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Data Article

Dataset describing maternal prenatal restraint stress effects on immune factors in mice

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ABSTRACT

Maternal immune dysregulation, caused by gestational psychological stress, infection, and other perturbations, results in altered offspring neurodevelopment and increases risk for psychiatric disorders. Prior work has found that multiple cytokines play critical roles in shaping offspring neurodevelopment after gestational stress, though how maternal psychological stress impacts maternal, placental, and fetal cytokine levels more broadly remains unclear. The purpose of the present study was to assess changes to IL-1β, IL-2, IL-4, IL-6, IL-10, IL-17A, IFNγ, and TNFα in a widely-used mouse prenatal restraint stress model. After repetitive restraint stress on gestational days 12–14, stressed dams had increased serum levels of IL-1β, IL-6, and IL-10. Embryonic day 14 IL-2 and IL-1β levels were decreased in prenatally stressed male fetal forebrain, while placental IL-2 was decreased by stress regardless of offspring sex. Placental and fetal forebrain IL-2 levels were negatively correlated. These data provide important insights into the immune changes that occur with prenatal restraint stress.

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Specifications Table

Subject	Neuroscience: Biological Psychiatry
Specific subject area	Prenatal restraint stress in a mouse model and subsequent immunologic impacts in pregnancy and on offspring.
Type of data	Tables and Figures
How the data were acquired	1. Direct weighing of animals and tissues 2. Polymerase chain reaction genotyping for sex 3. Pierce BCA Protein Assay (Thermo Fisher, Waltham, MA USA) 4. Bio-Plex Mouse Cytokine Group I 8-plex Assay (Bio-Rad, Hercules, CA USA) 5. High-sensitivity ELISA for mouse IL-6 and IL-17 (Invitrogen, Carlsbad, CA USA) 6. Bio-Plex Luminex 200 (Bio-Rad, Hercules, CA USA) 7. Bio-Plex Manager software (Bio-Rad, Hercules, CA USA) 8. Prism (GraphPad, La Jolla CA USA)
Data format	Raw and analyzed
Description of data collection	Maternal serum, placenta, fetal dorsal forebrain, and viscera were collected from CD-1 mice at gestational day 14 after prenatal restraint stress or not. Placentas and fetuses were weighed. Samples and data were collected in March and April of 2018.
Data source location	• Institution: University of Iowa Carver College of Medicine • City/Town: Iowa City • Country: USA • Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: 41.6628° N, 91.5452° W
Data accessibility	Repository name: Mendeley Data Identification number: 10.17632/5tfj857sb2.3 Direct URL to data: https://data.mendeley.com/datasets/5tfj857sb2/3

Value of the Data

- The dataset is useful because it can be used to identify trends in cytokine/chemokine levels across maternal-fetal domains with stress during pregnancy. Prenatal stress is a risk factor for neuropsychiatric disorders in children; cytokines/chemokines have been proposed as mechanisms for this neuropsychiatric risk. These data may be helpful in understanding the pathophysiology of neuropsychiatric disorders.
- The data will benefit researchers in immunology, development, neuroscience, and stress biology. The dataset demonstrates the detection limits and utility of multiplex ELISA in the setting of pregnancy and repetitive stress in a mouse model. Pairing these data with data on fetal growth, visceral cytokines for some cytokines, and fetal sex increases their interpretability and value.
- The data may inform or be used in future studies of maternal-fetal immunologic crosstalk in pregnancy and particularly in the setting of psychologic distress. In particular, studies may be informed by the finding here that cytokine levels in maternal serum, placenta, and fetal brain were uncoupled.
- The Mendeley dataset reveals poorly understood cytokine/chemokine changes across placenta, maternal serum, and fetal forebrain after a commonly-employed prenatal restraint stressor. Prior to this study, datasets were not available for this broad range of molecular inflammatory outcomes in multiple tissue compartments in which levels may differ due to active and passive mechanisms of transport.

- Our understanding of inflammatory changes with prenatal stress in this study was limited by technical range of the methodology; sample sizes had sufficient power overall but were potentially limited for specific measures.

1. Data Description

To understand how prenatal stress impacts fetal development, the maternal and fetal immune milieus, as well as the placenta that bridges them, must all be examined. Thus, in this study, we evaluated cytokine levels in CD1 mice at embryonic day 14 (E14) in maternal serum, placenta, fetal forebrain, and fetal viscera in both prenatally stressed (PS) and nonstressed (NS) conditions. PS dams were restrained in a plexiglass tube under bright lights for 45 min sessions during the light cycle (three times, 3–4 h apart, on E12 and E13 and once ending approximately 30 min before collection on E14). This design was used to capture both the chronic and acute effects of restraint stress on cytokine profiles. Resulting cytokine concentration and body and placental weight data are available here and on the Mendeley Data Repository [1].

A multiplexed ELISA approach was used to determine cytokine/chemokine levels across maternal serum, placenta, and fetal forebrain. The data for this study are posted to the Mendeley database [1] : http:// data.mendeley.com/datasets/5tfj857sb2/2. Raw analyte levels and levels normalized to total protein are available from two experimental cohorts. “OOR” indicates “out of range.”

Assessments of fetal bodyweight revealed no change by stress (NS=264.4 $\pm$ 6.13, PS=269.3 $\pm$ 3.69 mg; $n = 32$ NS, 53 PS; non-significant difference across all individuals and when grouped by litter). However, individual placental weight was significantly decreased in PS offspring placentae ($p<0.05$ across all individuals; NS=96.6 $\pm$ 2.83, PS=90.09 $\pm$ 1.83; $n = 32$ NS, 54 PS; unchanged when grouped by litter).

In Fig. 1, we show cytokine levels (raw data in Mendeley database) in maternal serum after prenatal restraint stress. Serum levels of IL-1β (t(7)= 3.206, $p = 0.015$), IL-6 (t(7)=3.694, $p = 0.008$), and IL-10 (t(7)= 3.709, $p = 0.008$) were significantly increased in PS dams relative to NS control levels by multi-plex ELISA (Fig. 1).

Fig. 2 displays cytokine levels across placenta and fetal tissues (raw data in Mendeley database). In placenta, IL-1β, IL-4, IL-10, and IL-17 levels were undetectable, but IL-2 was decreased by PS regardless of sex (Fig. 2A,B, male t(7)=2.982, $p = 0.020$ by *t*-test, female $p = 0.017$ by Wilcoxon signed rank test). Similarly, IL-2 was decreased by PS in male fetal forebrain ($p = 0.008$ Wilcoxon signed rank test, while IL-1β was trend-wise decreased (Fig. 2C, t(7)=2.045, $p = 0.080$ by *t*-test). Dorsal forebrain IL-1β and IL-2 levels both decreased with PS in males but not females, suggesting a role for sexual dimorphism of placenta in male vulnerability, as has been suggested previously [2]. All other cytokine levels were undetectable in E14 forebrain with this multiplex approach (Fig. 2C,D). Interestingly, we also found that placenta and forebrain levels of IL-2 were negatively correlated (Fig. 2E, $r=-0.434$, $p = 0.019$). We further assessed fetal viscera for changes in two pro-inflammatory cytokines with previous evidence for critical mediation of changes in neurodevelopment and brain function *in vivo*, IL-6 and IL-17 [3,4]. Via high-sensitivity ELISA of fetal viscera, neither IL-17 nor IL-6 levels were significantly changed by PS (Fig. 2F,G).

2. Experimental Design, Materials and Methods

We used a broad approach to cytokine detection (Bio-Rad, Hercules, California USA; Bio-Plex Mouse Cytokine Group I 8-plex Assay #Z60000D2EB including IL-1β, IL-2, IL-4, IL-6, IL-10, IL-17A, IFNγ, and TNFα and high-sensitivity ELISAs for mouse IL-6 and IL-17; Invitrogen, Carlsbad, CA USA). All offspring/placentas were genotyped for sex. Protein was isolated using a NET-N buffer, protease inhibitor (EDTA-free Complete Mini, Roche, Indianapolis, IN USA), and phosphatase inhibitor (PhosSTOP EASY pack, Roche) solution and microcentrifugation. From each of

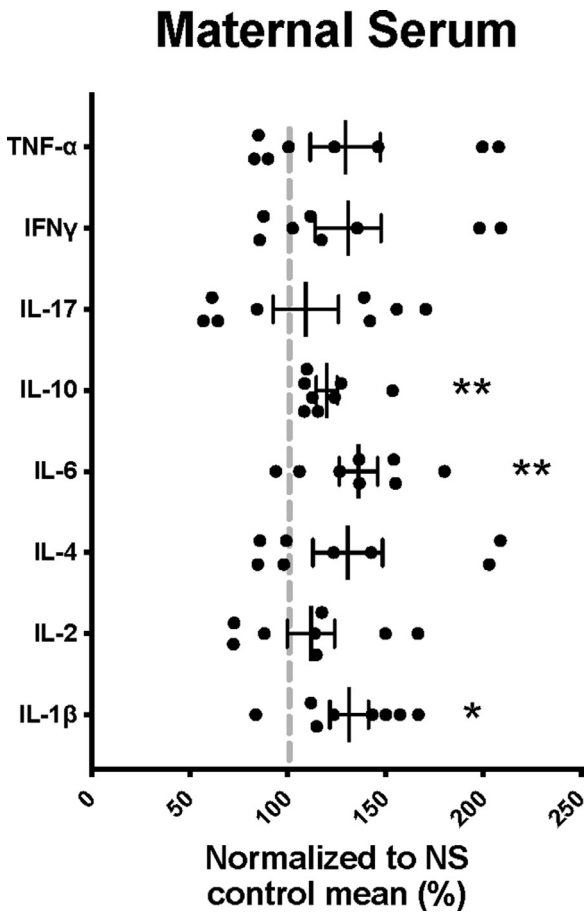

Fig. 1. Cytokine concentration ranges in this study and measured cytokine levels in maternal serum after prenatal restraint stress. Maternal serum cytokine levels after stress as a percentage of nonstressed controls levels. $n = 8$ prenatally stressed, 9 nonstressed dams. $^{*}p<0.05$, $^{**}p<0.01$ per t-test; means ± SEM displayed. Dashed gray line represents control nonstressed level for each cytokine (100%), to which prenatal stress samples were normalized.

eight NS and nine PS E14 litters from two cohorts collected at separate times the following samples were taken: maternal serum, one randomly selected placenta per sex per litter, microdissected dorsal forebrain (samples from each litter were pooled by sex across all litter offspring to achieve sufficient protein quantity), and one randomly selected fetal visceral tissue sample per sex per litter. Fetal viscera were used to provide tissue that would reflect non-brain, systemic levels of cytokines.

All samples were run in duplicate and normalized to total protein levels (Pierce BCA Protein Assay, Thermo Fisher, Waltham, MA USA). For each of two cohorts, one multiplex plate was run. Each plate contained samples and standards for each cytokine, which were assessed together on a Bio-Plex Luminex 200 (Bio-Rad) at the University of Iowa Flow Cytometry core. Cytokine concentrations were determined by Bio-Plex Manager software (Bio-Rad), based on manufacturer's instructions. To account for systematic differences between plate runs and cohorts, percent change (prenatal stress relative to nonstress) was calculated for each prenatal stress sample independently within each plate. These normalized percent change values were then combined across plates. Data were analyzed by student's t-test (parametric) or Wilcoxon signed rank test (nonparametric) and linear regression. Normality was assessed using the Kolmogorov-Smirnov

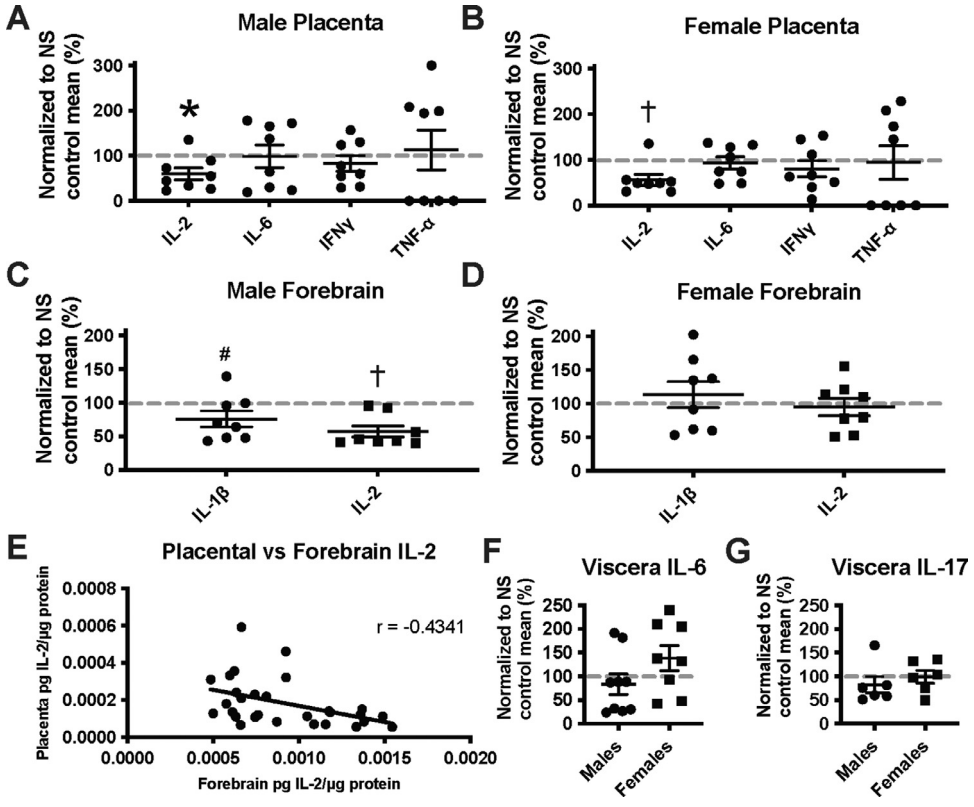

Fig. 2. Cytokine levels across placenta and fetal tissues. A-D) Cytokine levels in E14 fetal placenta and forebrain after prenatal restraint stress via multi-plex ELISA. All prenatal stress (PS) data are relative to mean nonstressed (NS) control levels. $N = 8$ PS, 9 NS litters. $^{*}p<0.05$, $\#p<0.1$ per t-test, $^{\dagger}p<0.05$ per Wilcoxon signed rank test. E) Significant negative correlation ($p = 0.019$) between placental and forebrain IL-2 per linear regression analysis. $N = 29$ pairs across sexes. F, G) Viscera levels of IL-6 and IL-17 via high-sensitivity ELISA. IL-6 $N = 7$ NS, 9 PS males; 8 NS, 8 PS females. IL-17 $N = 6$ both conditions, sexes. All results shown as mean ± SEM. TNFα data points at 0% were below detection limits. Dashed gray line represents control nonstressed level for each cytokine (100%), to which prenatal stress samples were normalized.

test in Prism (GraphPad, La Jolla CA USA). Outliers were defined as more than two standard deviations from the mean. $P<0.05$ was considered significant. Findings were displayed using Prism.

Ethics Statements

Experimental procedures involving animals were performed in accordance with University of Iowa Institutional Animal Care and Use Committee (IACUC) policies, the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978), and the ARRIVE guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Maternal prenatal restraint stress effects on immune factors in mice (Original data) (Mendeley Data).

CRediT Author Statement

Serena Banu Gumusoglu: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition; **Sara Victoria Maurer:** Data curation, Writing – review & editing, Project administration; **Hanna Elizabeth Stevens:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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