Immunology of gut microbiome and liver in non-alcoholic fatty liver disease (NAFLD): mechanisms, bacteria, and novel therapeutic targets

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. Most important contributors to its development are diet and obesity. Gut microbiome's importance for immune system and inflammatory pathways more widely accepted as an important component in NAFLD and other liver diseases' pathogenesis. In this article we review potential mechanisms of microbiome alteration of local and systemic immune responses leading to NAFLD's development, and how can modulate them for the treatment. Our review mentions different immune system pathways and microorganisms regulating metabolism, liver inflammation and fibrosis. We specifically point out TLR-4 as a potential key immune pathway activated by bacterial lipopolysaccharides producing pro-inflammatory cytokines in NAFLD. Also, we discuss three endotoxin-producing strains (*Enterobacter cloacae B29*, *Escherichia coli PY102*, *Klebsiella pneumoniae A7*) that can promote NAFLD development via TLR4-dependent immune response activation in animal models and how they potentially contribute to disease progression in humans. Additionally, we discuss their other immune and non-immune mechanisms contributing to NAFLD pathogenesis. In the end we point out gut microbiome researches' future perspective in NAFLD as a potential new target for both diagnostic and treatment.

Keywords: Non-alcoholic fatty liver disease, microbiome, lipopolysaccharide, endotoxin, TLR-4

Background

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term that includes different stages of a single disease. (Pouwels, Sakran et al. 2022) The main characteristic feature of NAFLD is hepatic steatosis with other causes excluded (e.g., viral infection, alcohol, autoimmune hepatitis, etc.).(Ando and Jou 2021) Most recent data showed that NAFLD has become the most common chronic liver disease worldwide with overall prevalence 29-35% among adults (Younossi, Koenig et al. 2016, Younossi, Golabi et al. 2023) which is much higher than previously thought. (Tian, Zhang et al. 2023) Its prevalence continues to grow rapidly all over the world (Riazi, Azhari et al. 2022) and including Russia (Bikbov, Gilmanshin et al. 2022). Higher rates of NAFLD incidence and prevalence are seen in males (Riazi, Azhari et al. 2022), and especially in patients with dyslipidaemia, obesity, metabolic syndrome, and Type 2 Diabetes Mellitus (T2DM). (Benedict and Zhang 2017) Right now NAFLD is considered a growing challenge for public health systems worldwide. (Lazarus, Mark et al. 2022)

NAFLD ranges from mild forms (such as steatosis, i.e., accumulation of fat in the liver without inflammation) to progressively more severe forms – non-alcoholic steatohepatitis (NASH). Fat accumulation promotes inflammation with subsequent fibrosis, cirrhosis and liver failure or even hepatocellular carcinoma (HCC). (Rinella, Neuschwander-Tetri et al. 2023) Although we currently understand its natural history (Nasr, Ignatova et al. 2018) and possible main underlying pathophysiological mechanisms (Parthasarathy, Revelo et al. 2020) (hepatocyte injury, inflammation and fibrosis) the NAFLD pathogenesis still remains unknown with many gaps. In the last 10 years the scientists proposed the "multiple hit" hypothesis in attempt to explain the causes of NAFLD initiation and progression (Buzzetti, Pinzani et al. 2016, Ziolkowska, Binienda et al. 2021). This hypothesis tries to explain the complex interplay between insulin resistance, adipokines (adipose tissue hormones), genetic predisposition, epigenetic factors, nutritional and lifestyle factors, and gut microbiota – all of them combined promotes lipotoxicity and oxidative stress with further mitochondrial dysfunction, inflammation and hepatic fibrosis (Ji, Yin et al. 2019, Parthasarathy, Revelo et al. 2020). Although, the exact proportions of environmental and genetic factors, various extra- and intrahepatic events that lead to different NAFLD phenotypes remain unknown to this day (Arab, Arrese et al. 2018). Our review article will primarily focus on the gut-liver axis and immune system interactions, how they initiate different pathological processes in the liver that potentially contribute to the NAFLD development and progression and how they can help in exploring new approaches in diagnostic and treatment based on gut microbiota modulation.

Gut-liver axis

The number of microorganisms in the gastrointestinal (GI) tract has been roughly estimated to be about 10^{14}, which is approximately 10 times more than the number of human cells and over 100 times the amount of microbiome genomic content compared to the human genome (Thursby and Juge 2017). They play an essential role in maintaining immune and metabolic homeostasis and also protecting against various pathogens in healthy organism (Jandhyala, Talukdar et al. 2015). Alterations in gut microbiota (also known as dysbiosis - imbalance between protective and harmful bacteria) have been implicated in many diseases' pathogenesis (Durack and Lynch 2019) including NAFLD. (Ji, Yin et al. 2019, Bruneau, Hundertmark et al. 2021). The relationships between gut microbiome and the liver are bidirectional: the gut is an important first entry port for many external environmental factors, while the liver is the first line to receipt and process these factors. Researches showed that the diet can shape and maintain the gut microbiota (Jandhyala, Talukdar et al. 2015, Thursby and Juge 2017) and alterations in the diet such as persistent food intake with high amount of saturated fats or fructose (Yu, Li et al. 2021)) promote gut microbiota changes which leads to barrier disruption in GI tract (Ji, Yin et al. 2019) and immune homeostasis (Zhou, Tripathi et al. 2021). Multiple studies have shown that patients with different NAFLD stages have altered gut microbiota (Ebrahimzadeh Leylabadlo, Ghotaslou et al. 2020, Albhaisi and Bajaj 2021, Luo, Chang et al. 2023). Gut microbiota was different both from healthy controls and between various NAFLD stages (steatosis vs. steatohepatitis fibrosis/cirrhosis vs. HCC) (Boursier, Mueller et al. 2016, Caussy, Tripathi et al. 2019, Kolodziejczyk, Zheng et al. 2019, Schwimmer, Johnson et al. 2019).

At the moment the exact mechanisms and pathways how the gut microbiota alterations affect NAFLD development and progression have not been completely understood (Parthasarathy, Revelo et al. 2020, Hrncir, Hrncirova et al. 2021, Oh, Gupta et al. 2023). Nevertheless, many studies show that potential mechanisms contributing to this are disruption in intestinal permeability (Mouries, Brescia et al. 2019)

and bacterial-derived ligands (e.g., LPS) (Zhou, Tripathi et al. 2021) and metabolites (e.g., short chain fatty acids, secondary bile acids) (Ebrahimzadeh Leylabadlo, Ghotaslou et al. 2020, Gupta, Min et al. 2022). These bioactive compounds get in the liver via a portal vein system and directly affect various liver cells (Brandl, Kumar et al. 2017). This initiates an immune response which propagates liver inflammatory and subsequent fibrosis.

About 90% of the microbiota in GI tract consists of two distinct phyla – Firmicutes and Bacteroidetes (De Filippis, Pellegrini et al. 2016). Different studies showed that advanced fibrosis in NAFLD/NASH was associated with Proteobacteria and Escherichia coli increase and at the same time decrease in Firmicutes (e.g., *Faecalibacterium prausnitzii*) (Loomba, Seguritan et al. 2017, Caussy, Tripathi et al. 2019). Patients with NASH or cirrhosis had a decrease in healthy microbiome bacteria such as Bacteroidetes and an increase in pathogenic bacteria such as Proteobacteria and Enterobacteriaceae species (Qi, Yang et al. 2020). This can point out their potential role in promoting liver inflammation. *Enterobacter cloacae* is another bacterium that can be involved in inducing inflammation and lipid accumulation in NAFLD was (Jin, Zheng et al. 2022).

Mechanisms of gut microbiome influence on NAFLD development

As we mentioned earlier gut microbiota and its various components and metabolites can be transported to the liver via portal vein system. Different liver immune cells (e.g., hepatic stellate cells (HSCs), Kupffer cells) interact with these gut-derived factors. Some of them are pro-inflammatory for these cells – lipopolysaccharide, lipoteichoic acid (LTA), and peptidoglycan. The more activation of liver immune cells by these bioproducts the more intense inflammatory response and fibrosis which lead to more severe liver damage intensifying NAFLD development. But some bacterial bioproducts such as tryptophan metabolites, short-chain fatty acids (SCFAs), carotenoids, bile acids, and phenolic compounds may actually decrease intensity of inflammation, immune cell response, oxidative damage, and lipogenesis in liver (Hrncir, Hrncirova et al. 2021).

Early animal NAFLD models showed which alterations gut microbiota increased liver inflammation and fibrosis (De Minicis, Rychlicki et al. 2014): increase in Gram-negative versus Gram-positive bacteria, a reduced ratio between Bacteroidetes and Firmicutes, and a significant increase in Gram-negative Proteobacteria. At that time the exact mechanisms have not been fully understood, but most likely they were mediated via Toll-like receptors (TLRs) activation on HSCs. This is indirectly supported by the data which show that liver inflammation similar to NASH can be achieved via TLR4 activation on HSCs (Bigorgne, John et al. 2016). Another thing that supports one of the crucial roles of TLRs in the pathogenesis of NAFLD is that they can also be activated by damage-associated molecular patterns released from injured cells and tissues, which may promote sterile inflammation (i.e., in the absence of microorganisms) in the liver. (Arab, Arrese et al. 2018) Another potentially important NAFLD pathogenesis components are inflammasomes which were shown to be tied to liver damage and fibrosis in NASH. (De Minicis, Rychlicki et al. 2014, Mridha, Wree et al. 2017)

Because of these factors, gut microbiota is considered one of the key if not the key element involved NAFLD pathogenesis. Exploring various signalling pathways of gut microbiome-derived factors on liver will help to elucidate new therapeutic strategies for NAFLD. The current article summarizes different mechanisms how different bacterial components and metabolites affect NAFLD development and progression. In addition, we would discuss what is known at the moment about

immune system-gut microbiome mechanisms in NAFLD because few articles have studied NAFLD patients' intestinal mucosa microbiota.

Immune mechanisms involved in NAFLD

Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are a steroid hormone receptor superfamily of transcription factors that are one of the main regulators of lipid and glucose metabolism and inflammation. (Christofides, Konstantinidou et al. 2021) The PPAR superfamily includes three subtypes – PPARα, PPARγ, and PPARβ/δ, with different organ and tissue distribution for each of them. Most commonly they can be found in the liver, but also in other tissues – adipose tissue and skeletal muscle. (Fougerat, Montagner et al. 2020, Christofides, Konstantinidou et al. 2021) One of the recently discovered PPARs' function includes normal homeostasis of intestinal tissue (Ning, Lou et al. 2019, Decara, Rivera et al. 2020) which can be altered in different gastroenterological diseases like inflammatory bowel diseases (both Crohn disease and ulcerative colitis) (Decara, Rivera et al. 2020) and in various liver diseases including NAFLD.

PPARs potentially contribute to the NAFLD development and progression mostly via fatty acid metabolism and lipogenesis regulation. (Fougerat, Montagner et al. 2020) PPARs can be activated by different ligands, such as prostaglandins and eicosanoids, fatty acids, and synthetic ligands. (Francque, Szabo et al. 2021)[45] All of the PPARs play important roles in liver inflammation (Fougerat, Montagner et al. 2020): PPARα downregulates pro-inflammatory genes, PPARβ/δ and PPARγ control M2 polarization of macrophage and PPARγ can promote anti-fibrotic effects. Also, all three PPARs regulate lipid and glucose metabolism in different organs and tissues (Wang, Nakajima et al. 2020): PPARα is the main regulator of lipid catabolism in the liver during fasting; PPARγ controls lipid storage in adipose tissue and adipocyte differentiation therefore increasing insulin sensitivity; PPARβ/δ induces glucose consumption and fatty acid synthesis in the liver and increase fat breakdown in muscle tissue. Many preclinical animal studies showed that all PPARs have protective effect on the liver by decreasing hepatic steatosis and liver inflammation. (Fougerat, Montagner et al. 2020, Francque, Szabo et al. 2021)

To sum up, PPARs are important for lipid and glucose metabolism regulation and potentially are involved in the NAFLD development and progression. PPARs' activation can lead to increased gene expression involved in fatty acid oxidation, lipogenesis, and inflammation, - all of which are implicated in NAFLD pathogenesis. Additionally, several studies showed PPAR agonists' potential as novel drugs to treat NAFLD. (Choudhary, Kumar et al. 2019, Francque, Szabo et al. 2021)

Pathogen-associated molecular patterns

Pathogen-associated molecular patterns (PAMPs) are various surface molecules of certain bacteria and some other microorganisms, that can be recognized by immune system, and triggering an inflammatory response. (Colak, Hasan et al. 2021) This PAMP-mediated immune response can contribute to NAFLD development and recent research article proposed that certain bacterial PAMPs are associated with an increased risk of NAFLD. (Schwimmer, Johnson et al. 2019, Huby and Gautier 2022, Li, Rempel et al. 2022, Nati, Chung et al. 2022)

Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS) is one of the most studied PAMPs involved in NAFLD pathogenesis. It is found on the surface of Gram-negative bacteria. (Zhou, Tripathi et al. 2021) Different studies have shown that LPS induces liver damage via the LPS-binding protein (LBP)-CD14 complex that in turn activates Toll-like receptor 4 (TLR4), promoting an inflammatory response (Wang, Tang et al. 2021) and increasing gut permeability (Nighot, Al-Sadi et al. 2017). Both events contribute to the development of NAFLD and specifically to NASH. (Sharifnia, Antoun et al. 2015)

TLR4 is one of the innate immune system's crucial components and plays the main role in recognition and response to bacterial and viral pathogens most notably it serves as a LPS sensor. (Sharifnia, Antoun et al. 2015) TLR4 is normally expressed in different cell types, including the liver cells like hepatocytes, Kupffer cells, HSCs and monocytes. (Sharifnia, Antoun et al. 2015) LPS activates Kupffer cells in the liver leading to increase in pro-inflammatory cytokines production (e.g., IL-18, IL-1β, and IL-12), which induces activity of cytotoxic T cells and Natural killer (NK) cells. (Kolodziejczyk, Zheng et al. 2019, Hrncir, Hrncirova et al. 2021, Gupta, Min et al. 2022) This innate immune system receptor can potentially be a link between gut microbiome and liver damage. As we already mentioned TLR4 signalling is involved in the inflammatory response and also induces metabolic disturbances promoting further liver damage in patients with NAFLD. (Belegri, Eggels et al. 2018, Shen, Wang et al. 2020) Although there are many other TLRs that can recognize other bacterial components (e.g., TLR2, TLR5, and TLR9) (Hug, Mohajeri et al. 2018, Sameer and Nissar 2021), their particular contribution to NASH development is poorly defined now. Picture 1 summarizes the various TLRs mechanisms involved in NAFLD pathogenesis.

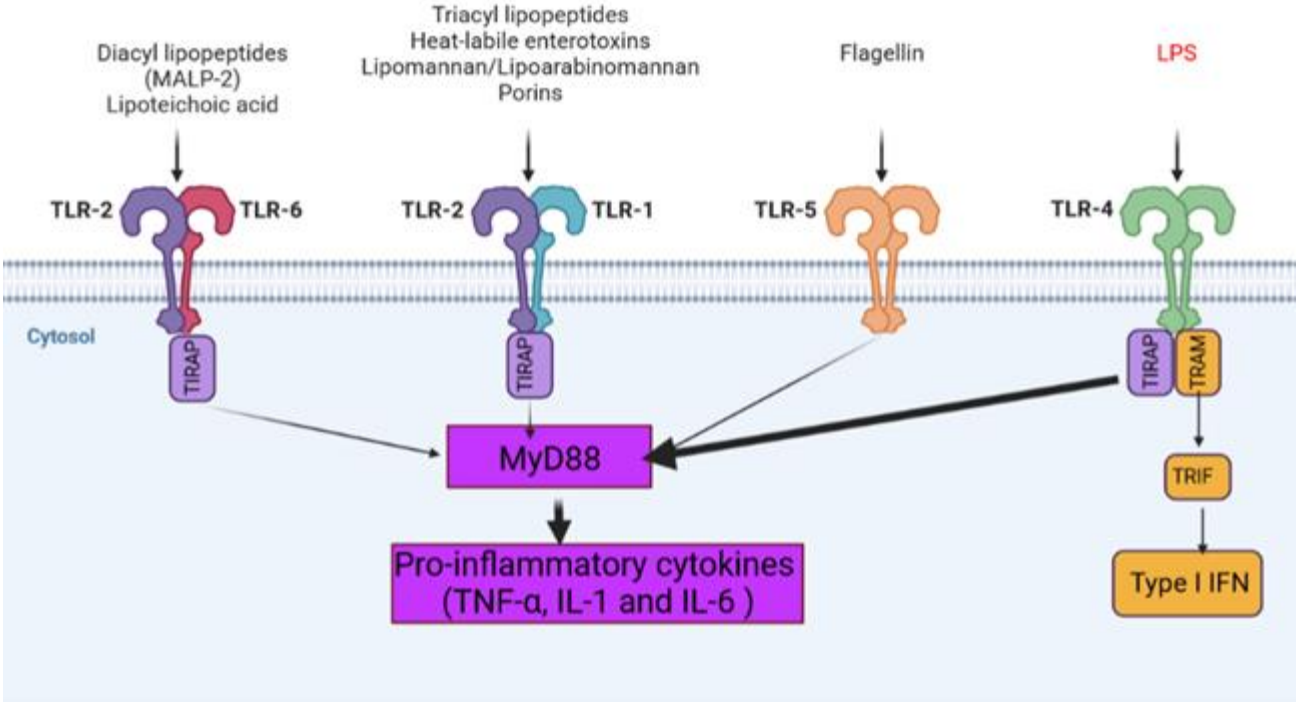

Figure 1. TLR bacterial ligands and signaling pathways.
Solid lines – most important pathways.
MALP-2: mycoplasma-derived macrophage activating lipopeptide 2; LPS: lipopolysaccharide; TIRAP, Toll/IL-1R (TIR)-domain-containing adaptor protein; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor protein inducing IFNβ; MyD88, myeloid

differentiation primary-response gene 88; IFN – interferon; IL-1 – interleukin-1; IL-6 – interleukin-6; TNF-α – tumor necrosis factor-alpha.
(This figure is created with BioRender.com).

There are two types TLR4 signalling pathways: the myeloid differentiation factor 88 (MyD88)-dependent and the MyD88-independent pathways. (Zhou, Tripathi et al. 2021) First pathway includes MyD88 which mainly transmits intracellular signals through the TIR domain and activates transcription factor nuclear factor-κB (NF-κB). (Fang, Zhou et al. 2021) After that NF-κB upregulates the expression of different inflammation-related genes and enhances various inflammatory cytokines' release (e.g., IL-1, IL-6, and TNF-α). (Sharifnia, Antoun et al. 2015, Fang, Zhou et al. 2021) Also this pathway was linked to progression to NASH and fibrosis. (Yang, Miura et al. 2017) The MyD88-independent pathway is dependent on Toll/IL-1 receptor domain-containing adaptor inducing interferon-β (TRIF), (Yang, Miura et al. 2017, Luo, Chang et al. 2023) which activates interferon regulatory factor 3 (IRF3) and induces interferon (IFN)-β and IFN-responsive genes expression. (Liu, Wu et al. 2022) In addition the MyD88-independent pathway promotes the late-phase activation of NF-κB and mitogen-activated protein kinase (MAPK). Both pathways lead to increased inflammation and liver steatosis which are the main features of NAFLD. (Katsarou, Moustakas et al. 2020) All of this indicates that LPS mostly affects the liver via LPS-TLR4 as a key pathway. Nevertheless, it needs to be mentioned that we still need to clarify what and how much specific bacteria with their bioproducts contribute to the liver inflammation in NAFLD.

Flagellin

Flagellin is another PAMP associated with NAFLD. (Jadhav and Cohen 2020, Han, Jiang et al. 2023) It also can be found on the surface of many different bacterial strains, and the data show that it damages the liver via the same two pathways mentioned above: hepatic fat accumulation and activation of an inflammatory response in the liver. These processes are main actors in the NAFLD pathogenesis. It was shown in mice that flagellin acting via vascular adhesion protein 1 (VAP-1) induced hepatic steatosis. (Toivonen, Vanhatalo et al. 2021) VAP-1 is a pro-inflammatory protein that is also involved in a bacterial lipopolysaccharide-induced inflammation and lipolysis in visceral adipose tissue. (Salmi and Jalkanen 2019) This in turn promoted liver fat accumulation.

There is also data showing that TLR5-flagelling interaction may actually have protective effect on the liver, as separate studies have shown that knock-down of TLR5 accelerates hepatic steatosis and increases susceptibility to liver injury and subsequent NASH development. (Vijay-Kumar, Aitken et al. 2010, Etienne-Mesmin, Vijay-Kumar et al. 2016) Another study has demonstrated that TLR5 activation-induced type 1 IFN signaling has both anti-inflammatory and antifibrogenic properties by preserving equilibrium between IL1b and IL1RN production which in turn have protective effect against liver fibrosis. (Zhou, Kim et al. 2020) This fact has chances to be one of the novel therapeutic strategies for new drugs development by modulating of TLR5 signaling to decrease the severity of liver fibrosis. Also, we should mention that flagellin can induce inflammation via cytosolic nucleotide oligomerization domain (NOD)-like receptors activation. (Tourlomousis, Wright et al. 2020) All of this information shows the importance of further investigation of flagellin and its receptors effects on NAFLD development and progression.

In addition to LPS and flagellin, there are other PAMPs which may be associated with NAFLD (Kolodziejczyk, Zheng et al. 2019, Katsarou, Moustakas et al. 2020, Khanmohammadi and Kuchay

2022): peptidoglycan, lipoteichoic acid, and teichoic acid. All these molecules are found on the surface of bacteria and are thought to be involved in NAFLD development.

To summarize, there is a bunch of evidence to suggest that certain PAMPs may play an important role in NAFLD pathogenesis. (Hrncir, Hrncirova et al. 2021) Additional research is required to confirm the link between these molecules and NAFLD, as well as to understand the exact mechanisms by which they may be involved. In our review article we would like to discuss LPS more thoroughly and identify what microorganisms use it as a main pathogenic factor in the development of NAFLD.

Lipopolysaccharide as main pro-inflammatory actor in NAFLD

Lipopolysaccharide (LPS) is the most studied gut-derived PAMP. It is the bacterial endotoxin, which can be found in the gram-negative bacteria outer membrane. NAFLD patients have higher levels of LPS in blood (Sharifnia, Antoun et al. 2015) which lead to steatohepatitis progression. (Kolodziejczyk, Zheng et al. 2019). Other studies have also shown that endotoxins like LPS can similarly activate TLR4 pathways to increase intestinal permeability. (Nighot, Al-Sadi et al. 2017, Kinashi and Hase 2021) Also we would like to mention that LPS levels progressively increased as steatosis developed further into steatohepatitis (Carpino, Del Ben et al. 2020, Kessoku, Kobayashi et al. 2021) which shows its importance as potentially key pro-inflammatory mediator in the pathogenesis of NAFLD progression.

Animal study showed that three endotoxin-producing strains, *Enterobacter cloacae B29*, *Escherichia coli PY102*, and *Klebsiella pneumoniae A7*, from the gut of morbidly obese volunteers with severe hepatic steatosis, when transplanted into the gut of germ-free mice on high-fat diet promoted NAFLD development via increased TLR4-dependent immune response. (Fei, Bruneau et al. 2020)[76]

According to our earlier discussion, LPS may be the most important PAMP stimulating NAFLD development and progression via TLR4-mediated pathways. There are several other bacteria associated with NAFLD (Ji, Yin et al. 2020), these three endotoxin-producing strains that we mentioned are potentially crucial for initiating disease progression. Consequently, we want to study further possible role of these bacteria in NAFLD development and progression, and most importantly what immune mechanisms they use to do that. The currently available data will be summarized in Table 1 and Picture 2.

Klebsiella pneumoniae A7

Klebsiella pneumoniae A7 is an opportunistic gram-negative bacterium associated with NAFLD. (Fei, Bruneau et al. 2020) The exact mechanisms how Klebsiella pneumoniae A7 affects NAFLD pathogenesis remain to be clarified, it can potentially be related to the mechanisms we discuss further.

The first potential mechanism is LPS production and further innate immune system activation and pro-inflammatory cytokines induction. (Fei, Bruneau et al. 2020) Additionally, K. pneumoniae A7 can also activate the Toll-like receptor 4 (TLR-4) pathway, which has been shown to be involved in hepatic steatosis development.

One of the articles has demonstrated that *Klebsiella pneumoniae A7* produces large amounts of endogenous alcohol that was linked to NAFLD progression in humans. (Yuan, Chen et al. 2019) Potentially this can be due to alcohol-mediated fatty acid oxidation inhibition in the liver with

concurrent lipogenesis induction, up-regulation of steatosis and inflammation pathways, and intestinal barrier disruption. (Wang, Mehal et al. 2021)

Finally, another potential mechanism is throught to be intestinal epithelial barrier disruption and initiation of a hepatic T helper 17 cell-mediated immune response with simultaneous pro-inflammatory genes up-regulation including serum amyloid A and interleukin-1β (IL-1β) in patients with Primary Sclerosing Cholangitis. (Trauner and Fuchs 2022) Similar mechanisms are highly likely to be involved in NAFLD development and progression.

Enterobacter cloacae B29

Enterobacter cloacae B29 is another bacterial species that has been recently associated with NAFLD. Unfortunately, the underlying mechanisms remain to be elucidated. It was previously associated with both to obesity and liver damage, (Keskitalo, Munukka et al. 2018)[81] which as we know both are associated with NAFLD, so it is safe to assume that these bacteria may also play a role in NAFLD pathogenesis. However, we still do not know how exactly *Enterobacter cloacae B29* contributes to NAFLD development and progression. One of the possible explanations is the bacteria's ability to breakdown dietary lipids, which in turn increases inflammatory response. (Keskitalo, Munukka et al. 2018) The article has shown that it promotes insulin resistant in animal models and additionally increased lipolysis and adipocyte hypertrophy with subsequent increased glycerol release. Although there were no signs of increased liver fat accumulation further analysis revealed higher AST activity and histology showed hepatic damage.

Another possible mechanism is through the increased production of pro-inflammatory molecules. *Enterobacter cloacae B29* has been found to produce molecules such as LPS, which can trigger an inflammatory response. This could also contribute to the development of NAFLD. We should specifically highlight, that LPS from Enterobacteriaceae exhibited a significantly higher endotoxin activity compared to other bacteria like Bacteroidetes even though the latter compromise more abundant group in the gut. (Lindberg, Weintraub et al. 1990)

NAFLD progression may be related to interactions between Enterobacter and high-fat diet. (Yan, Fei et al. 2016) It was shown that the PPARs signalling pathways were significantly activated. As we mentioned before, these pathways are mainly involved in inflammation and lipid, lipoprotein, and sterols metabolism. Alterations in these pathways were linked to obesity, insulin-resistance, and increased inflammation.

Another interesting fact is the Enterobacter’s ability can also increase flagellin-recognizing TLR5 expression (Keskitalo, Munukka et al. 2018) which is related to intestinal inflammation propagation. (Schwimmer, Johnson et al. 2019) However we need to say that no studies exploring interactions between Enterobacter flagellin and TLR5 have been conducted.

More research is needed to fully understand the role of *Enterobacter cloacae B29* in NAFLD. However, it becomes clearer that this bacterium and NAFLD are somehow connected.

Escherichia coli PY102

Escherichia coli (E. coli) is an important pathogen associated with NAFLD and has been found to colonize the liver and cause chronic inflammation. (Li, Hao et al. 2017) This bacterial infection is linked to hepatic fibrosis and is thought to be a key player in the NAFLD development. (Jiang, Wu et al. 2015, Loomba, Seguritan et al. 2017) It was also found that a trend of increase in *E. coli* in advanced fibrosis and also shown that alterations in gut microbiome specifically *E. coli* predominance occurs in earlier fibrosis stages and may even precede portal hypertension development. (Loomba, Seguritan et al. 2017)

The specific role of *E. coli PY102* strain in NAFLD pathogenesis is very complex and poorly defined as of now and remains to be further elucidated. It is believed that the presence of *E. coli PY102* triggers pro-inflammatory cytokines and chemokines production via LPS-TLR4 pathway activation, which in turn propagates inflammation and tissue damage in NAFLD. (Fei, Bruneau et al. 2020)

Another *E.coli* strain that can potentially contribute to NAFLD development is *E. coli NF73-1*. It can adhere to the intestinal mucosa surface and translocate into the liver parenchyma, which promotes inflammatory response via the of the TLR2-NF-KB/NLRP3-Caspase-1 signalling pathway activation mediating macrophage M1 polarization and further progression from NAFLD to NASH. (Zhang, Jiang et al. 2020) Although this strain is different from the one, we discussed above, we think *E. coli PY102* can potentially use the same mechanism.

Microorganism	**Mechanism of pathogenesis**	**References**
Klebsiella Pneumoniae A7	1. LPS 2. Alcohol 3. Th17 immunity 4. Epithelial barrier disruption	(Yuan, Chen et al. 2019, Fei, Bruneau et al. 2020, Trauner and Fuchs 2022)
Enterobacter cloacae B29	1. Obesity 2. Insulin resistance 3. Hepatic damage 4. LPS 5. Intestinal permeability 6. Flagellin	(Lindberg, Weintraub et al. 1990, Yan, Fei et al. 2016, Keskitalo, Munukka et al. 2018)
Escherichia coli PY102	1. LPS	(Fei, Bruneau et al. 2020)

Table 1. Bacteria mostly involved in the pathogenesis of NAFLD.
LPS – lipopolysaccharide; Th17 – T helper 17 cells.

Potential gut microbiome-modulating therapies for NAFLD

The potential of gut microbiome targeted therapies for NAFLD is an exciting and emerging field of research and as such, targeted therapies aimed at manipulating the microbiome may offer a novel treatment option. (Sharpton, Maraj et al. 2019)

One potential microbiome targeted therapy is probiotic supplementation. (Sharpton, Schnabl et al. 2021) Probiotics are live bacteria or yeast that can be taken orally to restore beneficial bacteria in the gut. Recent studies have suggested that probiotic supplementation may benefit NAFLD patients, as probiotics reduce fat accumulation in the liver and improve liver enzyme levels and, additionally, may reduce inflammation, which is another key factor in NAFLD. (Sharpton, Maraj et al. 2019)

Another very promising microbiome targeted therapy is fecal microbiota transplantation (FMT). FMT is basically transferring stool from a healthy donor to a patient with NAFLD, to restore normal gut microbiome. Recent randomized clinical trial has shown that FMT may benefit NAFLD, as it reduces fat accumulation in the liver and improves liver enzyme levels. (Xue, Deng et al. 2022)

Overall, gut microbiome targeted therapies for NAFLD are exciting and emerging fields of research. Antibiotic therapy, probiotic/synbiotic supplementation and FMT are potential therapies, beneficial for NAFLD. Nevertheless, we still have many blank spots so further research is required to understand the gut microbiome in NAFLD to develop safe and effective treatments.

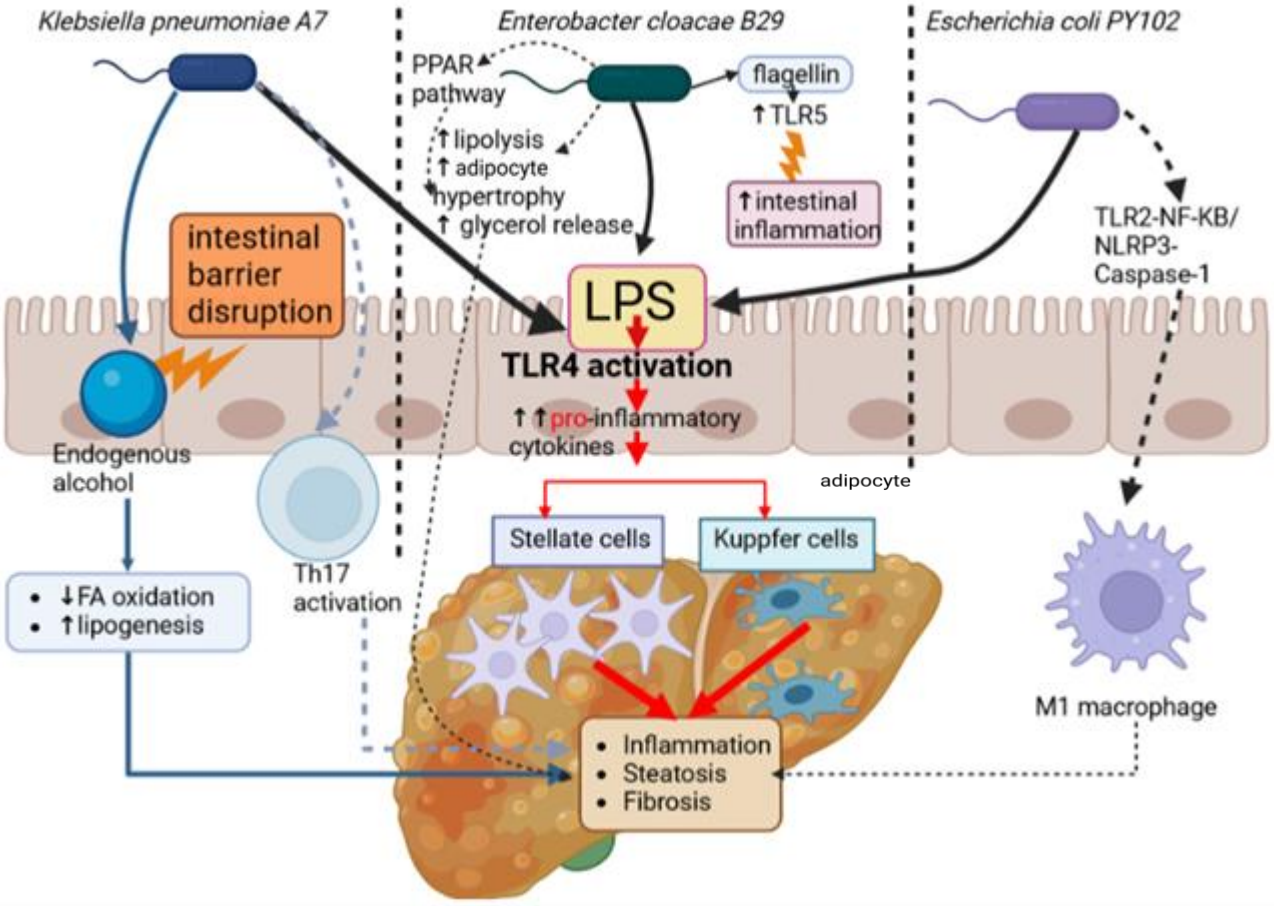

Figure 2. Schematic representation of gut microbiome influence on various immune pathways that contribute to the development and progression of NAFLD.
Solid lines – established pathways. Dashed lines – potential pathways.
LPS – lipopolysaccharide; TLR4 – Toll-like receptor 4; Th17 – T helper 17 cells; LPS – lipopolysaccharide; TLR5 – Toll-like receptor 5; TLR2-NF-KB/NLRP3-Caspase-1 – Toll-Like Receptor 2 nuclear factor kappa-B/ Nod-like receptor pyrin domain containing 3-Caspase-1 signaling pathway. (This figure is created with BioRender.com).

Concluding remarks

Overall, the immunology of gut microbiome-liver interactions in NAFLD patients is a complex and unresolved area of research. We showed that PPARs, various PAMPs and the gut microbiome potentially play a significant role in immune and inflammatory responses modulation in NAFLD and other chronic liver and gastrointestinal diseases. However, further studies are needed to optimise their use in clinical practice. It is crucial for developing safe and effective therapeutic strategies to identify the precise immunological pathways contributing to NAFLD development and progression. We believe that future research should focus on exploring the potential of using drugs targeting PPARs, PAMPs, and gut microbiome both for the treatment and prevention of NAFLD.

Conflict of interest

S.K. Gruzdev declares that he has no conflict of interest. I. V. Podoprigora, declares that she has no conflict of interest. O.A. Gizinger declares that she has no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Gruzdev Stanislav Konstantinovich. Review & Editing – Gruzdev Stanislav Konstantinovich, Podoprigora Irina Viktorovna and Gizinger Oksana Anatolievna; Supervision, Podoprigora Irina Viktorovna and Gizinger Oksana Anatolievna; Project administration, Podoprigora Irina Viktorovna and Gizinger Oksana Anatolievna. The first draft of the manuscript was written by Gruzdev Stanislav Konstantinovich and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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