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that increased susceptibility to K. pneumoniae is, in part, mediated by the intestinal microbiota, as animals recolonized with an alcohol-induced dysbiotic intestinal microbial community have significantly higher lung burdens of K. pneumoniae (5 × 104 CFU vs. 1 × 103 CFU) independent of EtOH. We also found that increased susceptibility in alcohol-dysbiosis recolonized animals was associated with a decrease in the recruitment and/or proliferation of CD4+ and CD8+ T-cells (1.5 × 109 cells vs. 2.5 × 109 cells) in the lung following Klebsiella infection. However, there were increased numbers of T-cells in the intestinal tract following Klebsiella infection, which may suggest that T cells are being sequestered in the intestinal tract to the detriment of host defense in the lung. Interestingly, mice recolonized with an alcohol-dysbiotic microbiota had increased intestinal permeability as measured by increased levels of serum intestinal fatty acid binding protein (55 vs. 30 ng/mL). Alcohol-dysbiotic microbiota also increased liver steatosis (Oil Red-O staining) and liver inflammation (>2-fold expression of IL-17 and IL-23). DISCUSSION/SIGNIFI-CANCE OF IMPACT: Our findings suggest that the commensal intestinal microbiota support mucosal host defenses against infectious agents by facilitating normal immune responses to pulmonary pathogens. Our data also suggest that increased intestinal permeability coupled with increased liver inflammation may impair the recruitment/proliferation of immune cells in the respiratory tract following infection. The role of the microbiota during host defense will be important areas of future research directed at understanding the effects of microbial dysbiosis in patients with AUDs.

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Essential amino acid supplementation improves lipid metabolism in older adults with elevated triglycerides

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OBJECTIVES/SPECIFIC AIMS: This study will assess the effect of essential amino acid (EAA) supplementation on plasma triglyceride (TG) in elderly adults. We will also explore the mechanisms mediating EAA mediated changes in fat metabolism and to suggest promising routes to refine therapy of hypertriglyceridemia. METHODS/STUDY POPULATION: In total, 7 nondiabetic male and female subjects ages 50-75 years with elevated plasma TG levels (130-500 mg/dL) were recruited to participate in an acute (5h) and long-term (8 wk) EAA supplementation study. We measured changes in regional and whole body fat metabolism, including changes in body composition, plasma TG levels, whole body fat metabolic rates, tissue mitochondrial respiratory capacity, and metabolomic profiles before and after supplementation. RESULTS/ANTICIPATED RESULTS: Long-term EAA supplementation decreased fasted plasma TG levels by 19% (p < 0.01). Metabolomics of skeletal muscle found acute EAA supplementation resulted in increased EAA metabolic products while long-term supplementation resulted in increased anaplerosis [flux into the tricarboxylic acid cycle (TCA) intermediate pool] and anaplerotic substrates [propionyl (p < 0.01) and succinyl (p < 0.01) carnitine] and intermediates of long-chain fatty acid metabolism [stearoyl (p < 0.01) and myristoyl (p < 0.05) carnitine]. However, tissue level respiratory capacity appeared to be unaffected by EAA supplementation. DISCUSSION/SIGNIFICANCE OF IMPACT: EAA supplementation has potential to improve lipid metabolism and plasma TG levels in non-diabetic older adults. Mitochondrial metabolomics suggest that insufficient TCA pool size may limit tissue fatty acid oxidation and may provide an additional route for nutritional therapy.

2290

Control of atherosclerosis regression by LXR α S198 phosphorylation

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OBJECTIVES/SPECIFIC AIMS: Accumulation of cholesterol-laden macrophages in arterial walls leads to atherosclerosis. LXRs induce expression of genes that are atheroprotective in macrophages including CCR7, a chemokine receptor that promotes their emigration from the plaque. CCR7 expression has been shown to be negatively regulated by phosphorylation of LXR α at S198 and is reduced in diabetic mice that show impaired plaque regression. I hypothesized that LXR α phosphorylation at S198 diminishes macrophage emigration from atherosclerotic plaque and contributes to impaired regression in diabetes. METHODS/STUDY POPULATION: Inducible LXR α S198A phosphorylation deficient knock in mouse were used as donors for bone marrow transplantation into mice prone to develop atherosclerosis. Plaques were developed by placing mice on western diet; and regression was induced by lowering their lipid levels.

Aortic plaques were then analyzed by using morphometric, histological, and molecular analyses in control and diabetic mice expressing either LXRlpha WT or LXRa S198A during regression. RESULTS/ANTICIPATED RESULTS: Surprisingly, lack of phosphorylation increased plaque macrophage content and impaired regression under normoglycemic condition; however, it did not exacerbate diabetic regression. Plaques in diabetic mice were associated with increased LXR α S198 phosphorylation. Consistent with this, LXR α phosphorylation is enhanced in macrophages cultured under hyperglycemic conditions indicating glucose-dependent regulation of LXR α phosphorylation. Monocyte trafficking studies reveal that lack of phosphorylation and diabetes independently increase recruitment of monocytes in the plaque that might contribute to increased macrophage content. Importantly, I found that diabetes also increases macrophage retention in the plaque, which is reversed in the absence of phosphorylation. We predict that this increased retention results from inhibition of emigration of plaque macrophages through enhanced phosphorylation in diabetes. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings suggest that inhibiting LXRα phosphorylation could be beneficial in diabetic atherosclerosis to reverse the accumulation of macrophages in the plaque. This study imparts insight on regulation of plaque macrophage trafficking through LXRα S198 phosphorylation.

2295

A novel in vivo zebrafish model of hematopoietic stem cell-driven regeneration of blood

Samima Sultana Habbsa, Mia McKinstry, Sara Payne, Christian Mosimann and Teresa Bowman

OBJECTIVES/SPECIFIC AIMS: Hematopoietic stem and progenitor cells (HSPCs) function to maintain steady state production of new blood cells and to rapidly respond to blood cell loss. Little is known regarding how HSPCs develop the ability to sense and respond to blood cell loss during embryogenesis. Gaining insight into the robust ability of HSPCs to regenerate blood during early development may allow us to develop therapies to rejuvenate this capacity at any stage. METHODS/STUDY POPULATION: We generated a new hematopoietic-specific and inducible cell ablation zebrafish model to uncover the origins of regenerative capacity in HSPCs during development. These transgenic zebrafish express a cyan fluorescent protein (CFP)-nitroreductase (NTR) fusion construct under the control of the draculin (drl) promoter (drl:CFP-NTR), which restricts NTR expression to blood cells. Co-expression analyses of drl:CFP-NTR with known markers of other blood types including HSPCs (runx I + 23:mCherry), erythroid cells (gata I:dsRed), and lymphoid cells (rag2:RFP), revealed drl:CFP-NTR was restricted to HSPCs and erythrocytes. To delineate the regeneration potential of embryonic HSPCs, we exposed drl:CFP-NTR transgenic zebrafish embryos to Metronidazole (MTZ), which results in selective ablation of only NTR-expressing blood cells. Embryos were treated from 2 to 3 days postfertilization and recovery of drl+ and gata I+ cells was evaluated over a 7-day recovery period. RESULTS/ANTICIPATED RESULTS: Following MTZ exposure, the nadir of drl+ cell ablation occurs at 2 days post MTZ (dpM) treatment. During the renewal phase of blood regeneration, we first observe recovery of drl+ cells by about 4 dpM, while more mature blood cells like gata I+ erythrocytes show a delayed recovery at about 6 dpM. Our results suggest that HSPCs can respond to injury as early as 2 days of life and that the HSPC-driven regeneration of embryonic blood cells occurs in a hierarchical fashion, similar to regeneration of the adult blood system. DISCUSSION/SIGNIFICANCE OF IMPACT: We have established a quantitative method for in vivo real-time monitoring of embryonic and larval blood regeneration. A significant advantage of our system is that we can use these insights to guide an in-vivo drug screen for factors that accelerate HSPCdriven blood regeneration in a complex biological environment.

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E-prescribing research participation: Feasibility of recruiting research participants using an EMR-integrated health information technology

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OBJECTIVES/SPECIFIC AIMS: To study the rate of recruitment to the Pulmonary Research Registry (PRR) at the University of Chicago using HealtheRx recruitment Versus usual practice. METHODS/STUDY POPULATION: CommunityRx is a health information technology, integrated with electronic medical record (EMR) platforms, that generates personalized referrals ("HealtheRxs") for community-based programs and services that