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Interleukin-15-mediated inflammation promotes non-alcoholic fatty liver disease

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ABSTRACT

Interleukin-15 (IL-15) is essential for the homeostasis of lymphoid cells particularly memory $CD8^+$ T cells and NK cells. These cells are abundant in the liver, and are implicated in obesity-associated pathogenic processes. Here we characterized obesity-associated metabolic and cellular changes in the liver of mice lacking IL-15 or IL-15Rα. High fat diet-induced accumulation of lipids was diminished in the livers of mice deficient for IL-15 or IL-15Rα. Expression of enzymes involved in the transport of lipids in the liver showed modest differences. More strikingly, the liver tissues of IL15-KO and IL15Rα-KO mice showed decreased expression of chemokines CCl2, CCL5 and CXCL10 and reduced infiltration of mononuclear cells. In vitro, IL-15 stimulation induced chemokine gene expression in wildtype hepatocytes, but not in IL15Rα-deficient hepatocytes. Our results show that IL-15 is implicated in the high fat diet-induced lipid accumulation and inflammation in the liver, leading to fatty liver disease.

1. Introduction

With the worldwide increase in the incidence of obesity, non-alcoholic fatty liver disease (NAFLD) is emerging as one of the most prevalent disorder of the liver [1–3]. NAFLD is mainly asymptomatic but has a wide pathological spectrum ranging from 'simple' steatosis to non-alcoholic steatohepatitis (NASH), which can progress to cirrhosis [4,5]. The main predisposing factors to NAFLD are obesity, diabetes, hyperlipidemia, and hypertension. Fat accumulation increases the susceptibility of the liver to factors that promote inflammation thereby precipitating NASH [6]. These factors include cytokine overproduction, lipid peroxidation, hepatocyte organelle (particularly mitochondria) malfunction and peroxisome proliferator-activated receptor (PPAR) dysfunction in the nucleus [5]. In the context of insulin resistance, the development of NAFLD depends on the interaction between the liver and peripheral tissues, including the skeletal muscle and adipose tissues, but the molecular mechanism is not fully elucidated. In insulin-resistant subjects, increased lipolysis in the adipose tissues increases the circulating free-fatty acids that are incorporated into hepatic triglyceride [7]. De novo hepatic lipogenesis is also upregulated by the activation of several lipogenic transcription factors, including sterol regulatory element-binding protein 1c [8].

Free fatty acid toxicity has been partially explained by endoplasmic reticulum (ER) stress and apoptosis induced by metabolites, including ceramides and diacyglycerols. ER stress as well as serum-free fatty acids, cytokines, etc., can activate c-Jun N-terminal kinase 1 (JNK1), which phosphorylate insulin receptor substrate 1 (IRS1) at an inhibitory site, and induce pro-inflammatory cytokines in target cells such as macrophages leading to insulin resistance [9]. NASH and insulin resistance are extensively related to a cytokine imbalance towards pro-inflammatory microenvironment in the liver. Bacteria translocated

Abbreviations: NAFLD, non-alcoholic fatty liver disease; IHL, intrahepatic lymphocytes; NASH, non-alcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; ER, endoplasmic reticulum.

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from the intestines, fatty acids derived from the diet and lipolysis in adipose tissue activate toll-like receptors (TLRs) in the liver, resulting in the activation of the cells of the innate immune system [10]. In NAFLD, proinflammatory cytokines such as TNFα activate the liver-resident macrophages, Kupffer cells (KC) [11], and their inactivation prevents the development of alcoholic fatty liver and NAFLD [5]. Furthermore, other immune cells in the liver such as NK and NKT cells contribute to the inflammatory process [12].

Interleukin-15 (IL-15) belongs to the γc family of cytokines [13]. The IL-15 receptor (IL-15R) complex consists of 3 subunits: the ligand-binding IL-15Rα chain (CD215), the β chain (CD122; also used by IL-2), and the common chain (γ_c – CD132; used by all IL-2 family cytokines) [14]. The biological activities of IL-15 are mostly mediated by the IL-15:IL-15Rα complex, produced by the same cell and '*trans-presented*' to responder cells expressing IL-15Rβγ_c [15,16]. IL-15 is well characterized as a growth factor for T lymphocytes and also influences many other immune cells [17,18]. Notably, IL-15 promotes the survival of memory $CD8^+$ T cells [19]. IL-15 also promotes the generation and maintenance NK cells, NKT cells, γδ T cells and certain T cell subsets in the gut [20,21]. IL-15 enhances survival of DCs and macrophages, and activates them to produce cytokines that facilitate a robust immune response [22–24]. The above-mentioned cell types are present in the liver in abundance [25] and influence the course of NAFLD [26,27]. In this study we show that absence of IL-15 or IL-15Rα prevents the development of NAFLD in mice.

2. Materials and methods

2.1. Mice

Wild type (WT) C57BL/6 mice were from Charles River. *Il15*$^{-/-}$ mice have been already described [28]. *Il15ra*$^{-/-}$ mice were purchased from Jackson Laboratory and bred into C57BL/6 background for more than ten generations. Mice were maintained in filter-topped cages in a specific pathogen-free facility and fed with standard chow diet and water unless specified otherwise. Only male mice were used in this study. All experiments were carried out with the approval of the institutional ethics committee.

2.2. Reagents

Abs against mouse CD3, CD8α, CD4, CD44, CD62L, NK1.1 conjugated to flurochromes were purchased from eBioscience (San Diego, CA), BD Biosciences (San Jose, CA) or Biolegend (San Diego, CA). Mouse CD1d tetramer pre-loaded with PBS57 (an α –GalCer analog – which work the same way as α – GalCer; CD1dPBS57) conjugated to PE was obtained from NIH tetramer core facility. Recombinant hIL-15 was obtained from R&D systems. Tissue culture media and FCS were obtained from Sigma.

2.3. Induction of NAFLD in mice

To induce hepatic steatosis, 8-weeks old WT, *Il15*$^{-/-}$ and *Il15ra*$^{-/-}$ mice, were maintained on high fat diet (HFD) (Research Diets, New Brunswick, NJ, USA; D12492: 20%kcal protein, 20% carbohydrate and 60% fat). Mice fed with normal control diet (NCD) were used as controls. Mice were maintained on NCD or HFD for 16 weeks before sacrifice. The weight of liver was measured at sacrifice. Aliquots of tissues were stored in formalin for histology, in OCT for immunohistochemistry and snap frozen in liquid nitrogen for RNA and protein extraction.

2.4. Isolation of intrahepatic lymphocytes (IHL) and flow cytometric analyses

At sacrifice, the livers were collected and rinsed with Krebs–Ringer–Buffer (KRB, 154 mM NaCl, 5.6 mM KCl, 5.5 mM Glucose, 20.1 mM HEPES, 25 mM $NaHCO_3$, pH 7.4). The liver tissues were digested in pre-warmed (37 °C) KRB supplemented with 2 mM $CaCl_2$, 2 mM $MgCl_2$, 300 CDU (casein digestion units)/mL Collagenase IV (Worthington) and 150 U/mL DNase I (Sigma) using gentle MACS Dissociator (Miltenyi Biotec) according to the instruction of the manufacturer. The homogenized liver samples were gently agitated on a rocking shaker for 30 min at room temperature. The tubes were left on a stand for 1 min to precipitate undigested liver tissue and the supernatants were passed through 40 μm cell strainer. Cells were resuspended in 25 ml of cold PEB buffer (0.5% Bovine serum albumin and 2 mM EDTA in Phosphate buffered saline (PBS)) and centrifuged at 50*g* for 5 min at 4 °C to eliminate contaminating hepatocytes. The supernatant was centrifuged at 300*g* for 10 min at 4 °C to collect the lymphocytes for FACS analysis [28]. Data was acquired on FACSCanto flow cytometer (BD Biosciences San Diego, CA) and was analysed using FlowJo software from TreeStar Inc (Ashland, OR).

2.5. Isolation and stimulation of primary hepatocytes

Primary hepatocytes isolation was carried out in 6–8 weeks-old mice as described previously [29]. Primary hepatocytes were starved overnight in DMEM-F12 with 0.1% FCS and stimulated with hIL-15 (20 ng/ml) for 12 h. Subsequently, the cells were washed twice and RNA extraction was performed.

2.6. Assessment of mitochondrial respiration in hepatocytes

The assays using Seahorse XF extracellular flux analyzer were carried out following the manufacturer's instructions. To determine how IL-15 deficiency changes the capacity of hepatocytes to oxidize FAs, we used the XF Palmitate–Bovine serum albumin (BSA) Fatty Acid Oxidation (FAO) substrate according to company's protocol. Palmitate-BSA FAO integrates the XF Cell Mito Stress Test with the BSA Control and XF Palmitate reagent. Primary hepatocytes were isolated as described in Section 2.4 and seeded in a 96 well plate. The day before the assay, the growth medium was exchanged for substrate-limited medium (DMEM, 0.5 mM Glucose, 1.0 mM GlutaMAX™ (life technologies), 0.5 mM carnitine and 1% FCS) and incubated ON. Next, cells were kept in FAO medium (Krebs–Henseleit Buffer (KHB) with 2.5 mM glucose, 0.5 mM carnitine and 5 mM HEPES) for 45 min and BSA control or Palmitate-BSA substrate was added. XF Cell Mito Stress Test analysis was performed.

2.7. Histology

At sacrifice, livers were fixed in formalin. Some tissue sections were kept frozen in OCT for immunohistochemistry or lipids staining. Lipid staining was carried out on tissue samples fixed in OCT using Sudan Black, a basic dye that combine with acidic groups in lipids compound, including phospholipids. The slides were washed in water and mounted with aqueous mounting media (VectaMount™). Images were taken using automatic tissue slide scanning (Hamamatsu NanoZoomer Digital Pathology (NDP) system).

2.8. Quantitative PCR

Snap-frozen liver samples were homogenized in TRIzol® (Life Technologies) using mixer mill MM 400 (Retsch, Hann, Germany).