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MOLECULAR METABOLISM

Non-alcoholic fatty liver and the gut microbiota

Stavros Bashiardes [1,4], Hagit Shapiro [1,4], Shachar Rozin [1,4], Oren Shibolet [2,3], Eran Elinav [1,*]

ABSTRACT

Background: Non-alcoholic fatty liver (NAFLD) is a common, multi-factorial, and poorly understood liver disease whose incidence is globally rising. NAFLD is generally asymptomatic and associated with other manifestations of the metabolic syndrome. Yet, up to 25% of NAFLD patients develop a progressive inflammatory liver disease termed non-alcoholic steatohepatitis (NASH) that may progress towards cirrhosis, hepatocellular carcinoma, and the need for liver transplantation.

In recent years, several lines of evidence suggest that the gut microbiome represents a significant environmental factor contributing to NAFLD development and its progression into NASH. Suggested microbiome-associated mechanisms contributing to NAFLD and NASH include dysbiosis-induced deregulation of the gut endothelial barrier function, which facilitates systemic bacterial translocation, and intestinal and hepatic inflammation. Furthermore, increased microbiome-modulated metabolites such as lipopolysaccharides, short chain fatty acids (SCFAs), bile acids, and ethanol, may affect liver pathology through multiple direct and indirect mechanisms.

Scope of review: Herein, we discuss the associations, mechanisms, and clinical implications of the microbiome's contribution to NAFLD and NASH. Understanding these contributions to the development of fatty liver pathogenesis and its clinical course may serve as a basis for development of therapeutic microbiome-targeting approaches for treatment and prevention of NAFLD and NASH.

Major conclusions: Intestinal host—microbiome interactions play diverse roles in the pathogenesis and progression of NAFLD and NASH. Elucidation of the mechanisms driving these microbial effects on the pathogenesis of NAFLD and NASH may enable to identify new diagnostic and therapeutic targets of these common metabolic liver diseases.

This article is part of a special issue on microbiota.

Keywords NAFLD; NASH; Microbiome; Liver

1. INTRODUCTION

Non-alcoholic fatty liver (NAFLD) is defined by pathologic accumulation of fat in the liver and is regarded the most common liver disease worldwide, with an estimated prevalence of around 25—30%. The prevalence of NAFLD is greatly increased in patients suffering from other pre-existing manifestations of the metabolic syndrome, such as obesity, type 2 diabetes, hyperlipidemia, and hypertension [1]. While NAFLD is generally asymptomatic, NAFLD patients feature an increased risk for development of other manifestations of the metabolic syndrome and accompanying complications such as cardiovascular diseases [2]. With that said, NAFLD can also occur in lean patients with normal BMI without abdominal obesity, and its prevalence is rapidly rising in countries such as India [3]. NAFLD and its associated manifestations were linked to elevated insulin resistance [3] and increased oxidized LDL to HDL ratio [4].

In up to 25% of NAFLD patients, the disease may evolve into a progressive form of liver disease named non-alcoholic steatohepatitis (NASH). NASH is defined as an inflammatory response to hepatic fat accumulation, resulting in chronic liver damage, scarring, and fibrosis. Continuous liver fibrosis may progress to cirrhosis, in which hepatocyte loss may lead to functional impairment [2]. Patients suffering of cirrhosis are predisposed to life risking complications including portal hypertension and increased risk for hepatocellular carcinoma [2]. Only limited options of pharmacotherapy are available for the treatment of NAFLD and NASH (vitamin K, metformin), and the advised treatment is a change in life style including weight reduction, enhanced exercise, and control of metabolic risk factors with glucose and lipid lowering agents [5,6].

While the pathogenesis of NAFLD is unknown, it is believed to involve abnormal lipid metabolism associated with obesity and the metabolic syndrome. Risk factors contributing to NAFLD development and progression include dietary fat consumption, genetic predisposition, excess visceral adiposity, insulin resistance, elevated serum free fatty acids, and excessive pro-inflammatory mediators. Additional liver intrinsic factors include modified hepatic glucose metabolism, insulin resistance, and altered lipid metabolism. Together, these factors lead to hepatic steatosis and, in some cases, chronic hepatic inflammation, lipotoxicity, and hepatocyte damage, which may progress into chronic hepatitis and cirrhosis [2].

Multiple animal models have been developed for the study of NAFLD and NASH. All models contain features common to some, but not all,

[1]Immunology Department, Weizmann Institute of Science, 76100 Rehovot, Israel [2]Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel [3]Department of Gastroenterology, Tel Aviv Medical Center, Tel Aviv 6423906, Israel

[4] Stavros Bashiardes, Hagit Shapiro and Shachar Rozin contributed equally to this work.

*Corresponding author. E-mail: eran.elinav@weizmann.ac.il (E. Elinav).

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NAFLD and NASH human manifestations. Dietary models include high-fat diet (HFD), methionine-choline deficient diet (MCDD), and high-fructose diet, all leading to NAFLD development and progression in rodents. HFD leads to the development of many metabolic syndrome-associated manifestations including ectopic accumulation of fat in the liver, leading to steatosis and associated insulin resistance, but in the absence of liver inflammation. Choline-deficient diet leads to decreased levels of VLDL and hepatic beta oxidation, resulting in accumulation of liver fatty acids and cholesterol, an intense inflammatory reaction, but little or no insulin resistance [7,8]. High-fructose diet induces steatosis, along with other metabolic abnormalities associated with this diet, such as weight gain, insulin resistance, and hyperlipidemia [9]. Genetic NAFLD models include mutations associated with NAFLD predisposition, such as the ones noted in the *PNPLA3* gene [10]. It is worth noting that there are phenotypic differences between *PNPLA3* associated mouse models (deletion, induced expression/insertion, and transgenic) highlighting a potential caveat of using genetic models to delineate mechanisms involved in human disease [11]. In homozygous carriers of *PNPLA3*, the prevalence of NAFLD is twofold higher as compared to non-carriers [12,13]. Another NAFLD risk gene is phosphatidylethanolamine N-methyltransferase (PEMT), which is involved in phosphatidylcholine synthesis. PEMT deficient mice fed with MCDD feature severe hepatic steatosis [14] that is partially recovered by choline supplementation [15]. Interestingly, loss of function PMET mutations were also found in some NAFLD subjects [16].

2. THE GUT MICROBIOME

Following the decoding of the human genome sequence [17], the visionary call for a second human genome project by Relman and Falkow [18] called for characterization of the genetic component of the microorganisms that colonize eukaryotes. A better understanding of microbial pathogenesis was a prelude to the realization of the importance of the microbiome to human physiology. Jeffrey Gordon and his group pioneered the understanding of factors affecting the structure of bacterial communities [19] and how microbial compositional structure may affect the risk of disease in mammals [20,21]. The microbiota communities, of which the gut microbiota is the most extensively studied, were found to play a crucial role in many aspects of development, metabolism and physiology [22–25]. Gut microbial composition is not homogeneous among individuals, and is characterized by a substantial inter-individual heterogeneity [26]. This represents a conundrum when searching for an association between disease and a deviation of bacterial community composition from the "normal" state. In a seminal study by the group of Jeffrey Gordon [27], a "core microbiome" was characterized on the basis of a particular gene and inferred metabolic pathway content, identified through metagenomics and parallel sequencing approaches [27]. Analyses encompassing characterization of microbiome composition predominantly on the basis of 16s rRNA sequence identity has indicated that the gut microbiome comprises over 1000 species of bacteria, with the most common Phyla including Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria [28]. Microbiota populations are compositionally dynamic, and its changes in microbial population structure can occur under multiple environmental, immune, and nutritional circumstances. Dysbiosis, in turn, can have profound effects on the host and has been associated with a number of human pathological conditions [27,29–32].

Germ free (GF) and antibiotic-treated mice models have become indispensable tools in determining the role and contribution of the microbiota to health and disease, including metabolic diseases such as NAFLD. GF mice are bred under sterile conditions, hence eliminating colonization by microorganisms. The procedure of generating these GF animals can be a challenge, technically and in terms of required infrastructure. In general, all steps in the process are designed to eliminate microorganism exposure. A donor pregnant female mouse is disinfected in a sterile hood and undergoes a C-section. The uterine sack is surgically removed, placed in liquid disinfectant, and transferred to a sterile GF isolator. In the isolator, the uterine sack is opened, and pups are removed and introduced to GF foster mothers. Following the initial procedure, the animals are raised within the sterile environment and are given sterile food and water. A more broadly applied alternative is microbiome depletion in mice through oral administration of wide spectrum antibiotics. In these models, introduction of specific communities or individual bacteria identified and isolated in healthy or in disease states into GF or antibiotic-treated mice is performed in order to study the microbiota contribution and function in normal host physiology and in disease progression [33–35].

Using metagenomics and GF platforms, significant associations have been made between compositional and functional alterations in the microbiome (termed dysbiosis) and the propensity to a variety of multifactorial diseases, including obesity and its associated metabolic abnormalities such as NAFLD [21] in animal models and in humans. Indeed, in obesity and its associated metabolic complications, evidence of the microbiome as a contributing factor has been repeatedly featured [20,36]. In these studies, HFD GF mice were found to gain less weight than conventional mice. Colonization of GF mice with microbiota from conventional mice resulted in replenished weight gain [20,36]. Genetically obese mice and HFD mice had a shift in their gut microbial composition to one that is able to harvest dietary energy at a higher capacity [21]. This obesity phenotype was transmissible upon fecal transplantation into GF mice, resulting in significantly enhanced weight gain and total body fat as compared to GF mice receiving fecal transplantation of lean mice [21]. Proposed mechanisms for these effects include increased microbiome ability and efficiency for carbohydrate metabolism and production of short chain fatty acid [20,21,36], reduction in conjugated bile acids [35], and augmented systemic and adipose inflammation [37]. It is worth noting that similar changes were identified in the gut microbiota of obese humans [38], although some of the results were conflicting among different studies [39,40].

3. ASSOCIATIONS BETWEEN DYSBIOSIS AND NAFLD

Portal blood flow constitutes an important link between the intestine and liver, with the majority of the liver blood supply derived from the intestine. The intestinal blood supply exposes the liver to a multitude of intestinal metabolites and food products [41]. In recent years, evidence suggested an involvement of the microbiota in NAFLD development [42]. An indication of this involvement may have come as early as 1982 when Drenick *et al.* [43] studied hepatic steatosis development in patients undergoing gastric bypass surgery that coincided with bacterial overgrowth. In this early study, a regression in hepatic steatosis was noted when patients were treated with the antibiotic metronidazole, suggesting a potential role of the gut microbial community in fatty liver development. Subsequently, small intestinal bacterial overgrowth has been shown to be more prevalent in patients with NASH than in healthy controls [44]. An accumulating number of studies in animal models and humans have followed to broaden our understanding of the microbiota role in the development and pathogenesis of NAFLD [42]. In the interest of clarity towards the taxonomic organization of

