Cytokine Indicators of Inflammation in Mice Fed a Western Type Diet

Emily Mortensen
*Utah State University, JL2885@yahoo.com*

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Cytokine Indicators of Inflammation in Mice fed a Western Type Diet

Emily Mortensen1, Eliza Owens2, Daphne Rodriguez2, Abby Benninghoff2,3

1 Dept. of Nutrition, Dietetics, and Food Science, Utah State University 2 Dept. of Animal, Dairy and Veterinary Sciences, Utah State University 3 Applied Nutrition Research, Utah Science and Technology Research Initiative (USTAR) Department, Utah State University 4Dept. of Biology, Utah State University

BACKGROUND

- Colorectal cancer is currently the second leading cause of cancer-related deaths in the United States with the risk increasing in individuals who suffer from colitis, inflammation of the colon lining, seen in Irritable Bowel Disease.

- Previous studies completed by our group have demonstrated that the Total Western Diet has a promoting effect on colitis-associated colorectal cancer (CACC) in mice leading to markedly increased colon inflammation as compared to mice consuming a healthy diet.

- Small, nonstructural proteins called cytokines are involved in the immune system. Several cytokines have been shown to be involved in the chronic inflammation that lead to the development of CACC.

- The amount of interleukins detected in tissues or in circulation may be used as a biomarker indicative of the inflammatory state of the organism.

OBJECTIVE

The objective of this study is to determine the blood concentrations of various cytokine biomarkers of systemic inflammation in mice fed either healthy diet (AIN93G) or a Western diet (TWD) prior to, during, and after colitis in mice and after colon tumors have developed.

HYPOTHESIS

We hypothesize that blood levels of IL-6 and IL-17 will increase in animals fed a TWD diet experiencing more severe colitis. In addition, levels of these cytokines will remain elevated through recovery and tumorigenesis of the disease. Both cytokines have been shown to have a positive correlation with colitis.

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- USTAR Applied Nutrition Research, Utah State University
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STUDY DESIGN

<table>
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<tr>
<th>Diets</th>
<th>Time Points of Interest</th>
<th>Diet Treatment</th>
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<tr>
<td>AIN</td>
<td>Initial (Day 21)</td>
<td>Day 21: Baseline after acclimation to experimental diets</td>
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<tr>
<td>TWD</td>
<td>Active colitis (Day 33)</td>
<td>Day 33: Immediately after AOM/DSS treatment</td>
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<tr>
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<td>Recovery (Day 47)</td>
<td>Day 47: 2 weeks after cessation of treatment</td>
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<tr>
<td></td>
<td>Tumor assessment (Day 112)</td>
<td>Day 112: Final time point: Various factors leading to tumorigenesis</td>
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</tbody>
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Mice

- C57BL/6J mice
- 263 Males and 272 Females
- 5 weeks of age

Experimental diets

- AIN93G: control diet that promotes rodent health
- Total Western Diet (TWD): promotes inflammation-associated colorectal carcinogenesis

TIME POINTS OF INTEREST

Day 21: Baseline after acclimation to experimental diets
Day 33: Immediately after AOM/DSS treatment
Day 47: 2 weeks after cessation of treatment
Day 112: Final time point: Various factors leading to tumorigenesis

Cancer Model

- 10 mg/kg azoxymethane (AOM) to initiate carcinogenesis on day 14 + 1% (w/v) dextran sodium sulfate to promote colon tumor development for 10 days

Blood Samples

- Blood samples were collected in Micro Z-gel tubes and spun at 10,000G for 5 minutes. Serum was collected and stored in -80°C. Samples were collected at necropsy times on days 7 (initial), 33 (Colitis), 47 (Recovery) and 112 (final).

METHODS

ELISA Assessment

- Concentration of the cytokines will be determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit obtained from ThermoFisher.
- A specific ELISA kit will be used for each respective cytokine.
- Cytokines of interest include: IL6, IL17

DATA ANALYSIS

- Data will be analyzed using a standard linear mixed model for the following:
  - Diet treatment
  - Time point
  - Diet x Time point
- Main comparisons of interest include AIN compared to the TWD at each of the four time points in order to determine if exposure to TWD exacerbates inflammation to the extent that biomarkers of inflammation were elevated in circulation.

EXPECTED RESULTS

- We expect blood levels of IL-6 and IL-17 to increase in animals fed a TWD diet indicating higher levels of colitis, and the levels to remain elevated through recovery and tumorigenesis. Prolonged inflammation in the colon lining can lead to an increased risk of developing CACC, as well as perforations in the colon. Symptoms of colon inflammation include diarrhea, loss of appetite, and malabsorption of nutrients and diet is a key factor affecting colitis.

CONTACT

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</tr>
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