

methotrexate (MTX) response in juvenile idiopathic arthritis (JIA) and in vitro. **METHODS/STUDY POPULATION:** A comparative metabolomic analysis was used to identify metabolomic markers and metabolic pathways associated with MTX activity in vitro and in vivo. Cell-based studies assessed metabolomic profiles in K562 erythroblastoid cells with or without MTX treatment. In vivo analysis utilized plasma samples from JIA patients treated with MTX (n=30) and included samples collected prior to the initiation of MTX and after 3-months of MTX treatment. Plasma samples were from an IRB-approved single center prospective cohort study of biomarkers of MTX response in patients with JIA and were stratified based on American College of Rheumatology pediatric (ACR Pedi) response criteria. Semi-targeted global metabolomic profiles including over 800 metabolites across three analytical platforms at the NIH West Coast Metabolomics Center at UC-Davis and were analyzed by univariate and multivariate analysis using MetaboAnalyst 3.0. **RESULTS/ANTICIPATED RESULTS:** In K562 cells, MTX treatment was associated with statistically significant changes in 550 of the 850 intracellular metabolites detected (false discovery rate less than 0.05). Major metabolic pathways inhibited by MTX included branched-chain amino acid metabolism, purine and pyrimidine biosynthesis, and lipid metabolism including the inhibition of arachidonic acid metabolism. In patients with JIA, far fewer plasma metabolites were significantly altered following the initiation of MTX and included only 15 of the 833 plasma metabolites detected. Interestingly, MTX treatment was associated with the inhibition of arachidonic acid synthesis, inhibition of purine metabolism, and a dramatic reduction in plasma levels of various exogenous metabolites. In particular, MTX treatment was associated reductions in known metabolic markers of intestinal microbiota metabolism, including: biotin and dehydrocholic acid. Further, stratification of patients based on ACR Pedi response demonstrated that clinical response was associated with a greater reduction in plasma dehydrocholic acid levels following the initiation of MTX. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This work demonstrates that MTX therapy is associated with a number of biochemical changes in vitro and in vivo, including: inhibition of purine metabolism, inhibition of arachidonic acid metabolism, and an apparent inhibition of gut microbiota metabolism. Most notably, inhibition of gut microbiota metabolism appears to demonstrate a relationship with the observed clinical efficacy of MTX in JIA.

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### **Mycobacterium bovis Bacille-Calmette-Guérin infection aggravates atherosclerosis**

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**OBJECTIVES/SPECIFIC AIMS:** The study aimed at assessing whether *M. bovis* BCG infection and inflammation exacerbates the development of atherosclerosis in *Ldlr*<sup>-/-</sup> mice. **METHODS/STUDY POPULATION:** Twelve-week old male *Ldlr*<sup>-/-</sup> mice (n=10) were infected with *M. bovis* BCG (0.3–3.0x10<sup>6</sup> colony-forming units (CFUs)) via the intranasal route, to simulate a natural respiratory route of infection. Mice were subsequently fed a western-type diet (WD) containing 21% fat and 0.2% cholesterol for 16 weeks.

Age-matched uninfected *Ldlr*<sup>-/-</sup> mice (n=10) fed with an identical WD served as controls. Mice were euthanized after 16 weeks of WD to examine atherosclerotic lesions in aortic root sections and en face aorta using Oil Red O staining. Plasma cholesterol and triglyceride levels were measured by enzymatic assays and lipoprotein distribution was assessed using fast protein liquid chromatography. Because of the important role of T cells and monocytes in atherosclerosis development, we assessed these cell subsets in blood using flow cytometry at 8 and 16 weeks. Experiments were conducted in duplicate. We used unpaired Student's t-test for group comparisons of numeric variables and flow cytometry data. **RESULTS/ANTICIPATED RESULTS:** *M. bovis* BCG infection significantly increased atherosclerotic lesions in en face aorta (plaque size per aorta area ratio; 0.15±0.13 vs. 0.06±0.02; P<0.01), but not in the aortic root. There were no significant differences in plasma cholesterol (1,160 mg/dL vs. 1,278 mg/dL; P = 0.36), triglycerides (340 mg/dL vs. 413 mg/dL; P = 0.28), or lipoprotein profiles between infected vs. uninfected mice at 16 weeks. *M. bovis* BCG increased circulating T lymphocytes (1,490 cells/uL vs. 1,227 cells/uL; P = 0.03) and monocytes (901 cells/uL vs. 414 cells/uL; P<0.01) within 8 weeks post-infection. When we assessed T lymphocyte subsets, *M. bovis* BCG infection increased total CD4<sup>+</sup> T cell counts (556 cells/uL vs. 416 cells/uL; P<0.01) but not CD8<sup>+</sup> T cells. No differences in the proportion of CD44<sup>+</sup>CD25<sup>+</sup> activated T lymphocytes were noted between groups. When we assessed monocyte subsets, *M. bovis* BCG infection increased the numbers of Ly6Chigh (709 cells/uL vs. 362 cells/uL; P<0.01) and Ly6Clow (145 cells/uL vs. 35 cells/uL; P<0.01) monocytes. Infection was associated with an increased proportion of Ly6Clow monocytes at week 8 (17% vs. 8%; P<0.01) and week 16 (19% vs. 5%; P<0.01), compared to uninfected mice. **DISCUSSION/SIGNIFICANCE OF IMPACT:** *M. bovis* BCG infection increased the extent of atherosclerosis formation in the aortas of WD-fed hyperlipidemic *Ldlr*<sup>-/-</sup> mice after 16 weeks. Lipid profiles were similar between infected and uninfected mice, and therefore do not explain the observed differences in atherosclerosis. Compared to uninfected controls, *M. bovis* BCG-infected mice exhibited increased CD4<sup>+</sup> T cell and monocyte driven inflammation. Interestingly, *M. bovis* BCG-infected mice had a higher proportion of non-classical Ly6Clow monocytes, suggesting a pro-atherogenic contribution of these cells in our model. Overall, our results support a pathogenic role of mycobacterial infection in atherosclerosis development and ASCVD.

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### **Olfactory habituation in schizophrenia**

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**OBJECTIVES/SPECIFIC AIMS:** The aim objective of this research work is the study of neurocognitive endophenotypes in the new classifications of mental disorders. **METHODS/STUDY POPULATION:** Neuropsychological tests, such as the UPSIT test were used. The population was composed by a sample of patients with a diagnosis of schizophrenia. **RESULTS/ANTICIPATED RESULTS:** Olfactory discrimination is a potential neurocognitive endophenotype in the study of schizophrenia research. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The aim impact of this research work is the study of Dimensional classifications of mental disorders.