NAFLD-related metabolic syndrome also presented similar findings [58,89,90]. In a clinical study of adults who are overweight and obese, the increased abundance of *A. muciniphila* was also observed to be involved in the effects of calorie restriction on metabolic disorders [58]. Another study of individuals with morbid obesity, who underwent weight-loss surgery (Roux-en-Y gastric bypass), showed that the abundance of *A. muciniphila*

Table 1 Alteration of *Akkermansia muciniphila* in NAFLD models and patients

Reference	Subjects/diets	Severity of NAFLD	Study group	Sample type (detection method)	Changes in *Akkermansia muciniphila* or *Akkermansia*
Animals					
Kim *et al.* (2020) [21]	Male C57BL/6N mice, HFD, 10 weeks	NAFL (H&E staining; serum ALT and AST↑)	1. Normal diet (ND + PBS, *n* = 5); 2. HFD + PBS (*n* = 5); 3. ND + *A. muciniphila* (*n* = 5); 4. HFD + *A. muciniphila* (*n* = 5)	Cecal contents (16S rRNA gene sequencing)	↓
Shi *et al.* (2021) [44]	Female C57BL/6 mice, saccharin or sucralose, 11 weeks	NASH (histopathological changes)	1. Control (*n* = 10); 2. neohesperidin dihydrochalcone (NHDC, *n* = 10); 3. saccharin (*n* = 10); 4. sucralose (*n* = 10)	Cecal contents (16S rRNA gene sequencing, metagenomic sequencing)	↓
Natividad *et al.* (2018) [48]	Male C57BL/6J mice, HFD, 9 weeks	NAFL (H&E staining; serum ALT and AST↑)	1. Control diet (CD, *n* = 10); 2. HFD (*n* = 10); 3. CD + *B. wadsworthia* (*n* = 10); 4. HFD + *B. wadsworthia* (*n* = 10)	Feces (16S rRNA gene sequencing)	↓
Hussain *et al.* (2016) [49]	Male C57BL/6 mice, HFD, 12 weeks	NAFL (Oil Red O staining)	1. Normal chow diet (NCR, *n* = 7); 2. HFD (*n* = 7); 3. HFD + orilistat (ORL, *n* = 7); 4. HFD + daesiho-tang (DSHT, *n* = 7)	Feces (qPCR)	↓ (tendency)
Lee *et al.* (2018) [50]	Male C57BL/6 mice, HFD, 12 weeks	NAFL (H&E staining)	1. ND (*n* = 5); 2. HFD (*n* = 5); 3. HFD-fed mice treated with a mixture of two *L. plantarum strains* (DSR, *n* = 5)	Cecal contents (16S rRNA gene sequencing, qPCR)	↓
Wang *et al.* (2019) [51]	Male C57BL/6 mice, HFD, 13 weeks	NAFL (H&E and Oil Red O staining; serum ALT ↑)	1. Normal control (NC, *n* = 6); 2. HFD (*n* = 6); 3. NC + puerarin (NC + PUE) (*n* = 6); 4. HFD + PUE (*n* = 6)	Feces (16S rRNA gene sequencing)	↓
Schneeberger *et al.* (2015) [52]	Male C57BL/6 mice, HFD, 16 weeks	Unknown	1. CT diet (CT, *n* = 6); 2. HFD (*n* = 6)	Cecal contents (qPCR)	Negative correlation with age and HFD feeding
Ye *et al.* (2018) [53]	Male C57BL/6 mice, methionine-choline-deficient (MCD) diet, 4 weeks	NASH (histopathological changes)	1. Control (*n* = 6); 2. MCD (*n* = 6)	Feces (16S rRNA gene sequencing)	↓
Moreira *et al.* (2018) [72]	Male C57BL/6 mice, HFD, 10 weeks	NASH (histopathological changes)	1. Control group (C); 2. C + liraglutide (C + L); 3. HFD; 4. HFD + liraglutide (HFD + L)	Feces (16S rRNA gene sequencing)	Liraglutide increased its abundance in HFD mice
Du *et al.* (2021) [73]	Kunming female mice, HFD, 23 weeks	NAFL (Oil Red O staining; serum ALT and AST ↑)	1. Normal chow (Chow, *n* = 6); 2. HFD (*n* = 6); 3. chow + betaine (Chow + B) (*n* = 6); 4. HFD + B (*n* = 6)	Feces (16S rRNA gene sequencing)	↓
Zhang *et al.* (2022) [74]	Male C57BL/6 mice, HFD, 12 weeks	NASH (histopathological changes; serum ALT/AST ↑)	1. Control group (Con, *n* = 5); 2. HFD (*n* = 5); 3. HFD + purified MDG (MDG-1. *n* = 5); 4. HFD + coarse MDG (MDG-C, *n* = 5); 5. HFD + inulin (Inu, *n* = 5); 6. HFD + antibiotics (Anti, *n* = 5)	Feces (metagenomic sequencing)	↓, negative correlation with most NAFLD parameters
Wang *et al.* (2021) [75]	Male C57BL/6J mice, HFD, 30 weeks	NASH (hepatic steatosis; hepatic TNFα, MCP-1, IL-6 and ROS ↑; serum ALT, AST, ALP, and γ-GT ↑; hepatic fibrosis)	1. NCD-fed mice (Control); 2. HFD-fed mice (Model); 3. HFD + metformin (Metf); 4. HFD + 100 mg/kg/day of PYOs (PYOs-L); 5. HFD + 300 mg/kg/d of PYOs (PYOs-H)	Cecal contents (16S rRNA gene sequencing)	Negative correlation with the development of NAFLD
Han *et al.* (2021) [76]	Male C57BL/6 mice, high fat/high sucrose (HFHS), 16 weeks	NAFL (H&E and Oil Red O staining; serum ALT and AST ↑)	1. Control group (Con, *n* = 5); 2. HFHS (*n* = 5); 3. HFHS + low-dose SMF (SMF-L, *n* = 5); 4. HFHS + high-dose SMF (SMF-H, *n* = 5)	Cecal contents (16S rRNA gene sequencing)	↑ (tendency)
Bao *et al.* (2021) [79]	Male C57BL/6J mice, HFD, 14 weeks	NASH (NAS scores ↑; hepatic IL-1β, IL-18, IL-6 and TNF-α ↑; hepatic macrophages ↑)	1. ND (*n* = 5); 2. HFD (*n* = 5); 3. ND + inulin (ND-INU, *n* = 5); 4. HFD + inulin (HFD-INU, *n* = 5)	Feces (16S rRNA gene sequencing)	↓

(*Continued*)

Reference	Subjects/diets	Severity of NAFLD	Study group	Sample type (detection method)	Changes in *Akkermansia muciniphila* or *Akkermansia*
Cui *et al.* (2020) [80]	Male Sprague–Dawley rats, HFD, 12 weeks	NASH (histopathological changes; serum ALT/AST ↑)	1. Control (n = 10); 2. HFD (model, n = 10); 3. HFD + metformin (positive control, n = 10); 4. HFD + Da-Chai-Hu (DCH, n = 10)	Feces (16S rRNA gene sequencing)	↓
Nakano *et al.* (2020) [81]	Male C57BL/6J mice, Western diet, 12 weeks	NAFL (Oil Red O staining; serum ALT ↑)	1. ND (n = 4); 2. ND + 2% BA group (NDBA, n = 2); 3. Western diet (WD, n = 4); 4. WD + 2% BA (WDBA, n = 4)	Feces (16S rRNA gene sequencing)	AST and ALT were positively correlated with family Verrucomicrobiaceae
Mu *et al.* (2020) [82]	Male C57BL/6J mice, HFD/F (high fat + 10% fructose solution), 8 weeks	NAFL (H&E staining; serum ALT, AST, AKP and hepatic ROS ↑)	1. Normal group (Normal); 2. HFD (Model); 3. L-carnitine; 4. HFD + low-concentration *L. fermentum CQPC06* (LCQPC06); 5. HFD + high-concentration *L. fermentum CQPC06* (HCQPC06); 6. HFD + *Lactobacillus delbrueckii* subsp. *Bulgaricus* (LDSB)	Feces (16S rRNA gene sequencing)	↓
Xie *et al.* (2020) [83]	Male C57BL/6J mice, Western diet, 12 weeks	NAFL (H&E staining; serum ALT and AST ↑)	1. ND (n = 4); 2. ND + 1% vine tea polyphenols (ND + 1% VTP, n = 4); 3. WD (n = 4); 4. WD + 0.5% VTP (n = 4); 5. WD + 1% VTP (n = 4); 6. WD + 2% VTP (n = 4)	Feces (16S rRNA gene sequencing)	↓
Nishiyama *et al.* (2020) [84]	Male ob/ob mice, standard diet, 4 weeks	NASH (histopathological changes; serum ALT/AST ↑)	1. C57BL/6J mice fed standard diet (WILD, n = 6); 2. ob/ob mice fed with standard diet (CONT, n = 6); 3. ob/ob mice fed with standard diet containing 5% bofutsushosan (BTS) (n = 6)	Feces (16S rRNA gene sequencing, qPCR)	Negative correlation with suppression of body weight gain
Li *et al.* (2020) [85]	Male C57BL/6J mice, HFD, 18 weeks	NASH (histopathological changes; serum ALT and AST ↑)	1. Normal chow diet (NCD, n = 8); 2. HFD (n = 8); 3. HFD + fed with 0.5% carboxymethylcellulose sodium (CMC-Na) (n = 8); 4. HFD + Sil (100 mg/kg, HFD + Sil group 1, n = 8); 5. HFD + Sil (300 mg/kg, HFD + Sil group 2, n = 8)	Cecal contents (16S rRNA gene sequencing)	↓
Régnier *et al.* (2020) [87]	Male C57BL/6J mice, high-fat and high-sucrose diet (HFHS), 8 weeks	NAFL (hepatic triglycerides ↑)	1. Control diet (CTRL, n = 10); 2. HFHS (n = 10); 3. HFHS + 0.3% (g/g) of rhubarb (RHUB, n = 10)	Feces (16S rRNA gene sequencing, qPCR)	Rhubarb promotes its growth in HFHS-fed mice
Juárez-Fernández *et al.* (2022) [88]	Wistar rats, HFD, 9 weeks	NAL (histopathological changes; serum ALT and AST ↑)	1. Control group (C) (n = 7); 2. HFD (n = 7); 3. C + quercetin (n = 7); 4. C + *A. muciniphila*; 5. HFD + quercetin (n = 7); 6. HFD + *A. muciniphila* (n = 8); 7. C + quercetin + *A. muciniphila* (n = 8); 8. HFD + quercetin + *A. muciniphila* (n = 8)	Feces (16S rRNA gene sequencing)	The colonization with *Akkermansia muciniphila* was associated with less body fat
Human					
Hoyles *et al.* (2018) [59]	Morbidly obese women	NAFL (diagnosed by histology)	1. No liver steatosis (n = 10); 2. liver steatosis 1 (n = 22); 3. liver steatosis 2 (n = 14); 4. liver steatosis 3 (n = 10)	Feces (metagenomic sequencing)	Increased trend in women with obesity. Verrucomicrobia and *Akkermansia* were significantly correlated with liver steatosis
Nistal *et al.* (2019) [60]	Adults with obesity	NAFLD (diagnosed by clinical, analytical and ultrasonographic data)	1. Twenty healthy adults (n = 20); 2. obese patients with NAFLD (n = 36); 3. obese patients without NAFLD (n = 17)	Feces (16S rRNA gene sequencing)	Reduced trend in patients with obesity with NAFLD

(*Continued*)

Reference	Subjects/diets	Severity of NAFLD	Study group	Sample type (detection method)	Changes in *Akkermansia muciniphila* or *Akkermansia*
Tsai *et al.* (2021) [61]	Patients with T2DM	NAFLD (diagnosed by ultrasonographic data)	1. Patients with T2DM with no or mild NAFLD (n = 80); 2. patients with T2DM with moderate or severe NAFLD (n = 83)	Feces (qPCR)	Decreased trend in patients with T2DM with moderate/severe NAFLD
Lee *et al.* (2021) [62]	Patients with NAFLD	NAFLD (diagnosed by abdominal ultrasound or computed tomography with elevated liver enzyme)	1. Healthy controls (n = 37); 2. NAFLD (n = 57)	Feces (16S rRNA gene sequencing)	↓
Özkul *et al.* (2017) [63]	Patients with NASH	NASH (diagnosed by histology)	1. Healthy controls (n = 38); 2. NAFLD (n = 46)	Feces (qPCR)	↓
Chierico *et al.* (2017) [64]	Children and adolescents	NAFL and NASH (diagnosed by liver ultrasound and percutaneous liver biopsy)	1. NAFL (n = 27); 2. NASH (n = 26); 3. obesity (n = 8) 4. case-controls (CTRLs, n = 54)	Feces (16S rRNA gene sequencing)	Decreased trend in pediatric patients with NAFLD
Pan *et al.* (2021) [65]	Children with obesity	NAFL (diagnosed by ultrasonography) and NASH (unknown diagnostic criterion)	1. NAFL (n = 25); 2. NASH (n = 25); 3. obese without NAFLD (n = 25)	Feces (16S rRNA gene sequencing)	↓
Schwimmer *et al.* (2019) [66]	Children who are overweight/obese	NAFLD (diagnosed by liver biopsy)	1. Overweight/obese children without NAFLD (n = 37); 2. children with NAFLD (n = 86, including 38 NAFL, 37 borderline NASH, and 11 NASH)	Feces (16S rRNA gene sequencing, metagenomic sequencing)	Decreased in children with NASH or moderate/severe fibrosis
Ponziani *et al.* (2019) [67]	Patients with NAFLD	NAFLD with cirrhosis (diagnosed by histological and/or clinical findings) and HCC (diagnosed by histology)	1. Patients with NAFLD-related cirrhosis and HCC (n = 21); 2. NAFLD-related cirrhosis without HCC (n = 20); 3. healthy controls (n = 20)	Feces (16S rRNA gene sequencing)	Decreased in NAFLD patients with cirrhosis
Dao *et al.* (2016) [58]	Adults who are overweight and obese	Unknown	1. Lower *A. muciniphila* abundance in baseline (Akk LO, n = 24); 2. higher *A. muciniphila* abundance in baseline (Akk HI, n = 25)	Feces (qPCR)	Akk HI group had lower AST and GGT and greatest benefits from dietary intervention
Liu *et al.* (2017) [68]	Patients with obesity	Obesity with elevation of serum ALT, AST, ALP, and GGT levels	1. Lean controls (n = 79); 2. individuals with obesity (n = 72)	Feces (metagenomic sequencing)	Enriched in lean controls; positive correlation with the concentration of circulating adiponectin
Kordy *et al.* (2021) [70]	Children and adolescents	NASH (diagnosed by liver biopsy)	1. NASH (n = 20); 2. control subjects (n = 20)	Feces (metagenomic sequencing)	↓

increased within 3 months after surgery and remained high 1 year later. The abundance increased in parallel with metabolic improvements [90]. In addition, *A. muciniphila* showed therapeutic potential for ethanol or drug-induced liver injury [22,23,25]. These findings suggested that *A. muciniphila* could be a potential target for the treatment of NAFLD.

Several studies have provided evidence for the beneficial role of *A. muciniphila* in the prevention of NAFLD (Table 2). In animal experiments, several studies have demonstrated the protective role of *A. muciniphila* in a short-term HFD-induced metabolic disorder mouse model [42,91]. Interestingly, a study reported that the oral administration of *A. muciniphila* (dose of 10^8–10^9 CFU/mL) could prevent HFD-induced NAFLD in mice, as reflected by decreased ALT levels and improvements in hepatic steatosis [21]. Rao *et al.* recently demonstrated that 6 weeks of *A. muciniphila* (2×10^7 CFU) treatment showed a therapeutic effect on NAFLD in mice [38]. They found that the withdrawal of *A. muciniphila* treatment after 4 weeks maintained the efficient persistence of its anti-NAFLD activities due to the reshaping of the gut microbiota [38]. Furthermore, antibiotic treatment decreased *A. muciniphila* abundance, accompanied by an exacerbation of NAFLD manifestations in HFD-fed mice. Co-treatment with antibiotics and *A. muciniphila* also demonstrated robust NAFLD-attenuating effects [38]. These findings showed the inhibition and therapeutic effects of *A. muciniphila* on NAFLD in animal models.

Notably, a clinical study of 32 humans who were overweight and obese addressed the therapeutic efficacy of *A. muciniphila* [18]. The results showed that pasteurized *A. muciniphila* treatment for 3 months reduced the levels of blood markers for liver dysfunction, including γ-glutamyltransferase (GGT) and AST [18]. They also confirmed the safety and tolerability of *A. muciniphila* in individuals with excess body weight at different doses of live (10^{10} or 10^9 CFU per day) or pasteurized *A. muciniphila* (10^{10} CFU per day) [18]. However, the above clinical study was performed in humans with obesity, not those with NAFLD, and enrolled a small number of individuals. Thus, future clinical interventions with more cases of obesity and even NASH in humans are needed to confirm and extend these findings.

Studies also reported the beneficial effects of *A. muciniphila* on the complications of NAFLD. For example, Higarza *et al.* reported that *A. muciniphila* administration reversed NASH-induced cognitive dysfunction, including spatial working memory and novel object recognition, in rats [92]. Another study further explained the potential mechanisms for these effects; that is, *A. muciniphila* restored microgliosis, neurodevelopment, and neuronal plasticity in the hippocampus of HFD-fed mice by enhancing gut barrier function [93]. In summary, these animal experiments and related human studies provided support for the role of *A. muciniphila* in the treatment or prevention of NAFLD.

Underlying mechanisms of action of *A. muciniphila* in NAFLD prevention

The importance of *A. muciniphila* in NAFLD has been investigated in recent decades. Yet, the exact mechanisms of action and the active components of *A. muciniphila* remain unclear. Hepatic fat accumulation and inflammation are two essential factors of NASH. Existing evidence suggested that *A. muciniphila* protects against NAFLD by alleviating hepatic steatosis (Fig. 2) and inflammation (Fig. 3).

Functional components of *Akkermansia muciniphila* that play a role in hepatic steatosis

In 2020, experts reached a consensus that metabolic dysfunction-associated fatty liver disease "MAFLD" is a more appropriate overarching term than NAFLD [94]. This term highlights the wide range of metabolic dysfunction phenotypes, including insulin resistance and abnormal lipid profiles, in NAFLD. In general, NAFLD coexists with obesity, diabetes, and dyslipidaemia, all of which interact with one another. A previous study indicated that impaired insulin signaling in adipose tissue contributed to NASH by dysregulating lipolysis, resulting in the excessive delivery of fatty acids to the liver [95]. The progression of hepatic steatosis to the state of inflammation is triggered by adipocytokine imbalances and lipotoxicity [96]. Hence, preventing steatosis and its related metabolic dysfunction is an appropriate therapeutic target for NASH.

Notably, current studies strongly demonstrated the protective potential of *A. muciniphila* on steatosis-related insulin resistance and lipid accumulation. Several studies have suggested that the beneficial effects of *A. muciniphila* on insulin resistance are partly due to the remission of metabolic endotoxaemia [19,41] or adipose tissue inflammation [41,97]. In 2013, Cani *et al.* demonstrated that supplementation with live, not heat-killed (121 °C, 225 kPa, 15 min) *A. muciniphila* (2×10^8 CFU) for 4 weeks could prevent HFD-induced insulin resistance and metabolic endotoxaemia by enhancing the gut barrier [19]. Later, they found that supplementation with pasteurized *A. muciniphila* (70 °C, 30 min) for 5 weeks had a better capacity to reduce endotoxaemia (also called LPS) and adipose tissue inflammation in HFD mice than that with live *A. muciniphila* [41]. They also identified an active ingredient called Amuc_1100 and demonstrated a similar efficacy of 3 μg Amuc_1100 on insulin sensitivity [41].

Table 2 Efficacy of *Akkermansia muciniphila* in treating NAFLD and other liver injuries

Reference	Diet	Subjects	Diseases	Dosages	Time	Colonization (detection method)	Study group	Efficiency of treatment
Animals								
Kim *et al.* (2020) [21]	HFD	Male C57BL/6N mice	NAFL (H&E staining; serum ALT and AST levels ↑)	Live, 10^8–10^9 CFU/mL	10 weeks	Obviously increased abundance (16S rRNA gene sequencing)	1. Normal diet (ND + PBS, $n = 5$); 2. HFD + PBS ($n = 5$); 3. ND + *A. muciniphila* ($n = 5$); 4. HFD + *A. muciniphila* ($n = 5$)	Serum TG and ALT levels↓; the gene expression of hepatic TG synthesis and inflammatory factor IL-6↓
Rao *et al.* (2021) [38]	High-fat and high-cholesterol (HFC) diet	Male C57BL/6J mice	NASH (histopathological changes)	Live, 1×10^8 CFU/ mL, 200 μL, every other day	6 weeks	Increased by 20-fold (qPCR)	1. HFC ($n = 5$–8); 2. HFC + *A. muciniphila* ($n = 5$–8)	Hepatic steatosis/ inflammatory (indicated by HE staining) ↓, serum ALT/AST/ALP↓, and hepatic genes expression related to steatosis and inflammation↓
Higarza *et al.* (2021) [92]	High-fat, high cholesterol diet (HFHC)	Male Sprague–Dawley rats	NASH-induced cognitive damage	Live, 10^9 CFU, 100 μL, daily	4 weeks	No differences (16S rRNA gene sequencing and qPCR)	1. NC group ($n = 8$); 2. HFHC + PBS ($n = 8$); 3. HFHC + *Lacticaseibacillus rhamnosus* GG (HFHC + LGG, $n = 8$); 4. HFHC + *A. muciniphila* CIP107961(HFHC + AKK, $n = 8$)	HFHC-induced cognitive dysfunction (including impaired spatial working memory and novel object recognition) ↓
Human								
Depommier *et al.* (2019) [18]	/	/	Overweight/obese insulin-resistant volunteers	Live (10^{10} or 10^9 CFU per day) or pasteurized *A. muciniphila* (10^{10} CFU per day)	3 months	Unknown	1. Placebo group ($n = 11$); 2. pasteurized bacteria group ($n = 12$); 3. live bacteria group ($n = 9$)	Blood markers for liver dysfunction including γ-glutamyltransferase (GGT) and AST↓
Other liver diseases								
Grander *et al.* (2018) [22]	Lieber-De-Carli diet containing 1–5 vol.%	Female WT mice	Alcoholic liver disease (ALD)	Live, 1.5×10^9 CFU, 200 μL, every other day	2 day or 15 days or 6 days	Obviously increased abundance (qPCR)	1. Pair fed (Ctrl); 2. Pair fed + *A. muciniphila* (Ctrl + A.muc); 3. ethanol fed (EtOH); 4. ethanol fed + *A. muciniphila* (EtOH + A.muc)	Acute ethanol-induced hepatic injury and inflammation↓; chronic ethanol-induced hepatic inflammation and steatosis↓
Keshavarz *et al.* (2021) [23]	HFD (+ CCl_4 injection)	Male C57BL/6 mice	Liver injury (liver fabrosis)	10^9 CFU/200 μL live or pasteurized *A. muciniphila,* 50 mg/200 μL Evs, daily	4 weeks	Obviously increased abundance (qPCR)	1. Healthy control animals (ND, $n = 5$); 2. HFD/CCl_4 + PBS (PBS, $n = 5$); 3. HFD/CCl_4 + live *A. muciniphila* (Am) ($n = 5$); 4. HFD/CCl_4 + pasteurized *A. muciniphila* (Pam, $n = 5$); 5. HFD/CCl_4 + EV (EV, $n = 5$)	Serum liver enzymes↓; hepatic inflammation and fibrosis markers↓
Wu *et al.* (2017) [25]	Normal chow diets (+ concanavalin A injection)	Male C57BL/6 mice	Liver injury (resembling autoimmune liver diseases and virus hepatitis)	Live, 3×10^9 CFU, 200 μL, daily	14 days	Obviously increased abundance (16S rRNA gene sequencing)	1. *A. muciniphila* + Con A (Akk, $n = 7$); 2. PBS + Con A (Control, $n = 7$); 3. PBS + PBS (Normal, $n = 8$)	Serum ALT and AST↓; liver histopathological damage↓

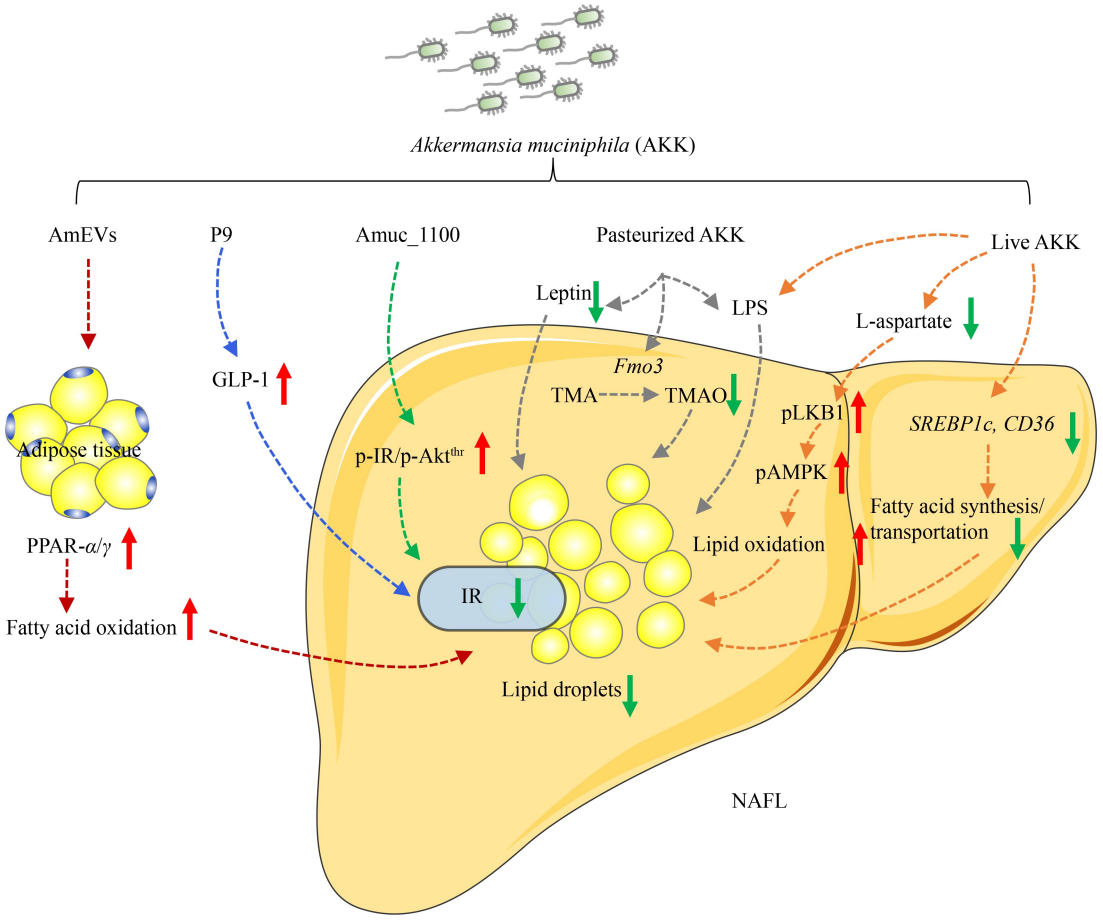

Fig. 2 Potential mechanism by which *A. muciniphila* alleviates hepatic fat accumulation in NAFLD. Live or pasteurized *A. muciniphila* and its active ingredient (Amuc_1100, P9 and AmEVs) alleviate steatosis and its related insulin resistance. Abbreviations: AKK, *A. muciniphila*; AmEVs, *A. muciniphila*-derived extracellular vesicles; GLP-1, glucagon-like peptide-1; IR, insulin resistance; LPS, lipopolysaccharide; TMAO, trimetylamine oxide.

In 2018, Chelakkot *et al.* isolated the active ingredient called AmEVs and found that 2 weeks of feeding AmEVs could improve 12-week HFD-induced glucose intolerance in mice [26]. Consistently, AmEVs (10 μg) significantly decreased the expression of inflammatory markers in adipose tissue [97]. According to these studies, the improvement in insulin resistance by *A. muciniphila* is negatively linked with a reduction in circulating LPS, and the latter could be responsible for the enhancement of intestinal permeability. Live or pasteurized *A. muciniphila*, Amuc_1100, and AmEVs were reported to strengthen the intestinal barrier function in HFD-induced metabolic dysfunction in mice [19,26,41,97].

In addition to its ability to improve insulin resistance, *A. muciniphila* could considerably regulate lipid metabolism in animals [98–102] and humans [18]. For instance, in mice with metabolic dysfunction induced by HFD, oral administration of *A. muciniphila* (2×10^8 CFU) downregulated the expression of genes related to fatty acid synthesis and transport in liver/muscle and further reduced fat deposition in the liver and muscle [99]. *A. muciniphila* also activated hepatic genes related to lipid oxidation (*LPL*, *PCG-1α*, *CPT-1β*, *UCP2*, and *LXR*), lipid transportation (*FATP4* and *FAT/CD36*), and cholesterol transportation (*LDLR*) and increased the levels of hepatic proteins related to energy expenditure (*pLKB1*, *pAMPK*, and mitochondrial complexes I, II, IV, and V) in the liver of NAFLD mice. These alterations contribute to the improvement of fat accumulation [38]. AmEVs were as effective as *A. muciniphila* in improving lipid profiles [97,103]. In a prospective study of individuals who were overweight/obese, live and pasteurized *A. muciniphila* showed therapeutic efficacy on dyslipidaemia, but pasteurized *A. muciniphila* was superior to live *A. muciniphila* in lowering serum total cholesterol levels [18]. The latest identified P9 protein of *A. muciniphila* could improve glucose intolerance by promoting GLP-1 secretion by intestinal endocrine cells and reduce hepatic lipid accumulation in mice fed with HFD for 8 weeks [42]. Overall, existing evidence confirmed the protective role of *A. muciniphila* and its active components in steatosis-related metabolic dysfunction (Fig. 2), which may, at least partly, account for the mechanism of action of *A. muciniphila* in NAFLD improvement.

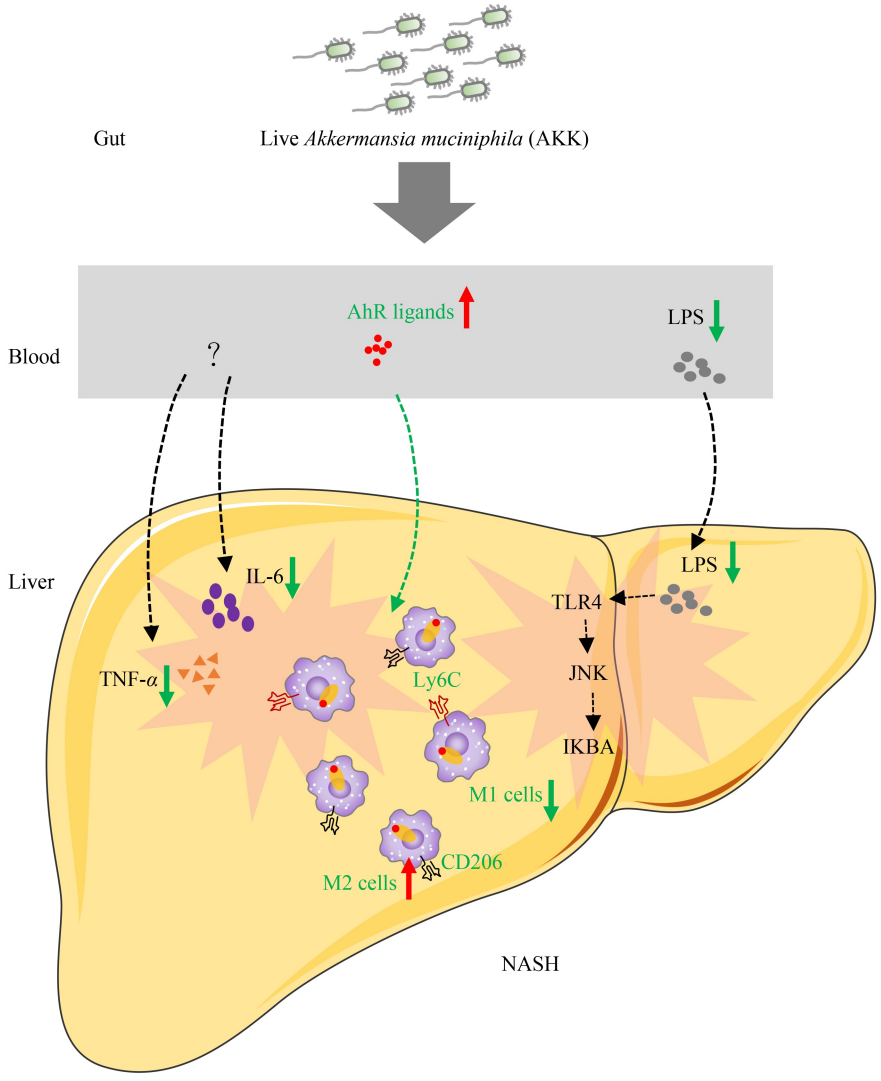

Fig. 3 Potential mechanism by which *A. muciniphila* prevents hepatic inflammation in NAFLD. *A. muciniphila* regulates hepatic inflammation and the immune response in NAFLD via multiple potential mechanisms. The current focus on the mechanisms of *A. muciniphila* in improving hepatic inflammation mainly centers on the production of inflammatory factors, LPS signals, and macrophages. Abbreviations: AKK, *A. muciniphila*; AhR, aryl hydrocarbon receptor; LPS, lipopolysaccharide; M1/2 cells; macrophage type 1/2; TLR4, toll-like receptor 4.

Modulation of hepatic innate immune response in NAFLD by *A. muciniphila*

NASH is the inflammatory form of NAFLD, and it is characterized by hepatic inflammation and fibrosis. It is regarded as a major cause of liver cirrhosis. In addition to steatosis, hepatic inflammation in NAFLD is triggered by multiple mechanisms [96]. In fact, whether NASH is always preceded by NAFL is not certain [4], suggesting the existence of other potential mechanisms of NASH development. The gut microbiota affects immune homeostasis and metabolism in the liver, thereby affecting NAFLD progression [6,7]. Emerging evidence indicated that *A. muciniphila* regulates hepatic inflammation and the immune response in NAFLD via multiple potential mechanisms.

Signals of A. muciniphila downregulate hepatic inflammation

The signals of proinflammatory cytokines, such as IL-6 and TNF-α, are critically involved in the pathophysiology of human NAFLD. These cytokines trigger the production of other cytokines and recruit inflammatory cells in the liver [104]. Interestingly, the levels of IL-6 and TNF-α, which are negatively correlated with decreased *A. muciniphila* abundance in the gut, were reduced in the serum and liver of mice with sucralose-induced NAFLD [44]. Notably, some studies reported that *A. muciniphila* prevented the hepatic expression of the proinflammatory factor IL-6 in HFD-fed [21] or high-fat and high-cholesterol diet-fed [38] NAFLD mice. Similarly, in HFD/CCl_4-induced fibrotic mice, not only

live *A. muciniphila* but also pasteurized *A. muciniphila* and its AmEVs could reduce the levels of serum IL-6 and TNF-α and downregulate hepatic TNF-α expression [23]. These findings indicated a link between *A. muciniphila* and hepatic proinflammatory factors (IL-6 and TNF-α, Fig. 3). However, further analysis is needed to explore the original cells of the proinflammatory factors and how *A. muciniphila* communicates with these hepatic proinflammatory factors in the progression of NAFLD.

A. muciniphila and gut-derived LPS signaling in liver

LPS is a typical pathogen-associated molecular pattern (PAMP) that activates the hepatic innate immune response [40]. A high level of serum LPS, hepatocyte LPS localization, and upregulation of downstream TLR4 signaling have been observed in patients with NASH [105,106]. A study showed that *A. muciniphila* reduced LPS signaling (including JNK and IKBA) in the liver of chow diet-fed mice [99]. *A. muciniphila* was reported to reduce circulating LPS levels by enhancing gut barrier function in HFD-induced models of metabolic disorders [19]. In other models of liver injury, *A. muciniphila* was found to downregulate LPS levels and its related signaling pathway. For instance, *A. muciniphila* could suppress LPS production by regulating the gut microbiota in mice with immune-induced liver injury [25]. The inhibitory effect of live and pasteurized *A. muciniphila* and AmEVs on liver TLR4 expression was also observed in HFD/CCl_4-induced fibrotic mice [23]. Furthermore, a cell experiment demonstrated that live/pasteurized *A. muciniphila* and AmEVs could markedly inhibit the gene expression of LPS-targeted receptors (*TLR4*) and even fibrosis markers (*a-SMA*, *TIMP1*, *Col1a1*, and *TGF-β*) in hepatic stellate cells [23]. These findings suggested a potential mechanism by which *A. muciniphila* acts on liver inflammation in NAFLD by regulating the LPS signaling pathway (Fig. 3). However, whether *A. muciniphila* specifically affects the expression of TLR4 in hepatic stellate cells or other immune cells in NAFLD remains unclear.

A. muciniphila and TLR2 signaling in liver

In addition to the PAMPs of the microbiota, microbial metabolites, especially BAs and tryptophan metabolites, are implicated in immune system functions [107]. Gut-derived signals could activate the hepatic innate immune response, contributing to the development of NASH [40,108]. Live and pasteurized *A. muciniphila* and its AmEVs were also reported to downregulate hepatic TLR2 expression in HFD/CCl_4-induced fibrotic mice [23]. TLR2 knockout suppressed the progression of NASH [109]. *A. muciniphila* was hypothesized to downregulate TLR2 expression and further prevent HFD-induced liver inflammation (Fig. 3). Of course, these findings warrant further investigation in NAFLD models with TLR2 knockout.

A. muciniphila and macrophages in liver

Macrophages are one of the most important types of innate immune cells in the liver, and they have been shown to play an important role in NAFLD in human and animal models [110]. *A. muciniphila* or Amuc_1100 could reduce the infiltration of macrophages in the colon [33]. However, the specific mechanism by which the microbial signaling of *A. muciniphila* affects hepatic macrophages in NAFLD remains unclear.

Interestingly, recent studies suggested the importance of *A. muciniphila* in modulating the network between AhR and macrophages against NASH. The modulation of AhR in macrophage polarization was confirmed in other models of immune disorders [111–113]. Previous studies have discussed the anti-inflammatory actions of AhR in NAFLD [114–116]. For instance, Lin *et al.* demonstrated that liver-specific AhR knockout aggravated HFD-induced hepatic inflammation in mice [115]. The depletion of *A. muciniphila* abundance together with a reduction in liver AhR ligands was observed in saccharin/sucralose-induced NAFLD mice [44]. Live and pasteurized *A. muciniphila* and Amuc_1100 could upregulate AhR targeted genes (including *CYP1A1*, *IL-10*, and *IL-22*) [36], and *A. muciniphila* was regarded as a key contributor to tryptophan metabolism (which provides AhR ligands) [36,117]. These results indicated that *A. muciniphila* may protect against NASH, partly by regulating the dialog between AhR and macrophages (Fig. 3). Whether and how *A. muciniphila* affects hepatic macrophage polarization of NAFLD warrant further research.

Notably, in other models of liver injury, the current focus on hepatic immune cells regulated by *A. muciniphila* is neutrophils and macrophages [22,25]. For instance, in a model of acute and chronic alcoholic hepatitis, *A. muciniphila* decreased neutrophil infiltration (MPO^+) in the liver, showing its preventive and therapeutic efficacy [22]. *A. muciniphila* was also reported to protect against immune-mediated liver injury by reducing the accumulation of hepatic immune cells, including neutrophils ($Ly6G^+$) and macrophages ($F4/80^+$), in mice [25]. Despite these advanced findings, some limitations exist in current studies targeting the functional mechanism of *A. muciniphila* in NAFLD. First, *A. muciniphila*-regulated inflammatory cells were only quantified by immunohistochemical staining methods. Thus, more accurate methods, such as flow cytometry, are needed. Second, how *A. muciniphila* communicates with hepatic immune cells in NAFLD remains unclear. Taken together, these findings provided additional knowledge on

how *A. muciniphila* functions in the processes of liver immunity of NAFLD, that is, it likely acts by inhibiting the microbiota-related innate immune response in the liver.

In addition, *A. muciniphila* exhibited protective effects against liver fibrosis, which is the advanced stage of NAFLD. Administration of live and pasteurized *A. muciniphila* and extracellular vesicles of *A. muciniphila* for 4 weeks prevented HFD/CCl_4-induced liver fibrosis [23] and hepatic stellate cell (LX-2 cell) activation [118]. These findings indicated the promise of the application of *A. muciniphila* for treating liver fibrosis. However, further studies are needed to confirm the effects of *A. muciniphila* or its derivatives on the progression of NAFLD to fibrosis and its exact functional mechanism.

Conclusions and perspectives on the role of *A. muciniphila* in NAFLD

Numerous studies mostly reported the decreased abundance of *A. muciniphila* in animals with NAFLD induced by HFD, MCD, and saccharin/sucralose diets (summarized in Table 1). However, the alterations of *A. muciniphila* in patients with NAFLD (with histopathological diagnosis) rather than those who are overweight/obese still need further investigation. Current studies linked the increased abundance of *A. muciniphila* with beneficial effects in NAFLD therapy (summarized in Table 2). Different NAFLD interventions also boost *A. muciniphila* abundance. Consistently, the administration of *A. muciniphila* directly benefits the prevention and therapy of NAFLD. Present animal experiments strongly supported the efficacy of *A. muciniphila* in treating hepatic steatosis and improving glucose and lipid metabolism in NAFLD. However, the efficacy of *A. muciniphila* in treating patients with NAFLD has not been assessed, although few clinical studies have reported its application in patients with obesity and T2DM [13,18], largely due to less evidence for the underlying mechanism by which *A. muciniphila* acts on hepatic manifestations (especially inflammation) in NAFLD.

In summary, much of the current knowledge on the mechanisms of *A. muciniphila* in NAFLD relies on research *in vitro* and in animal models. This study summarized the potential mechanisms in accordance with the current findings (Figs. 2 and 3). First, convincing evidence exists for the ability of *A. muciniphila* to improve hepatic lipid accumulation in the process of NAFLD. In addition to live and pasteurised *A. muciniphila*, the active components of *A. muciniphila*, such as Amuc_1100, AmEVs, and P9 protein, have demonstrated similar actions, and more components have

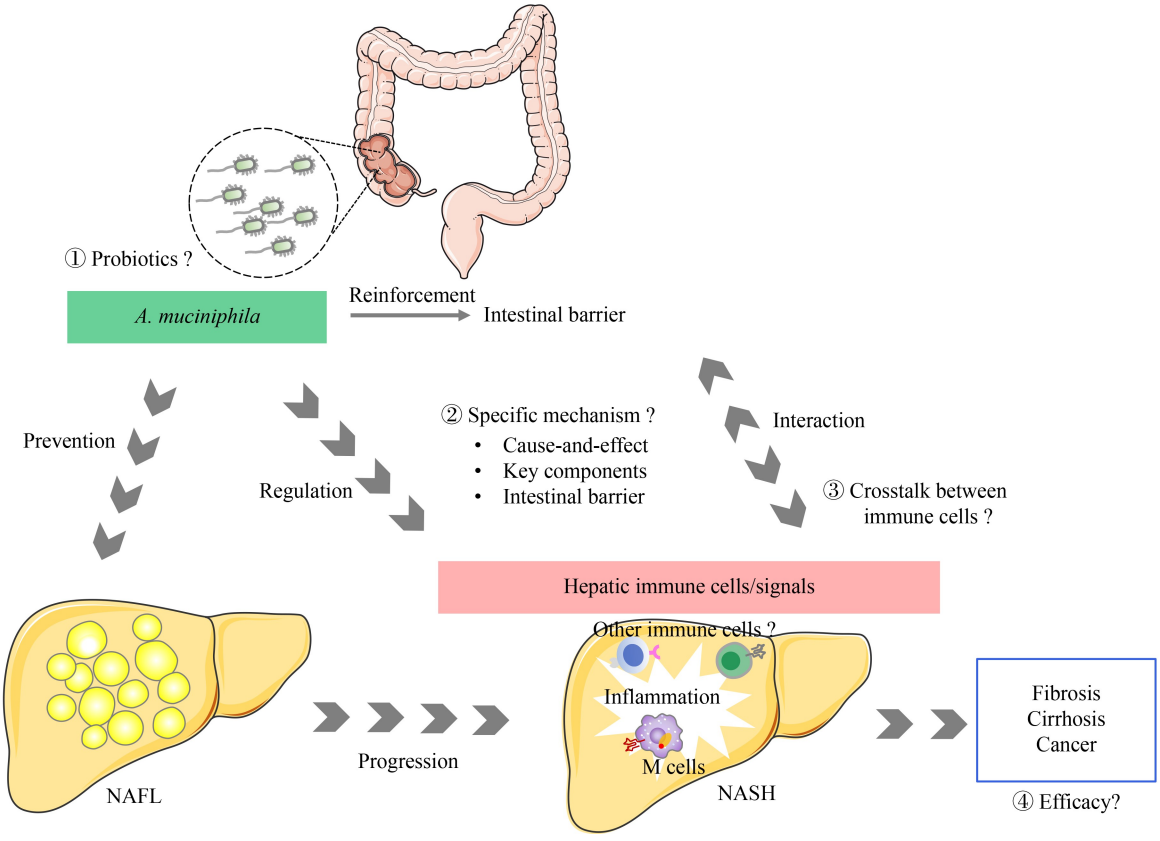

Fig. 4 Perspectives on the regulation of NAFLD by *A. muciniphila.*

yet to be discovered. Second, *A. muciniphila* protects against hepatic inflammation in NAFLD by regulating the hepatic immune response, which may be related to the production of inflammatory factors (IL-6 and TNF-α), LPS signals, and macrophages. The current focus on the regulation of *A. muciniphila* on the hepatic immune response of NAFLD is relatively limited.

Some concerns on the regulation of *A. muciniphila* on the hepatic immune response of NAFLD need to be resolved (Fig. 4). First, a cause-and-effect evidence that *A. muciniphila* acts on immune cells/signals in NAFLD improvement remains to be gathered. A combination of germ-free mice (or antibiotic clearance) and *A. muciniphila* supplementation (or symbiotic microbial consortia transplantation) could help clarify the role of *A. muciniphila*. Second, whether and how these immune cells/signals interact with each other in the process of which *A. muciniphila* prevents NAFLD remain elusive. The importance or necessity of immune cells/signals needs to be confirmed by gene knockout mice. Third, the specific mechanism involved in the crosstalk between the discovered immune signals/cells and *A. muciniphila* remains unknown. What are the key components of *A. muciniphila*? Do the reported active components of *A. muciniphila* have similar efficacy in preventing NASH? Given the contribution of the intestinal barrier in the development of NAFLD, is the efficacy of *A. muciniphila* in the hepatic immune response entirely based on its gut barrier protection functions? Finally, does *A. muciniphila* present the same benefits for fibrosis or cancer that could progress from NAFLD? The understanding of the underlying mechanisms of action could pave the way for the application of *A. muciniphila* in treating patients with NAFLD and other related liver injuries. A notable detail is that most of the present studies were performed in animal NAFLD models that do not completely reflect human NAFLD. Thus, the results require further validation and investigation. In addition, clinical trials on the safety and effectiveness of *A. muciniphila* in treating human NAFLD are urgently needed.

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Compliance with ethics guidelines

Yuqiu Han, Lanjuan Li, and Baohong Wang declare no competing financial interests. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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