The effect of TCDD on cytokine production during the progression of insulitis in NOD mice

Tuan Pham
Dr. Nancy Kerkvliet
Environmental and Molecular Toxicology
Oregon State University
HHMI Summer 2012
September 15, 2012
Type 1 Diabetes

• An autoimmune disease

• T-cells attack pancreatic β- cells, causing the destruction of insulin producing cells.
  • T-cells inappropriately recognize molecules on pancreatic β- cells.

• This infiltration of T-cells of the pancreatic islets is called insulitis.

Healthy Pancreas

Pancreas with diabetes
The NOD (non-obese diabetic) mice spontaneously develop Type 1 Diabetes (T1D).

Early progression of insulitis can be observed at 7 weeks of age.

When blood glucose level is higher than 250 mg/dl, NOD mice are considered diabetic.

Incidence of diabetes occur 90-100% in NOD females by 30 weeks of age
  • In contrast, only 40-60% in NOD males
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

- A persistent environmental contaminant.

- TCDD is a potent immunosuppressant and suppresses diabetes in the NOD model.
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

Incident of Diabetes Over 30 Weeks Period.

Goal

• Since insulitis is marked by the inflammation of the pancreas, we want to observe whether TCDD suppresses type 1 diabetes by altering cytokine level.
  • Cytokines are cell signaling molecules used in intercellular communication
    • IFN-γ- pro-inflammatory
    • IL-17- pro-inflammatory
    • IL-22- tissue protective

• **Prediction:** IFN-γ and IL-17 production are suppressed in TCDD-treated mice and IL-22 is induced.
Experimental Design
Animal Groups

- There were two treatment groups, TCDD-treated and vehicle-treated.
  - Animals were treated every other week starting at 7 weeks of age and ending at 15 weeks.
  - The first dose is 50 \( \mu \text{g/kg} \) while the other doses are 15 \( \mu \text{g/kg} \)

- Mice were overdosed with \( \text{CO}_2 \) and serum was collected at the designated age.

- Blood glucose levels were taken at the time of initiation (7 weeks of age) and at the time of sacrifice.
Animal Groups

![Bar graph showing cumulative diabetes incidence (%) vs. age (weeks) with key symbols indicating VEH and TCDD doses.]

1st TCDD Dose

Week 7

Key
- Sacrificed
Method- ELISA (Enzyme-linked immunosorbent assay.)

- ELISA is a tool to quantify the target cytokines (IFN-γ, IL-17, and IL-22) in serum.
  - Coat surface with capture antibody.
  - Block nonspecific binding sites.
  - Add serum sample to the plate.
  - Add Detection antibody to sample.
  - Apply HRP-linked (enzyme) antibody.
  - Apply substrate

*plate is washed after each step*
A standard curve is generated from known amounts of IFN-γ, IL-17 or IL-22.

From the standard curve, a linear equation is produced

\[ y = mx + b, \text{ where} \]
\[ y = \text{OD (optical density)} \]
\[ x = \text{concentration (pg/mL)} \]
Results
**IFN-γ ELISA**

- $y = 0.00073x + 0.19598$
- $R^2 = 0.94835$

### Standard

![Graph showing standard OD and concentration in log scale]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration Detected (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8wk</td>
</tr>
<tr>
<td>VEH</td>
<td>BDL</td>
</tr>
<tr>
<td>TCDD</td>
<td>BDL</td>
</tr>
</tbody>
</table>

- 1:2 dilution of serum was used

*BDL: Below detectable limit*

*VEH: vehicle*
Serum

- Naturally, serum contains a considerable amount of proteins and other substances
  - Albumin
  - Electrolytes
  - Hormones
- These proteins can inhibit binding between the target cytokine and its capture antibody.
Troubleshooting - Interference by serum components

- **Spike-and-recovery:** A known amount of analyte is added into the natural test sample matrix.
  - Serum was diluted: 1:2, 1:10, 1:50, 1:100, 1:500, 1:1000.
  - Each serum dilution was spiked with 1000 pg/mL of IFN-γ.
Troubleshooting Result

- Concentration Detected (pg/mL)
- Serum Dilution

Graph showing the concentration detected at various serum dilutions. The spiked sample is indicated with a star.
# IFN-γ ELISA (1:50 dilution)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration Detected (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8wk</td>
</tr>
<tr>
<td>VEH</td>
<td>BDL</td>
</tr>
<tr>
<td>TCDD</td>
<td>BDL</td>
</tr>
</tbody>
</table>

*BDL: Below detectable limit
*VEH: vehicle
IL-17 ELISA (1:50 dilution)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration Detected (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8wk</td>
</tr>
<tr>
<td>VEH</td>
<td>BDL</td>
</tr>
<tr>
<td>TCDD</td>
<td>BDL</td>
</tr>
</tbody>
</table>

*BDL: Below detectable limit  
*VEH: vehicle

Standard

![Standard Plot](image)
## IL-22 ELISA (1:50 dilution)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration Detected (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8wk</td>
</tr>
<tr>
<td>VEH</td>
<td>BDL</td>
</tr>
<tr>
<td>TCDD</td>
<td>BDL</td>
</tr>
</tbody>
</table>

*BDL: Below detectable limit
*VEH: vehicle

**Standard**

- OD in Log Scale
- Concentration in Log Scale

![Graph showing standard curve with linear and log-scale axes, demonstrating the relationship between concentration and optical density](graph)
Blood Glucose Level

* VEH: vehicle
Conclusion

• IFN-γ, IL-17 and IL-22 were not present in the serum, suggesting cytokine production was not altered.

• Based on data, the autoimmune response was not systemic, but likely to be localized to the pancreas.
Future Investigation

- Examining the pancreas and pancreatic lymph nodes through immunohistochemistry.
Acknowledgement

• Dr. Nancy Kerkvliet
  • Members of Kerkvliet Lab
• Dr. Kevin Ahern
• Howard Hughes Medical Institute
• Oregon State University Honors College