HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

IL-17 Signaling Accelerates the Progression of Nonalcoholic Fatty Liver Disease in Mice

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Inflammation plays a central pathogenic role in the pernicious metabolic and end-organ sequelae of obesity. Among these sequelae, nonalcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in the developed world. The twinned observations that obesity is associated with increased activation of the interleukin (IL)-17 axis and that this axis can regulate liver damage in diverse contexts prompted us to address the role of IL-17RA signaling in the progression of NAFLD. We further examined whether microbe-driven IL-17A regulated NAFLD development and progression. We show here that IL-17RA$^{-/-}$ mice respond to high-fat diet stress with significantly greater weight gain, visceral adiposity, and hepatic steatosis than wild-type controls. However, obesity-driven lipid accumulation was uncoupled from its end-organ consequences in IL-17RA$^{-/-}$ mice, which exhibited decreased steatohepatitis, nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase enzyme expression, and hepatocellular damage. Neutralization of IL-17A significantly reduced obesity-driven hepatocellular damage in wild-type mice. Further, colonization of mice with segmented filamentous bacteria (SFB), a commensal that induces IL-17A production, exacerbated obesity-induced hepatocellular damage. In contrast, SFB depletion protected from obesity-induced hepatocellular damage. *Conclusion*: These data indicate that obesity-driven activation of the IL-17 axis is central to the development and progression of NAFLD to steatohepatitis and identify the IL-17 pathway as a novel therapeutic target in this condition. (HEPATOLOGY 2014;59:1830-1839)

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Obesity has become a full-fledged pandemic.[1] Inflammation is central to the pathogenesis of the metabolic and end-organ sequelae of obesity, including nonalcoholic fatty liver disease (NAFLD).[2] NAFLD represents a spectrum of disorders that range from nonalcoholic steatosis (NAFL) to nonalcoholic steatohepatitis (NASH) to cirrhosis.[3] In the U.S., one-third of adults have evidence of steatosis. The prevalence of NASH is lower, affecting 2%-3% of adults and children.[4] That said, up to 25% of those with NASH progress to cirrhosis.[5] Despite its clinical and public health significance, the immunopathogenesis of NAFLD, in particular those mechanisms leading to development of steatohepatitis, remains underdefined.

Interleukin (IL)-17A and IL-17F are closely related cytokines that play essential roles in barrier immunity to bacterial and fungal infection.[6] Dysregulated production

Abbreviations: ALT, alanine transaminase; HFD, high-fat diet; LPS, lipopolysaccharide; NAFL, nonalcoholic fatty liver disease; NAFLD, NASH, nonalcoholic steatohepatitis; SFB, segmented filamentous bacteria.

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Received May 14, 2013; accepted September 2, 2013.

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Supported by an R01 DK099222, a Pilot Grant under NIH P30 DK078392, and a CCHMC Trustee Grant (to SD). I.T.W.H. received funding from NIH grants AI075159, HD07463, GM063483, and a Fellowship from the Albert J. Ryan Foundation. Gnotobiotic mice were supported by P40 OD010995, P30 DK34987 and the Crohn's and Colitis Foundation of America.

of these IL-17 family members also appears to drive inflammatory pathology in various autoimmune diseases.[6] Both IL-17A and IL-17F signal through a complex consisting of IL-17RA and IL-17RC. In addition, IL-17E signals through a complex consisting of IL-17RA and IL-17RB.[6] The ability of IL-17 signaling to induce the production of cytokines and neutrophil chemokines is central to their biological effects.

Obesity is associated with increased IL-17A production in humans.[7] Mirroring this, T helper (Th)17 cell expansion is observed in obese mice.[8] In response to caloric excess, mice lacking IL-17A exhibit protection from glucose dysmetabolism, despite increased weight gain.[9] The IL-17 axis has also been linked to hepatic injury: an increased frequency of IL-17A-expressing cells (and increased IL-17A expression) in diverse human liver diseases and mouse models of liver injury.[10] Further, IL-17A neutralization or genetic deletion of IL-17A has been shown to be protective in mouse models of acute hepatitis,[11] drug-induced liver injury,[12] and lipopolysaccharide (LPS)-induced liver injury during high-fat diet stress.[13] IL-17RA is widely expressed in the liver and IL-17 drives neutrophil chemokine expression by such cells.[10] Of note, an increased hepatic neutrophil/lymphocyte ratio is predictive of NAFLD progression,[14] and neutrophilic inflammation has been described in human NASH.[15]

Given that IL-17 is induced during obesity, and given the observed linkage between IL-17 induction and hepatic inflammation and damage, we hypothesized that activation of the IL-17 axis might causally contribute to NAFLD pathogenesis. The current studies provide, to our knowledge, the first evidence for a critical role for IL-17RA signaling in driving NAFLD progression, as well as the first report of a role for microbe-driven IL-17 production in exacerbating hepatocellular damage in NAFLD. Specifically, genetic deletion of IL-17RA as well as antibody-mediated neutralization of IL-17A led to protection from steatohepatitis, nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activation, and hepatocellular damage in mouse models of obesity-induced NAFLD. Furthermore, colonization of mice with segmented filamentous bacteria (SFB), a bacterium known to augment IL-17 production, exacerbated and accelerated hepatocellular damage, whereas depletion of SFB led to protection from obesity-induced hepatocellular damage.

Materials and Methods

Mice. All mice were on a C57BL/6 background. All studies were approved by the Cincinnati Children's Hospital Medical Center (CCHMC) Institutional Animal Care and Use Committee (IACUC). Wild-type (WT) mice and IL-17RA$^{-/-}$ mice (Amgen) were bred at CCHMC. For SFB colonization/depletion studies, mice were obtained from Jackson Labs or Taconic Farms.

Obesity Models. Diets: Mice were fed either a high-fat diet (HFD; Research Diets #D12492) or a chow diet (Chow; LAB Diet #5010). Glucose dysmetabolism: Fasting glucose and insulin and glucose tolerance testing were quantified after an overnight fast. Liver: Hepatic triglycerides were quantified using Triglyceride Reagent and Triglyceride Standards (Pointe Scientific); serum alanine transaminase (ALT) levels were quantified using ALT Reagent and Catatrol I and II (Catachem); lipid peroxidation was quantified using 4-hydroxynonenal (4-HNE) enzyme-linked immunosorbent assay (ELISA) reagents (Cell Biolabs)—all according to the manufacturer's instructions.

Histology. Liver and gut tissue were fixed in 10% buffered formalin and stained with hematoxylin and eosin (H&E).

Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR). Tissue samples were homogenized in TRIzol, RNA was extracted, reverse transcribed to complementary DNA (cDNA), and subjected to qPCR analysis (Light Cycler 480 II; Roche). For bacterial quantification and colonization, fecal and intestinal DNA was extracted by way of TissueLyser (Qiagen)-mediated lysis and levels of 16S DNA were quantified by qPCR.

SFB Colonization/Depletion. Mice were colonized by way of oral gavage of fecal material dissolved in phosphate-buffered saline (PBS). SFB depletion was

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DOI 10.1002/hep.26746

Potential conflict of interest: Nothing to report.

Additional Supporting Information may be found in the online version of this article.

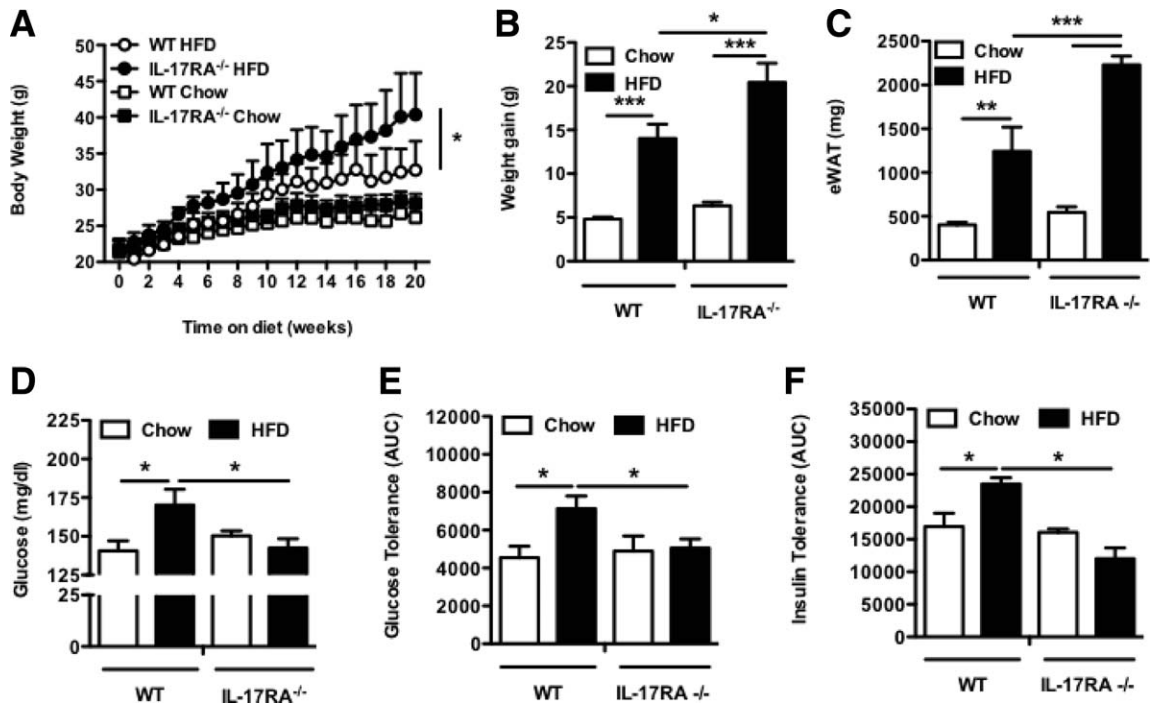

Fig. 1. IL-17RA deficiency exacerbates HFD-induced obesity but protects from glucose dysmetabolism. Eight-week-old male IL-17RA$^{-/-}$ mice and WT controls (n = 4-12/condition) were placed on an HFD or chow. (A) Body weight. (B) Body weight gain over 20 weeks. (C) Epididymal white adipose tissue (eWAT) weight. (D) Fasting glucose. (E) Glucose tolerance test. (F) Insulin tolerance test. A representative of two separate experiments. (A), AUC ANOVA *$P < 0.0001$; (B,C) and (E,F), ANOVA *$P < 0.001$; (D), ANOVA *$P < 0.03$; (A-F), Tukey's correction *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$.

achieved by way of vancomycin treatment (0.5 g/L; Fisher) in drinking water.

Hepatic Immune Infiltration. Single cell suspensions were isolated as described[16] and stained with directly conjugated monoclonal antibodies or isotype controls (all eBioscience). Data were collected using an LSRII flow cytometer and analyzed by FlowJo software.

Limulus Amebocyte Lysate (LAL) Assay. Systemic endotoxin levels were quantified using the LAL assay (Lonza) according to the manufacturer's instruction.

In Vivo *Cytokine Capture Assay.* Systemic IL-17A, tumor necrosis factor (TNF), IL-6, and interferon-gamma (IFN-γ) levels were detected using the *in vivo* cytokine capture assay,[17] employing biotinylated capture antibodies (eBioscience).

Statistical Analysis. Data were analyzed by analysis of variance (ANOVA) followed by Tukey's correction, unpaired Student *t* test, or by comparing intercepts of linear regression equations implemented in Prism 5a (GraphPad Software). For data failing

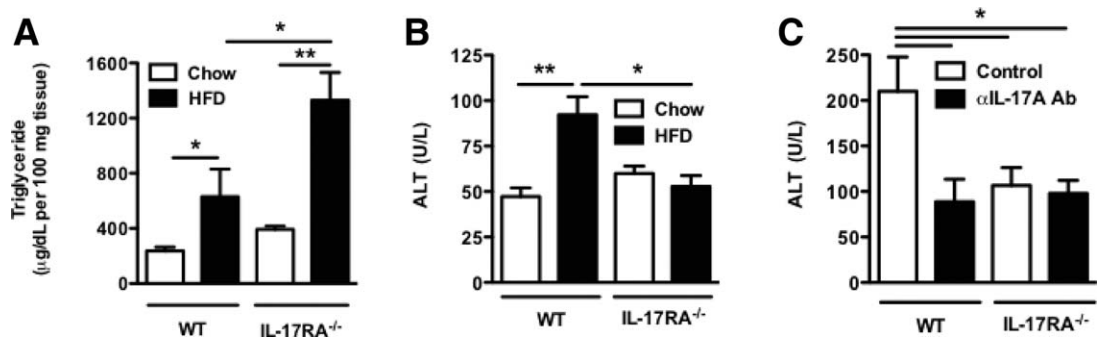

Fig. 2. IL-17A signaling by way of IL-17RA exacerbates hepatocellular damage during HFD-induced obesity. Eight-week-old male IL-17RA$^{-/-}$ mice and WT controls (n = 4-12/condition) were placed on an HFD or chow. (A) Hepatic triglyceride levels. (B) Serum ALT levels. Analysis done after 20 weeks of HFD feeding. (C) Neutralization of IL-17A protects from HFD-induced hepatocellular damage. Mice were treated intraperitoneally once a week for 3 weeks, with 250 μg/mouse of anti-IL-17A or isotype control. Treatment started at week 12 of HFD stress and ALT was quantified at week 15. (A,B) Representative of two separate experiments. (C) A single experiment. (A), ANOVA *$P < 0.001$; (B), ANOVA *$P < 0.003$; (C), ANOVA *$P < 0.02$. (A-C), Tukey's correction *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$.

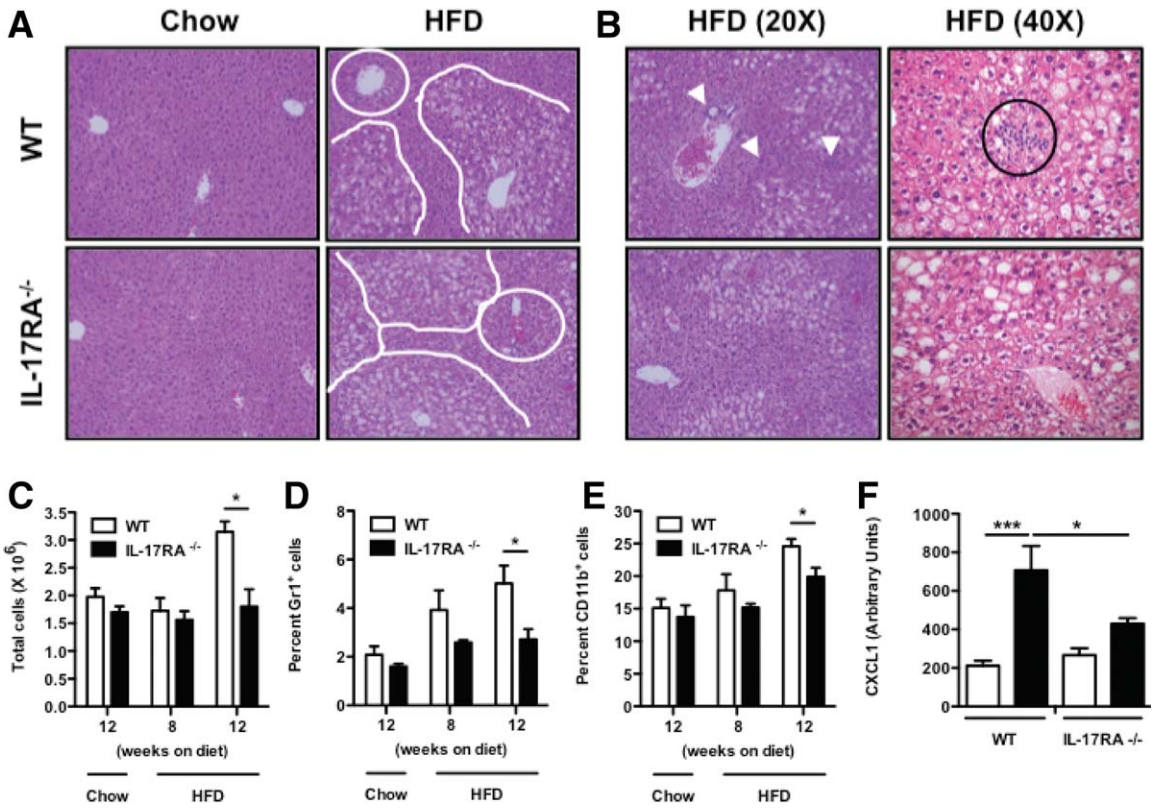

Fig. 3. Increased steatosis but decreased steatohepatitis in IL-17RA$^{-/-}$ mice on an HFD. Representative liver histology (H&E staining) after 20 weeks on chow or HFD. (A) Marked difference in the amount and type of steatosis between WT and IL-17RA$^{-/-}$ mice on HFD. HFD stress drives steatosis, mostly located in zone 3 (outlined in white), with sparing of zone 1 (white circles) in WT controls. In contrast, IL-17RA$^{-/-}$ mice show increased overall steatosis that is predominantly located in zones 2 and 3. No histological difference is observed on RD. (B) Decreased steatohepatitis in IL-17RA$^{-/-}$ mice. Multiple foci of lobular inflammation with clusters of inflammatory cells (white arrows) in the livers of WT mice on HFD. Higher magnification (40×) depicts the inflammatory focus in WT mice (black circle). Hepatic immune infiltration (C) total cells (CD45$^+$), (D) CD45$^+$Gr1$^+$ cells, and (E) CD45$^+$CD11b$^+$ cells. (F) Hepatic qRT-PCR expression of neutrophil recruiting chemokine, CXCL1. (C,D), Student *t* test; (F) ANOVA $*P < 0.004$; Tukey's correction $*P < 0.05$, $***P < 0.001$.

($\alpha = 0.01$) the D'Agostino-Pearson Omnibus test for normality, the Mann-Whitney test or Kruskal-Wallis test with Dunn's multiple testing correction was used. All values are represented as means ± SE.

Results

Integral Role of IL-17 Signaling in the Pathogenesis of NAFLD. To define the contribution of IL-17 signaling to the progression of NAFLD, IL-17RA$^{-/-}$ and WT mice were challenged in a standard model of HFD-induced obesity—a model that induces obesity along with steatosis and hepatocellular damage. Under these conditions, IL-17RA$^{-/-}$ mice exhibited significantly increased body weight and weight gain, white adipose tissue (WAT) weight, and systemic leptin levels relative to WT controls (Fig. 1A-C; Supporting Fig. 1A). However, IL-17RA$^{-/-}$ mice were protected from glucose dysmetabolism (fasting hyperglycemia, glucose intolerance, and insulin resistance; Fig. 1 D-F) and, despite significant increases in hepatic triglyceride deposition (Fig. 2A) and a trend towards increased total liver weight (Supporting Fig. 1B), were protected from hepatocellular damage, as quantified by analysis of serum ALT concentrations (Fig. 2B). No differences in daily food intake were observed between WT and IL-17RA$^{-/-}$ mice subjected to HFD stress (Supporting Fig. 1C). Of note, antibody-mediated neutralization of IL-17A conferred protection from hepatocellular damage in WT mice challenged with an HFD, specifically implicating IL-17A, among the diverse IL-17 family member ligands for IL-17RA, in obesity-related hepatocellular damage (Fig. 2C).

To assess the robustness of these findings, we employed an additional model of NAFLD: challenge with a high-carbohydrate, high medium-chain trans fat diet (HFHCD), a model that induces a broader spectrum of NAFLD.[16] Challenge of WT mice with an HFHCD resulted in increased IL-17A and decreased IL-17RA hepatic messenger RNA (mRNA) expression,

along with increased recruitment of Gr-1^{+} cells into the liver (data not shown). As seen with HFD challenge, IL-17RA$^{-/-}$ mice exhibited increased body weight and weight gain, adiposity, hepatic weight, and hepatic triglyceride deposition, but were protected from glucose dysmetabolism and hepatocellular damage (Supporting Fig. 2). Further, kinetic analysis of serum ALT levels revealed that the lack of IL-17RA led to sustained protection from hepatocellular damage (Supporting Fig. 2L). Taken together, these data provide strong evidence that the IL-17 axis regulates disease progression in obesogenic mouse models of NAFLD.

Regulation of Hepatic and Systemic Inflammatory Responses by the IL-17 Axis During Obesity. We also examined the progression of steatosis to steatohepatitis in WT and IL-17RA$^{-/-}$ mice. Of interest, despite increased weight gain and hepatic triglyceride accumulation, IL-17RA$^{-/-}$ mice were resistant to the development of steatohepatitis, as determined by the absence of foci of inflammatory cells in IL-17RA$^{-/-}$ compared to WT controls (Fig. 3A,B). Similarly, HFD-challenged IL-17RA$^{-/-}$ mice exhibited decreased infiltration of immune cells in the liver—something attributed to reduced infiltration of Gr-1^{+} cells and CD11b^{+} cells (Fig. 3C-E; Supporting Figs. (3 and 4)). Further, reduction in immune infiltration correlated with reduced hepatic expression of mRNA for the neutrophil chemokines CXCL1 and CXCL2, CXCL12, G-CSF along with mRNA for Alox5, an enzyme critical for production of the neutrophil chemoattractant LTB4 (Fig. 3F; Supporting Fig. 5). Of note, HFD-challenged IL-17RA$^{-/-}$ mice also exhibited decreased mRNA expression of markers of myeloid cell infiltration and expression of other inflammatory mediators (Supporting Fig. 5).

Although the molecular mechanisms that regulate hepatocellular damage during NAFLD are not well understood, the most favored hypothesis suggests that steatosis sensitizes hepatocytes to subsequent inflammatory- and/or reactive oxygen species (ROS)-driven oxidative stress-mediated hepatocellular injury and fibrosis.[18] Of note, it is well established that: (1) IL-17A mediates neutrophil-dependent hepatocellular damage[19]; (2) IL-17A can drive ROS production[20]; and (3) infiltrating neutrophils and/or inflammation-associated ROS production can induce hepatic parenchymal cell injury.[21,22] Therefore, as activated neutrophils and Kupffer cells produce high amounts of ROS by way of NADPH oxidase cascade, we quantified hepatic expression of NADPH oxidase components in WT and IL-17RA$^{-/-}$ mice. As shown, IL-17RA$^{-/-}$ mice on HFD had significantly decreased hepatic

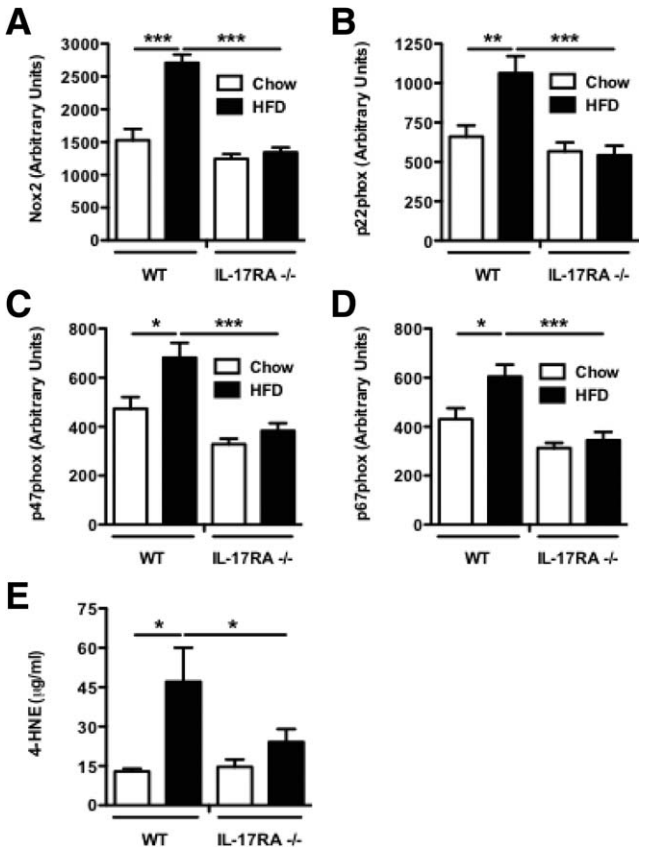

Fig. 4. IL-17RA$^{-/-}$ mice exhibit decreased expression of enzymes driving hepatic ROS production during HFD stress. Hepatic mRNA expression of NADPH oxidase components and hepatic lipid peroxidation after 20 weeks on diet, (A) Nox2, (B) p22phox, (C) p47phox, (D) p67phox, and (E) 4-HNE. Represents a single experiment; (A-E), ANOVA $*P < 0.0001$; Tukey's correction $*P < 0.05$, $**P < 0.01$; $***P < 0.001$.

Nox2, p22^{phox}, p47^{phox}, and p67^{phox} mRNA expression, along with decreased lipid peroxidation as quantified by hepatic 4-HNE concentrations compared to WT controls (Fig. 4). These data suggest that the IL-17 axis, by regulating hepatic immune infiltration/inflammation, also modulates hepatic NADPH oxidase-dependent ROS production and lipid peroxidation—providing a clear downstream candidate mechanism for IL-17-mediated exacerbation of hepatocellular damage in this context.

HFD feeding is reported to increase circulating endotoxin levels,[23] something also associated with NASH pathogenesis.[24] We thus quantified serum endotoxin concentrations in WT and IL-17RA$^{-/-}$ mice fed chow and HFD, and examined the ability of IL-17RA$^{-/-}$ mice to respond to LPS challenge. As shown, although HFD feeding did increase systemic endotoxin levels, significant differences between WT and IL-17RA$^{-/-}$ were not observed (Fig. 5A). It is noted that systemic measurements may miss differences in portal vein endotoxin

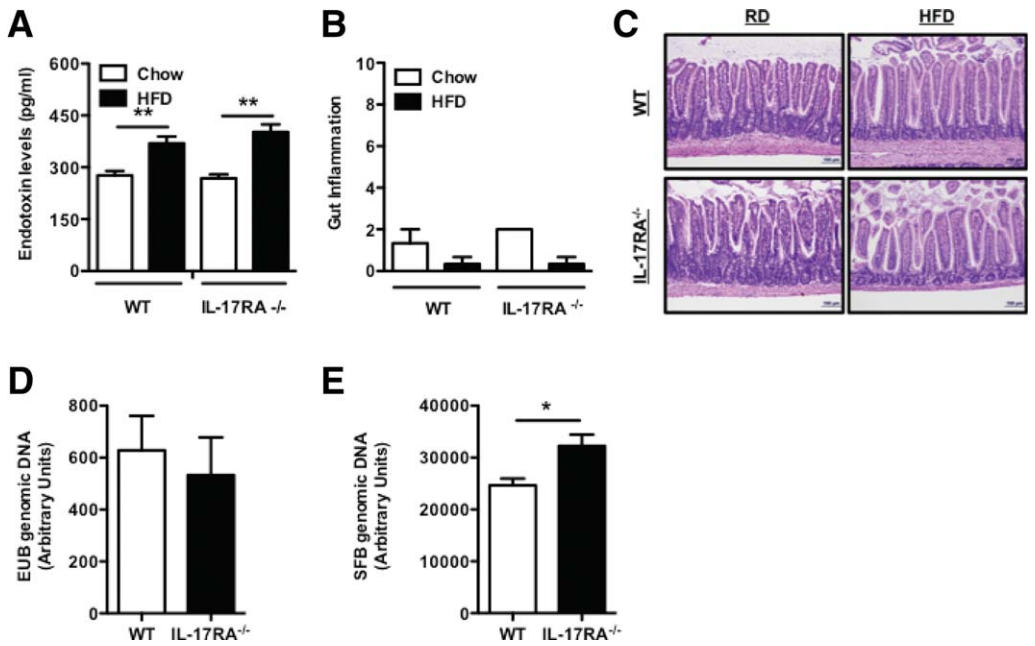

Fig. 5. Lack of IL-17RA does not alter metabolic endotoxemia or result in frank intestinal inflammation during HFD stress, but does lead to alterations in the gut microbiota. (A) Systemic endotoxin levels after 20 weeks on diet (n = 4-12/condition). (B) IL-17RA$^{-/-}$ mice and WT controls (n = 3/condition) were placed on an HFD or chow for 16 weeks and intestinal inflammation was quantified. (C) Representative histology (H&E staining) of quantified intestinal tissue. (D) Total Eubacterial DNA levels in the terminal ileum of 6-week-old chow-fed WT and IL-17RA$^{-/-}$ mice (n = 6-7). (E) SFB levels. Represents a single experiment. (A,E), Student *t* test *$P < 0.05$; **$P < 0.01$.

levels. We further examined whether IL-17RA signaling regulates LPS-driven production of proinflammatory cytokines during HFD stress. IL-17RA$^{-/-}$ mice had comparable levels of proinflammatory and immunoregulatory cytokines following LPS challenge when fed chow diet—as quantified by a cytokine capture assay that integrates *in vivo* cytokine production over the 24-hour period of challenge.[17] However, under HFD stress, IL-17RA$^{-/-}$ mice exhibited decreased levels of LPS-stimulated proinflammatory and immunoregulatory cytokines compared to WT controls (Supporting Fig. 6). In addition, as IL-17RA is required for maintenance of baseline neutrophil counts in mice,[25] we examined whether this contributed to protection from NAFLD progression. Although IL-17RA deficiency was associated with decreased peripheral blood neutrophil and monocyte concentrations at baseline, such differences were obviated by obesogenic-diet stress (Supporting Fig. 7). Together, these suggest that IL-17 signaling leads to increased responsiveness to inflammatory stimuli and recruitment of neutrophils and/or myeloid cells to the liver during obesogenic dietary stress.

Contribution of SFB to IL-17 Induction in NAFLD Models. The role of IL-17 signaling in intestinal barrier immunity and inflammation is well established.[6] Thus, we examined whether the observed differences between the IL-17RA$^{-/-}$ and WT mice were secondary to alterations in intestinal inflammation. Histological examination of the small and large intestines revealed no inflammatory infiltrates during either HFD or HFHCD challenge (Fig. 5B,C; Supporting Fig. 8).

Variations in the intestinal microbiota have previously been associated with both obesity and NAFLD.[26] Indeed, a (transferable) dysbiotic microbiome has been shown to contribute to the development of steatosis and hepatocellular damage.[27] To date, however, the move from association to causation has not been made. In other contexts where gut-derived IL-17 significantly contributes to physiology (gut immune system maturation) or pathophysiology, an individual pathobiont has been causally implicated: SFB is capable of inducing robust IL-17A production in the gut, with local and systemic consequences.[28,29] Not surprisingly, given the described role for SFB-mediated induction of Th17-associated barrier immunity, IL-17RA$^{-/-}$ mice exhibited a significant increase in the relative abundance of SFB, without marked differences in total Eubacteria, as determined by qPCR for 16S rDNA (Fig. 5D,E).

Since both SFB colonization and obesity have been described to induce IL-17 production,[9,28] we sought to determine whether such effects were additive. As shown in Fig. 6A, SFB-negative WT mice exhibited increased serum IL-17A subsequent to HFD challenge. Notably, SFB colonization augmented IL-17A

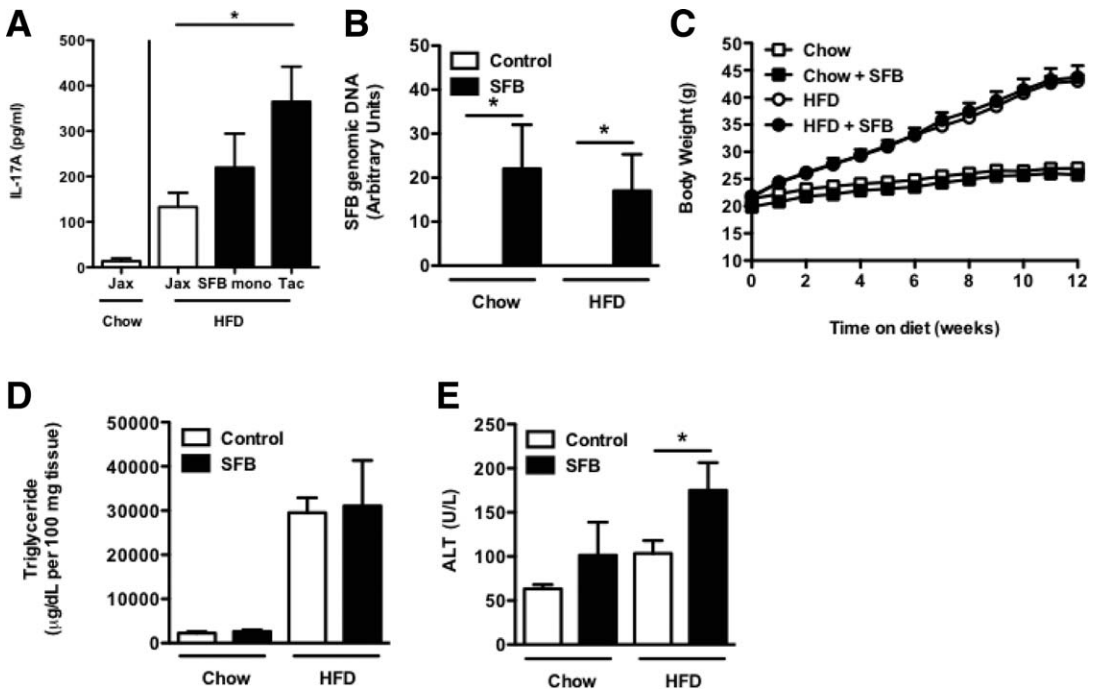

Fig. 6. SFB colonization elevates serum IL-17A and induces obesity-associated hepatocellular damage. Mice from Jackson Labs were colonized by way of oral gavage with either autologous SFB-negative fecal material from Jackson Labs (Jax) or exogenous SFB-positive fecal material from C57BL/6 mice from Taconic Farms (Tac) or from C57BL/6 mice monocolonized with SFB (SFB Mono). (A) IL-17A levels determined after 12 weeks of HFD challenge. (B) Fecal SFB levels at the initiation of HFD feeding. (C) Body weight. (D) Hepatic triglyceride levels. (E) Serum ALT levels. Represents a single experiment. (A) (n = 3 chow group; n = 7-11/condition HFD groups). (C-E) (n = 6-9/condition). ANOVA; $*P < 0.03$; Tukey's correction $*P < 0.05$. (B,E), Student *t* test $*P < 0.05$.

production in mice placed on an HFD. SFB-positive mice exhibited IL-17A levels in excess of those observed in SFB-negative mice. This was true regardless of the source of SFB—whether from the complete fecal microbiota of WT mice obtained from Taconic Farms,[28] or that from SFB mono-associated mice.[30]

In order to examine the effects of SFB-induced augmentation of IL-17A production on obesity-associated hepatocellular damage, SFB-negative WT mice were colonized with SFB. Colonization was confirmed/quantified by qRT-PCR on fecal pellets (Fig. 6B). With HFD challenge, despite similar body weights and hepatic triglyceride accumulation, these mice exhibited a significant increase in markers of hepatocellular damage (Fig. 6C-E), indicating that SFB colonization augments hepatocellular damage in this model. Of note, no other marked phenotypic differences were observed in SFB colonized mice in comparison with mock-colonized controls (Supporting Fig. 9).

To examine the converse, we ablated SFB colonization in WT mice using vancomycin, an antibiotic shown to reverse SFB-mediated induction of Th17 polarization *in vivo*.[31] Such antibiotic treatment is, perforce, nonspecific, and may lead to broad disruption of microbial community structure.[32] Indeed, vancomycin-treated mice exhibited cecal enlargement (Supporting Fig. 10), a finding observed with a variety of antibiotic treatments.[28] Nonetheless, vancomycin treatment led to decreased serum IL-17A levels (Fig. 7A), depleted SFB colonization (Fig. 7B), and reduced HFD-mediated hepatocellular damage (Fig. 7E), without affecting body weight or hepatic triglyceride accumulation (Fig. 7C,D). No other phenotypic differences were observed in vancomycin-treated mice when compared with control-treated mice (Supporting Fig. 10).

We similarly sought to assess the extent to which SFB-associated increases in IL-17A production might affect the development of hepatocellular damage in the context of excess energy intake during chow feeding. For this, $Lepr^{db/db}$ mice were colonized with SFB. We found that SFB-colonized $Lepr^{db/db}$ mice exhibited relative protection from the development of obesity (Fig. 8A), something consonant with the known role of IL-17A in restraining weight gain in diet-induced obesity.[9] In contrast, SFB-colonized $Lepr^{db/db}$ mice achieved parity with their SFB-negative counterparts with regard to the level of hepatocellular damage, despite their relative leanness (Fig. 8B). Direct comparison of weight and serum ALT concentrations

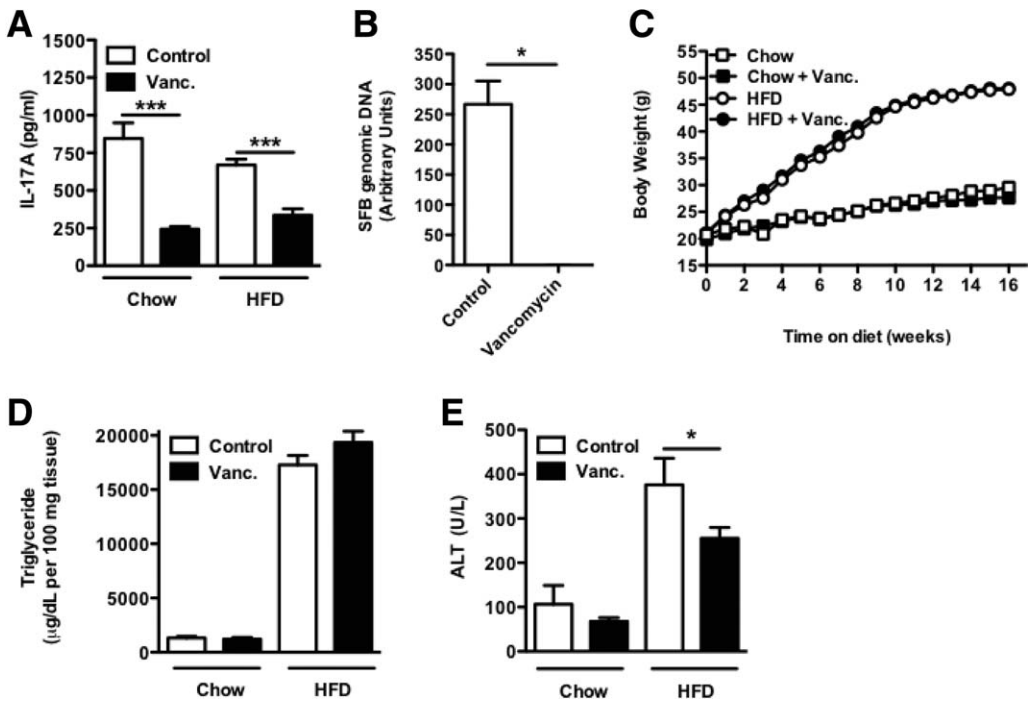

Fig. 7. SFB depletion reduces serum IL-17A and suppresses obesity-associated hepatocellular damage. Mice from Taconic Farms (n = 6-29/condition) were treated with vancomycin in their water or mock-treated and fed either HFD or chow for 16 weeks. (A) Serum IL-17A levels. (B) Fecal SFB levels at the initiation of HFD feeding. (C) Body weight. (D) Hepatic triglyceride levels. (E) Serum ALT levels. A representative of two separate experiments. (A,B), Student *t* test $*P < 0.05$; $***P < 0.001$. (E), Mann-Whitney test $*P < 0.05$.

demonstrated significant SFB colonization-associated augmentation of hepatocellular damage in Lepr$^{db/db}$ mice (Fig. 8C). Of interest, SFB-colonized Lepr$^{db/db}$ mice did not eat less than their mock-colonized counterparts and did not exhibit other substantial phenotypic differences (Supporting Fig. 11).

Discussion

Taken together, our data represent the first evidence for a critical role for IL-17RA signaling in driving NAFLD progression. Specifically, our data suggest a model in which induction of the IL-17 axis exacerbates and accelerates hepatocellular damage in the context of obesity and established steatosis, driving the progression from steatosis to steatohepatitis. Our data also underscore the potential for specific components of the gut microbiota to drive this progression: SFB colonization augments systemic IL-17A concentrations, associated with increased hepatocellular damage, in the context of obesity.

Our data imply that SFB mediates its effects on murine NAFLD by way of its effects on induction of IL-17 signaling, consonant with the role of SFB in

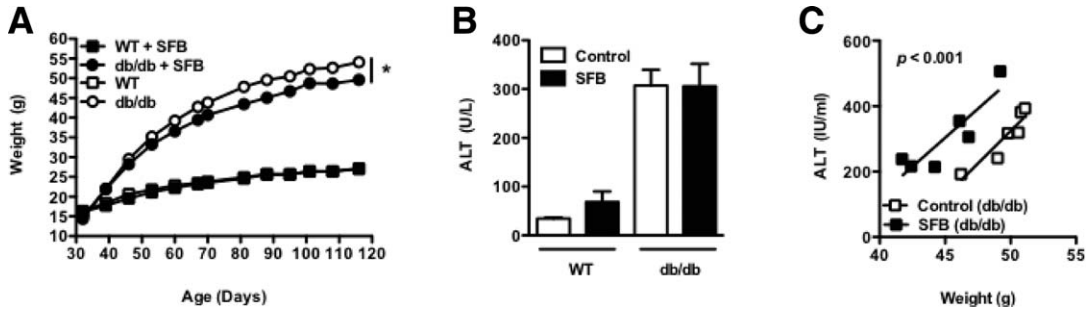

Fig. 8. SFB colonization of chow-fed genetically obese, Lepr$^{db/db}$ mice protects from weight gain but accelerates hepatocellular damage. (A) Body weight. (B) Serum ALT levels. (C) Hepatocellular damage (ALT) expressed as a function of body weight. A representative of two separate experiments (n = 5-6/condition). (A), AUC ANOVA $*P < 0.0001$; Tukey's correction $*P < 0.01$. (C), $P < 0.001$; linear regression line intercepts are significantly different.

driving extraintestinal pathology through activation of the IL-17 axis in other mouse models of inflammatory disease.[28] Current data suggest that SFB is a pathobiont in humans as it is in mice, being developmentally regulated in both species.[33] If SFB plays a similar role in human NAFLD, it may be that individuals with hepatic steatosis who develop progressive NAFLD might do so as a consequence of first developing maladaptive immune responses and/or dysbiosis early in life. Examples of similar developmental predispositions have been cataloged for other pathophysiologic states.[34] Further, based on our findings, it is a reasonable hypothesis that the classes and/or species of bacteria that drive to hepatocellular damage in human NAFLD likely also induce IL-17. Of interest, infection with *Clostridium difficile,* a close relative of SFB, induces IL-17[35] and has also been associated as an independent risk factor for adverse outcomes in patients with cirrhosis.[36]

Further, our data lay the foundation for future investigation of the key cellular and molecular mechanisms underlying the contributions of the IL-17 axis and the gut microbiota to NAFLD progression. Specifically, the relevant source(s) of IL-17A production, the critical IL-17RA-expressing cell type(s), the critical immune-infiltrating cells in the liver, and the underlying cellular effector mechanisms remain to be defined. IL-17A is predominantly produced by Th17 cells, with contributions from other $\alpha\beta$ T cell subsets.[6] Many of these cell types are abundant within the liver itself or in close apposition to it. In terms of the receptor, a plethora of liver-resident cells express IL-17RA.[10] Our current understanding of NASH pathogenesis is most compatible with an important role for IL-17RA expression by Kupffer cells or hepatic stellate cells but not hepatocytes, hepatic endothelial cells, or neutrophils.[37-39] However, future studies using cell type-specific deletion of IL-17RA will be essential for determining the critical IL-17RA-expressing cell type(s) important in driving the progression of NAFL to NASH.

Our data also suggest that IL-17RA signaling regulates neutrophil and monocyte recruitment to the liver during HFD stress—something associated with altered activation of the NADPH oxidase cascade, lipid peroxidation, and, presumably, hepatocellular damage.[7,25,26] These findings lay the foundation for future studies focused on defining the role of neutrophils and monocyte-derived cells, as well as the role of ROS and mitochondrial damage, in IL-17 axis-driven NASH pathogenesis.

The relevance of these data from mouse models to human disease needs validation. Of note, the association of IL-17 axis activation with diverse human liver diseases,[10] along with the association of single nucleotide polymorphisms (SNPs) in and near genes important to the IL-17 axis (e.g., STAT4 and RORA) in humans with increases in serum levels of a marker of hepatobiliary disease, both suggest the relevance of the IL-17 axis in human NAFLD.[40,41] Further, in humans it has been shown that: (1) NASH correlates with elevated expression of IL-17[13]; (2) neutrophilic inflammation of the liver[15] and an increased hepatic neutrophil/lymphocyte ratio is predictive of the progression of NAFLD to NASH[14]; and (3) patients with NASH exhibit structural abnormalities in hepatocyte mitochondria and exacerbated ROS production.[42]

In sum, our findings implicate IL-17 signaling as a causal contributor to the progression of NAFLD to steatohepatitis, identify a specific species of commensal bacteria that is capable of driving both IL-17 production and hepatocellular damage, and suggest novel candidate therapeutic approaches for NASH. The latter is of significant relevance as efforts are under way to move anti-IL17 therapeutics into the clinic[43] for the treatment of autoimmune inflammation, while oral vancomycin has been shown to reduce serum markers of hepatocellular damage in primary sclerosing cholangitis,[44] another inflammatory condition leading to cirrhosis. Targeting of the IL-17 axis by way of either of these approaches may represent a novel therapeutic approach to NAFLD.

Acknowledgment: We thank M. Kirby, M. Bower, J. McAlees, C. Minges, J. Ashworth, J. Dunn, and N. Boespflug for experimental assistance, and A. Imaoka and Y. Umesaki for providing SFB monocolonized material.

Author Contributions: ITWH, TES, DAG, SS, LMF, MC, RS, KAS, and SD participated in data acquisition, analysis and interpretation. RBS, SX, and RK provided materials and technical support and participated in critical review of the article. ITWH, CLK, and SD participated in the conception and design of the study and wrote the article. CLK and SD obtained the funding. All authors have reviewed the article and approve the final version.

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