Environ Health Perspect

DOI: 10.1289/EHP7699

Supplemental Material

DNA Methylation Patterns in $CD4^+$ T Cells of Naïve and Influenza A Virus-Infected Mice Developmentally Exposed to an Aryl Hydrocarbon Receptor Ligand

Catherine G. Burke, Jason R. Myers, Christina M. Post, Lisbeth A. Boule, and B. Paige Lawrence

Table of Contents

Figure S1. Water consumption and body weight change in mice treated with DNA methylation altering drugs. Mice were developmentally exposed to peanut oil vehicle (Veh) or TCDD (1 μg/kg BW). At 21 days, 25 female offspring of vehicle dams, and 17 female offspring of TCDD dams were randomly assigned to 3 treatment groups: normal vivarium water (control), water containing S-adenosylmethionine (SAM; 0.5 mg/mL), or water containing Zebularine (Zeb, 0.2 mg/mL). (A) The amount of water consumed per mouse per day prior to infection. Water consumption was measured every 2 days. The amount of water consumed per mouse was calculated by recording the amount of water consumed per cage divided by the total number of mice within that cage (4-5 mice per cage). (B) Weekly weight gain from 3 weeks of age until infection. (C-E) Body weight after IAV infection in (C) all groups, (D) vehicle groups, and (E) TCDD groups was recorded for 9 days. No mice died from the infection. The number of mice in each group was as follows: control water vehicle (8), control water TCDD (5), SAM water vehicle (8), SAM water TCDD (6), Zeb water vehicle (9), and Zeb water TCDD (6). All data shown denote the mean ± SEM. Data were analyzed by 2-way ANOVA, and F values are shown in each graph.

Figure S2. $CD4^+$ T cell purity. $CD4^+$ T cells were isolated using MojoSort Mouse $CD4^+$ T cell kit (BioLegend, San Diego, CA). (A) Purity of isolated $CD4^+$ T cells was determined using flow cytometry. The FACS plots depict cell enrichment from two samples, and the number on each plot denotes the mean (±SEM) purity of $CD4^+$ T cells from infected offspring of dams treated with vehicle control or TCDD. (B) $CD4^+$ T cell purity for individual samples from vehicle and TCDD offspring that were naïve or infected.

Figure S3. Differential gene expression in $CD4^+$ T cell from mice exposed developmentally to TCDD or control and infected with influenza A virus. Pregnant mice were administered vehicle or TCDD (1 μg/kg body weight) or peanut oil vehicle control on gestational days (GD) 0, 7, 14, and 2 days after parturition (PND2). At 8-10 weeks of age, offspring were infected with influenza A virus (IAV; strain HKx31). Nine days after infection, $CD4^+$ T cells were purified, RNA isolated, and RNA-sequencing (RNA-seq) was performed. The number of differentially expressed genes (DEGs) was assessed and presented in a volcano plot.

Figure S4. Comparison of cellular functions and pathways during IAV infection in offspring developmentally exposed to vehicle control or TCDD. Pathways were ranked by p-value, and 20 pathways in $CD4^+$ T cells from infected vs. naïve offspring in (A) Vehicle and (B) TCDD exposure groups. Position of dots denotes relative rank in the other exposure group. The y-axis shows the pathway rank within the (A) Vehicle or (B) TCDD. A positive change in rank order indicated that pathway was found at a higher rank in the other exposure group. Open circles represent pathways that were ranked among the top 20 for both vehicle and TCDD exposure groups. In (A), filled circles represent pathways only in the vehicle. In (B), filled circles represent pathways only in the TCDD. Abbreviations: V, vehicle, T, TCDD, i, infected, n, naïve.

Figure S5. Correlation of changes in DNA methylation and gene expression. (A-F) Scatter plots show the correlation between change (Δ) in DNA methylation and fold change in differentially expressed genes (DEG) in $CD4^+$ T cells from (A-C) vehicle or (D-F) TCDD exposed offspring prior to and after infection. Correlations in (A,D) all genomic regions, (B,E) promoters, and (C,F) introns are shown. Black lines show the R2 and p-value for all DEGs (all circles). Blue lines show the R2 and p-value for DEGs with an inverse correlation between gene expression and DNA methylation change (open circles). The percentage of open circles is indicated above each plot.