Diabetic Complications Consortium

Application Title: Macrophage Dysfunction in Diabetic Urinary Tract Infection

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1. Project Accomplishments:

The goal of this project was to investigate how high fat diet and ACSL1interact to influence macrophage function and the host response to diabetic urinary tract infection (UTI). In this study we demonstrated that 4 months of high fat diet (HFD) produced weight gain and glucose intolerance equally in WT mice and macrophage ACSL1 KO mice (mACSKO). In response to a UTI with uropathogenic E. coli HFD fed mice developed more robust pyuria, had smaller urinary volumes, and cleared the initial bactiuria more readily. However, HFD fed mice also had increased tissue edema, inflammation, and bladder cell proliferation; suggesting that systemic metabolic abnormalities could lead to altered tissue remodeling. In general, the influence of ACSL1 loss-of-function in macrophages was modest with diet driving most of the differences observed between the mice. Studies using infected bone-marrow derived macrophages did reveal that release of the inflammasome-regulated cytokine IL-1β was decreased in cells deficient in ACSL1. The findings from this study will lead to further investigation about how diet modulates bladder inflammation and risk of UTI complications including recurrent infections and malignancies.

2. Specific Aims:

Specific Aim 1. To delineate the interplay between fatty acid overload and macrophage ACSL1 in inflammation activation in response to uropathgenic E. coli.

Results: To address this question we incubated bone marrow-derived macrophages with uropathogenic *E. coli* and analyzed IL-1β release at early and late time points after infection. Our data consistently revealed decreased IL-1b secretion from mACSKO macrophages compared to wild type at both 6 and 24 hours after exposure to pathogenic bacteria. We also demonstrated that ACSL1 is localized to the mitochondrial and likely contributes to the inflammasome activation response through modulating mitochondrial lipid properties. Although provocative, additional experiments will be necessary to further define the mechanism by which ACSL1 influences inflammasome activation.

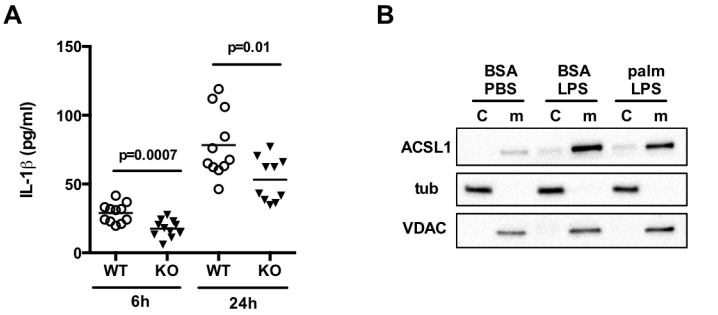

Figure 1. Loss of ACSL1 in macrophages reduces inflammasome activation in response to E. coli. . (A) WT and mACSKO (KO) bone marrow derived macrophages were infected with 1x10^6 CFU of *E. coli* and IL-1β release was quantifed at 6h and 24h post-infection by ELISA. (**B**) WT macrophages were treated with control (BSA-PBS) or LPS (100 ng) with or without palmitate (250 μM) for 16h after which cytosolic (c) and mitochondrial (m) fractions of cell lysates were prepared. Western blotting was performed for ACSL1. Tubulin and VDAC were used as fractionation controls for cytosolic and mitochondrial preparations, respectively.

Specific Aim 2. To investigate the consequences of macrophage ACSL1 deficiency in diabetic UTI

Results: We performed two independent experiments where WT or mACSKO mice were fed standard diet (STD) or HFD for 16 weeks after which they were infected with uropathogenic E.coli. Urine, serum, and tissue analysis were performed at 1 and 3 days post-infection. Our study revealed similar weight gain and glucose intolerance between WT and mACSKO mice after 4 months of diet. Interestingly, serum triglycerides were consistently lower in mACSKO mice (Fig. 2). After infection, HFD fed mice had more leukocytes in the urine and lower bacterial titers. Consistent with this, tissue analysis revealed more inflammation and edema at both early and late time points in HFD fed mice, (Fig. 3). In conjunction with increased bladder inflammation, epithelial proliferation was also enhanced in HFD fed mice, particularly at day 3 after infection. These observations suggest that HFD feeding leads to persistent local inflammation after infection. Interestingly, this augmented inflammatory response could promote urinary tract complications such as recurrent infections and bladder cancer both of which occur in humans with type II diabetes. In general, the impact of

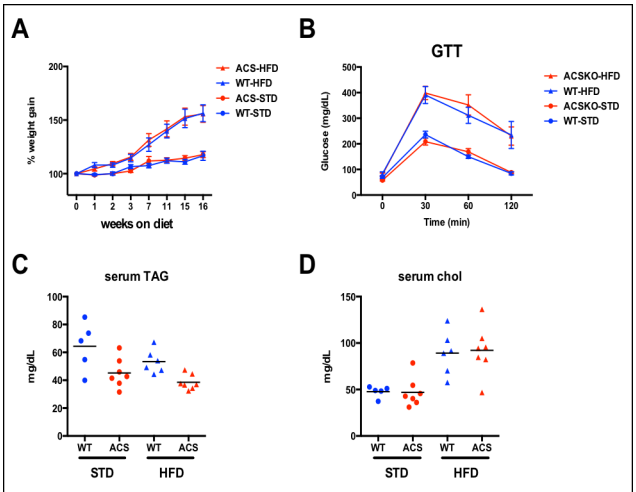

Figure 2. Metabolic characterization of WT and mACSKO mice after HFD. (A) WT and mACSKO (ACS) mice were fed a standard diet (STD) or high fat diet (HFD) for 16 weeks during which weights were obtained on a weekly basis. The % change from baseline weight is shown. (**B**) Upon the completing 16 weeks of HFD, mice were injected with glucose IP and seriel blood glucose determinations were made to assess glucose tolerance. (**C, D**) Serum was collected from mice 24h infection with E. coli and the levels of triglyceride (TAG) and cholesterol (chol) were quantified. For A and B, each dot represents the mean ± SEM. For C and D, each dot represents one animal.

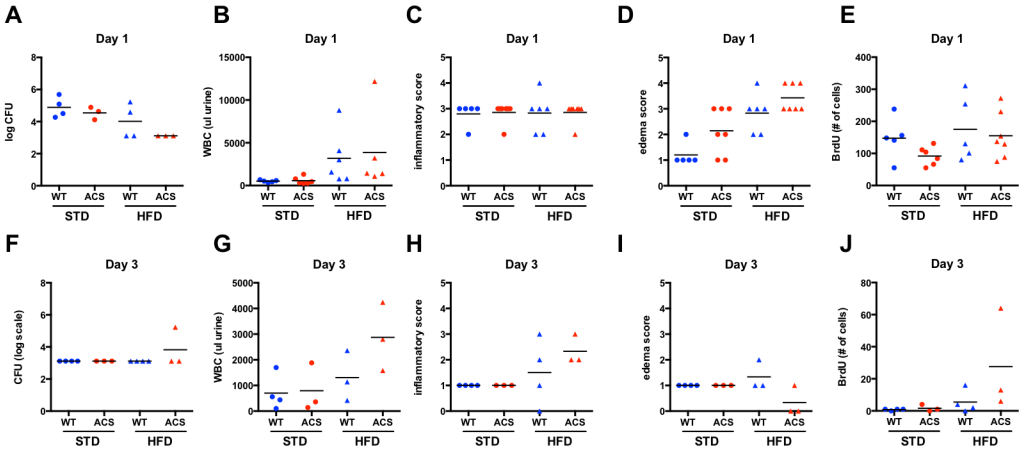

Figure 3. Bladder infection and inflammation comparison at early and late time points after infection with E. coli. (A-J) WT and mACSKO (ACS) mice were fed a standard diet (STD) or high fat diet (HFD) for 16 weeks after which they were infected intraurethrally with E. coli. Urine samples and bladder tissue was collected at day 1 and day 3 after infection. Sampels were analyzed for urine CFUs (**A,F**), urine white blood cells (WBCs; **B,G**), tissue inflammation (**C,H**), tissue edema (**D,I**), and bladder epithelial cell BrdU staining (**E,J**). Each dot represents one mouse.

ACSL1 deficiency was mild with a trend towards increased tissue edema and bladder cell proliferation in the HFD cohorts. Ongoing components of this aim include tissue RNA and immunohistochemical analysis of bladder tissue.

3. Publications:

There are not currently any publications that have resulted from this study; however, both the in vitro and in vivo components of our investigation are being followed up on for the purposes of publication and additional grant funding.