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

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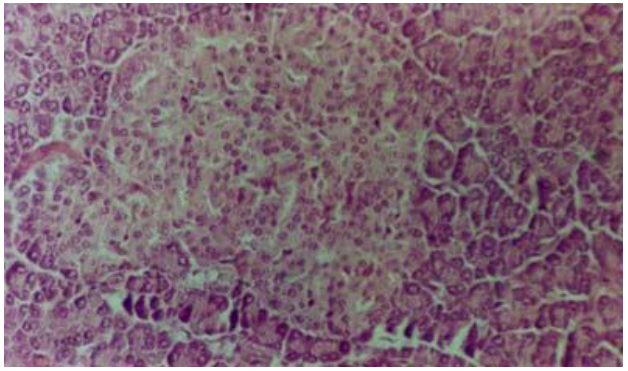
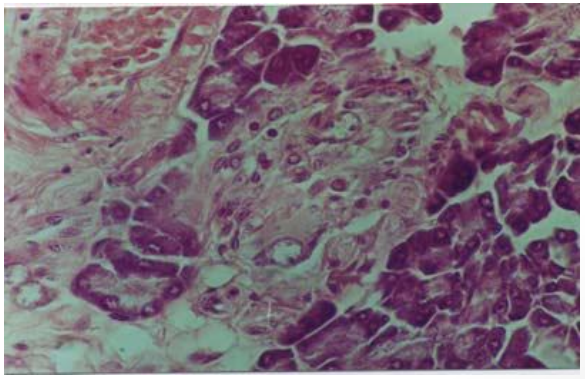
Type 1 Diabetes

- 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.



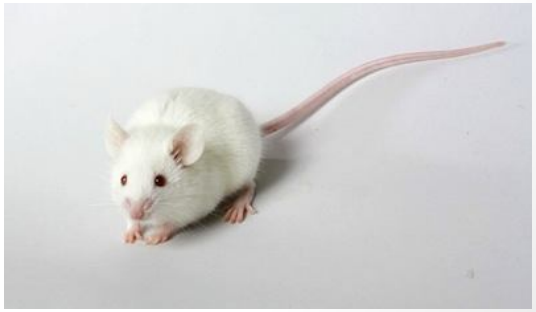
Healthy Pancreas



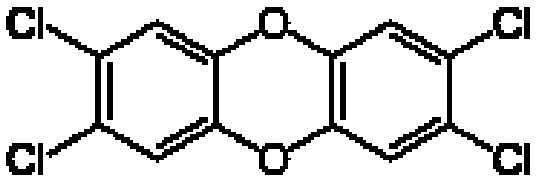
Pancreas with diabetes

NOD Model

- 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

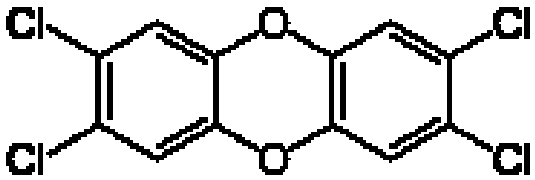


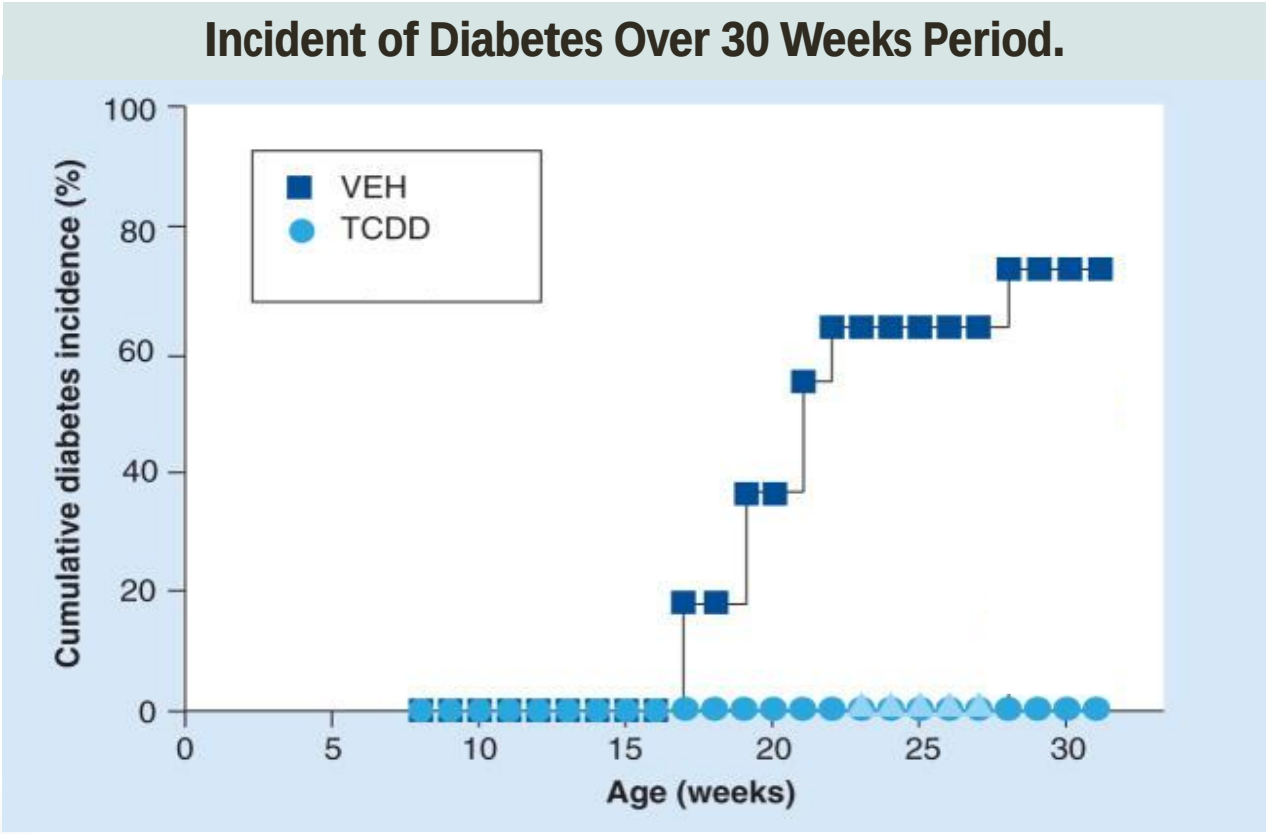
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)



- A persistent environmental contaminant.
- TCDD is a potent immunosuppressant and suppresses diabetes in the NOD model.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)





Kerkvliet, N. I., et al, 2009. *Immunotherapy 1:539*.

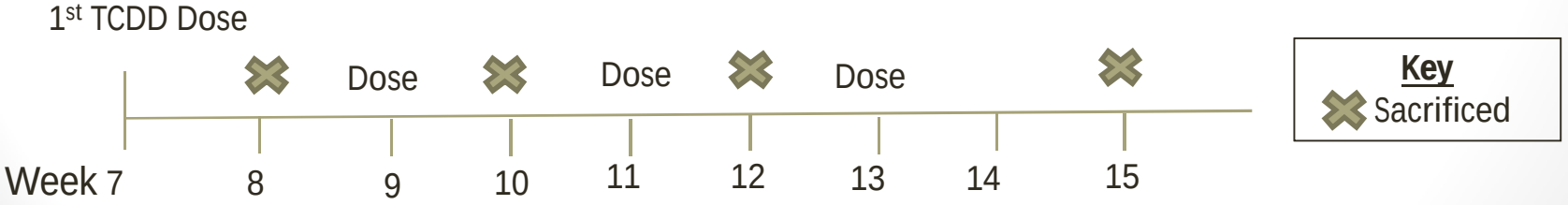
Goal

- 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.

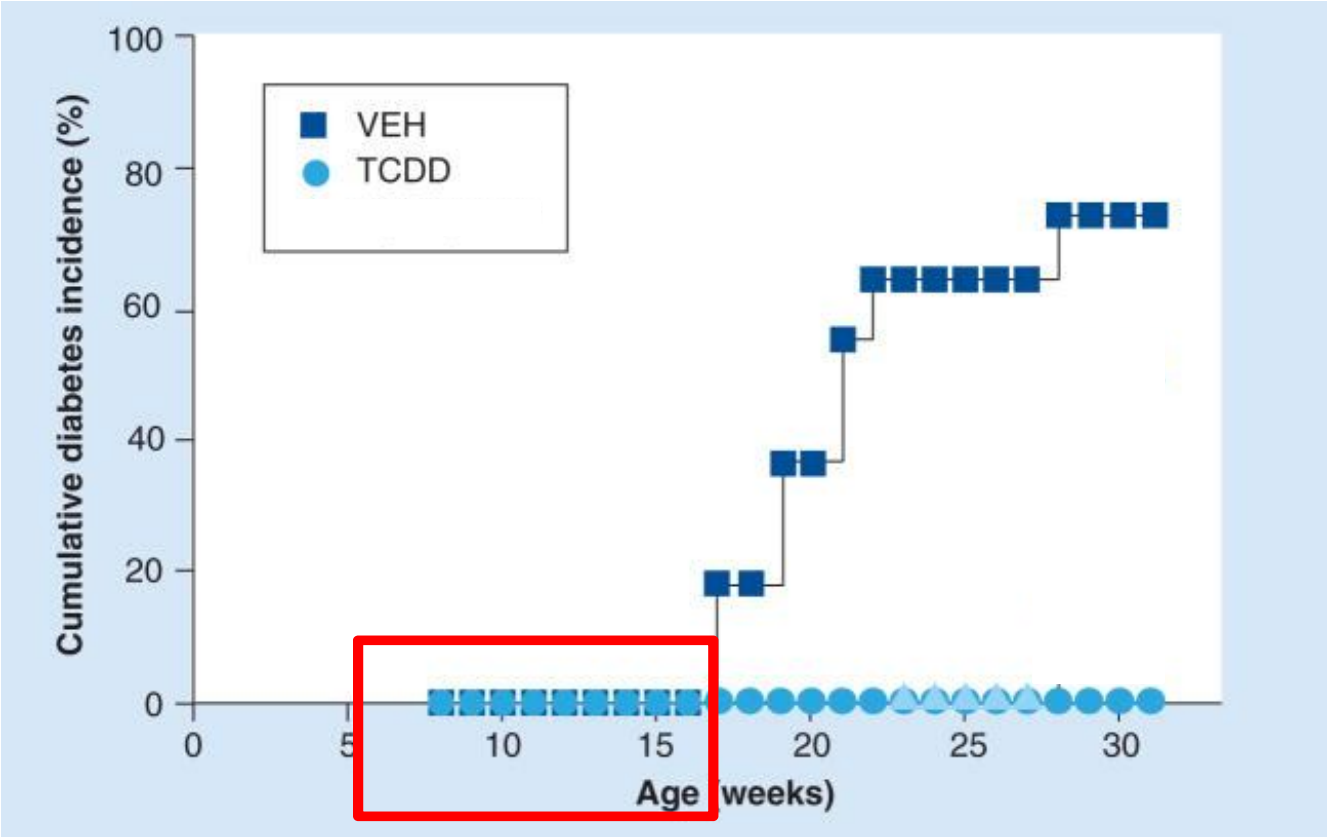
Experimental Design

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 μg/kg while the other doses are 15 μg/kg
- Mice were overdosed with 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



1st TCDD Dose

Dose Dose Dose

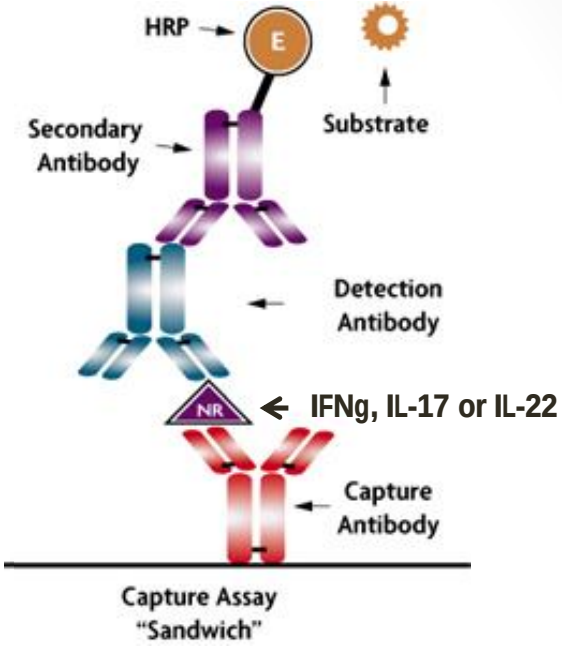
Week 7 8 9 10 11 12 13 14 15

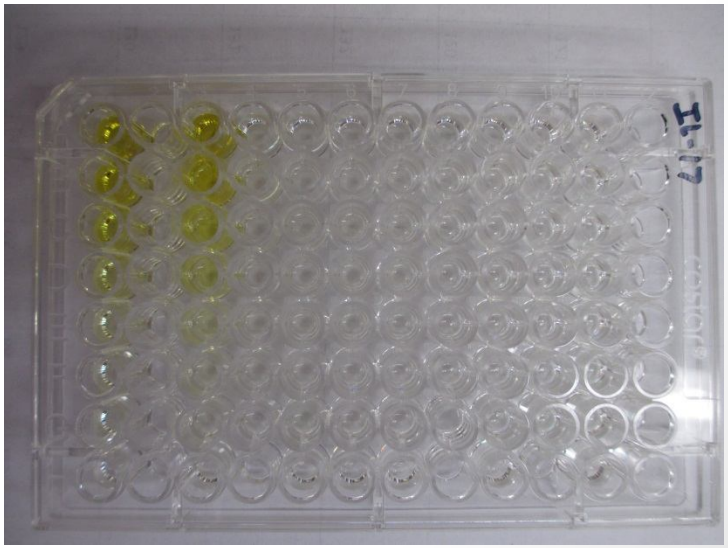
Key
Sacrificed

Method- ELISA (Enzyme-linked immunosorbent assay.)

- ELISA is a tool 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*



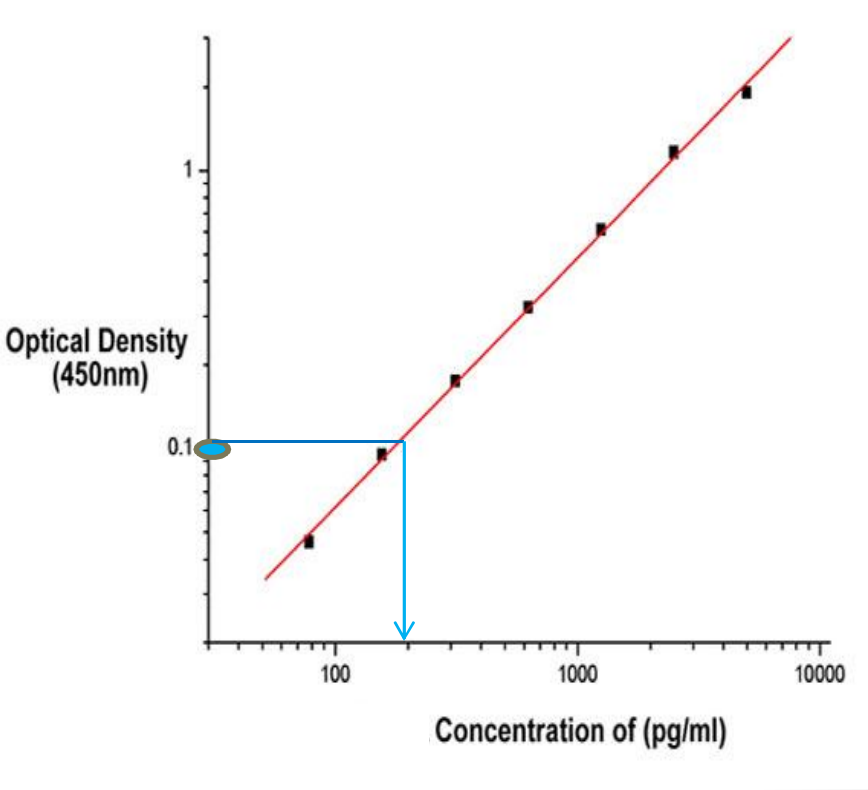


Data Analysis

- 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***, *where*

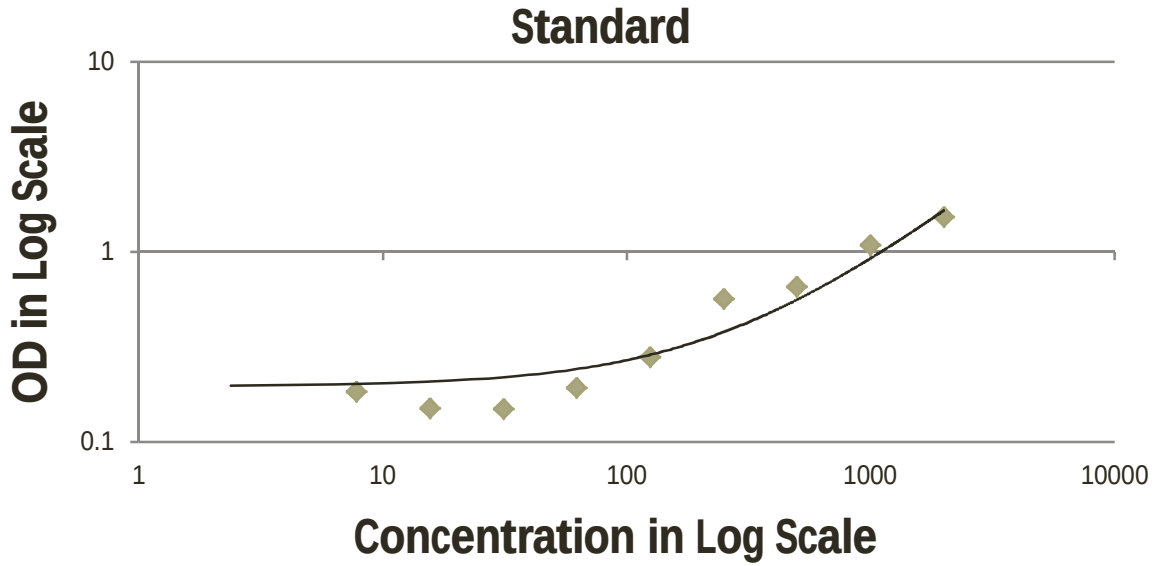
y= OD (optical density)

x= concentration (pg/mL)



Results

IFN-γ ELISA



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

Treatment	Concentration Detected (pg/mL)			
	8wk	10wk	12wk	15wk
VEH	BDL	BDL	BDL	BDL
TCDD	BDL	BDL	BDL	BDL

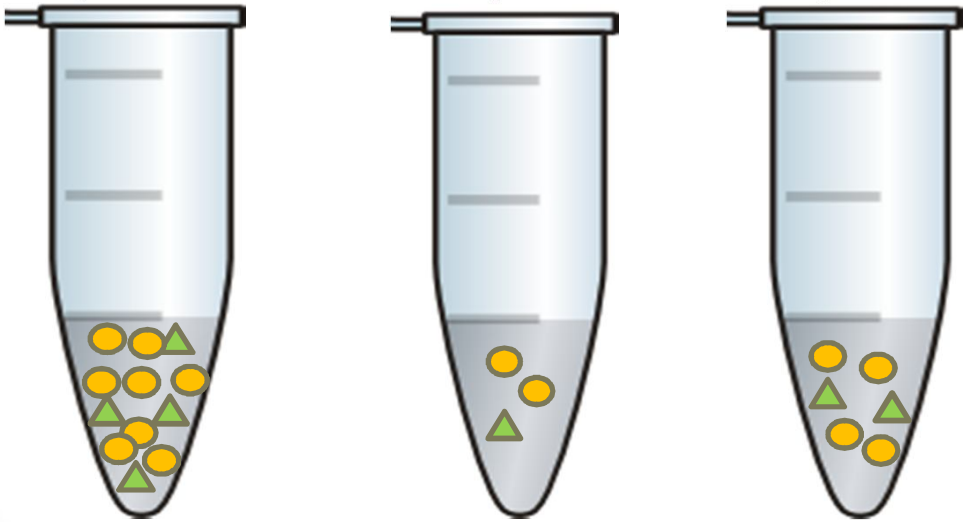
- 1:2 dilution of serum was used

**BDL: Below detectable limit*

**VEH: vehicle*

Serum

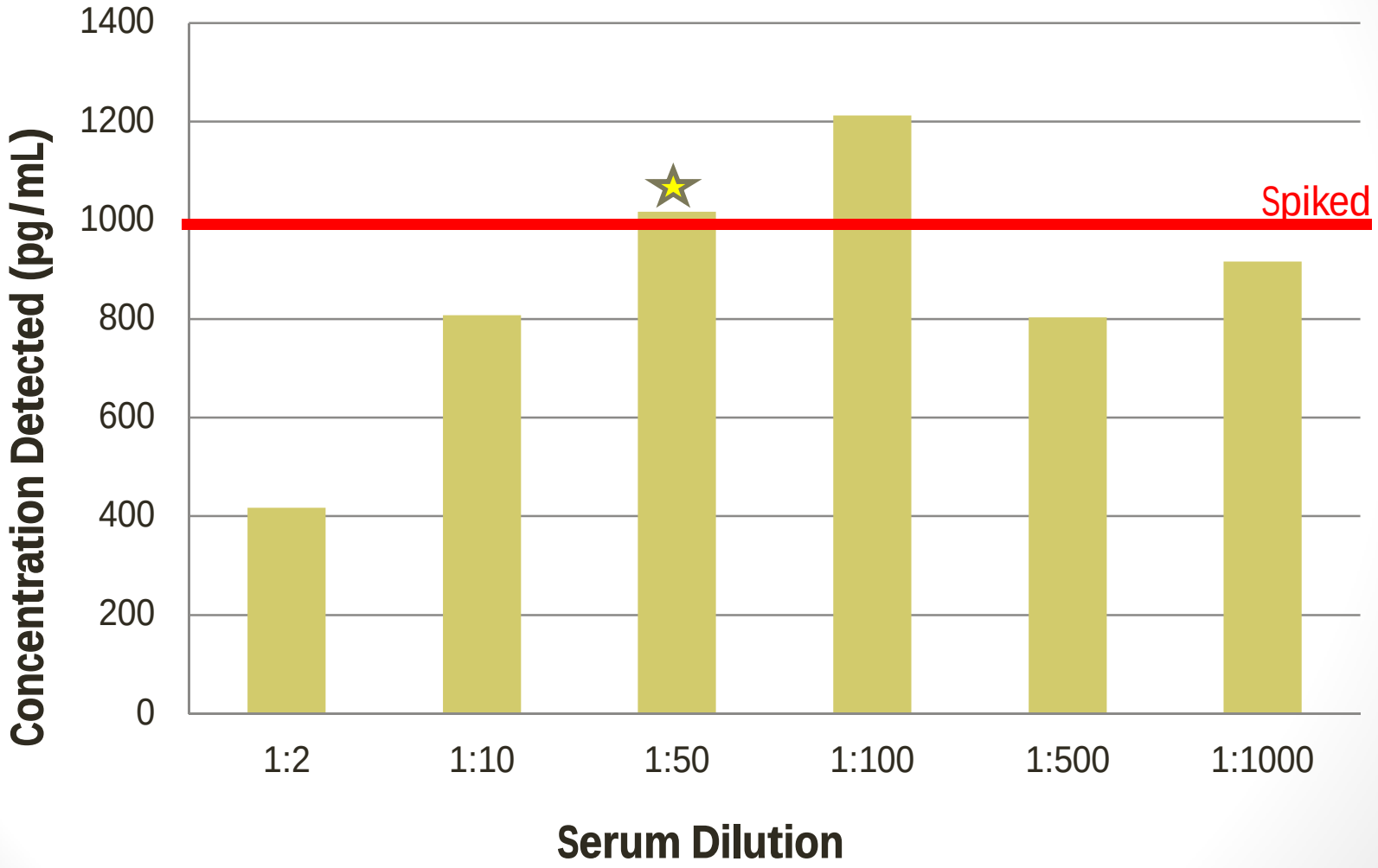
- Naturally, serum contains a considerable amount of proteins and other substances
  - Albumin
  - Electrolytes
  - Hormones
- These proteins can inhibit binding between the target cytokine and its capture antibody.



Troubleshooting- Interference by serum components

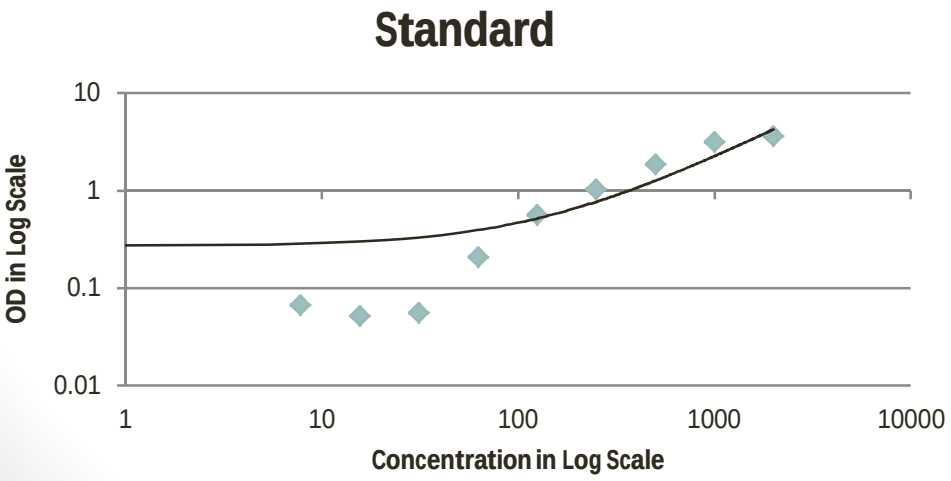
- **Spike-and-recovery:** A known amount of analyte is added into the natural test sample matrix.
  - Serum was diluted: 1:2, 1:10, 1:50, 1:100, 1:500, 1:1000.
  - Each serum dilution was spiked with 1000 pg/mL of IFN-γ.

Troubleshooting Result



IFN-γ ELISA (1:50 dilution)

Treatment	Concentration Detected (pg/mL)			
	8wk	10wk	12wk	15wk
VEH	BDL	BDL	BDL	BDL
TCDD	BDL	BDL	BDL	BDL

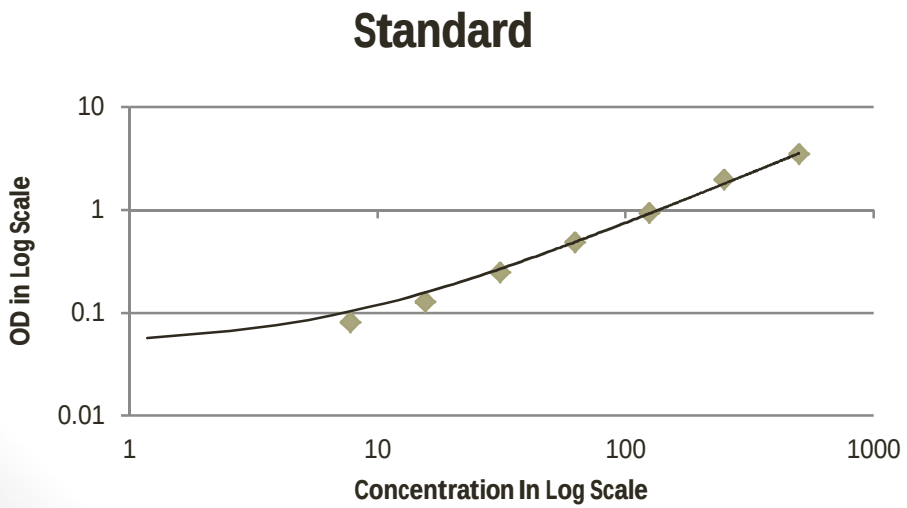


**BDL: Below detectable limit*

**VEH: vehicle*

IL-17 ELISA (1:50 dilution)

Treatment	Concentration Detected (pg/mL)			
	8wk	10wk	12wk	15wk
VEH	BDL	BDL	BDL	BDL
TCDD	BDL	BDL	BDL	BDL

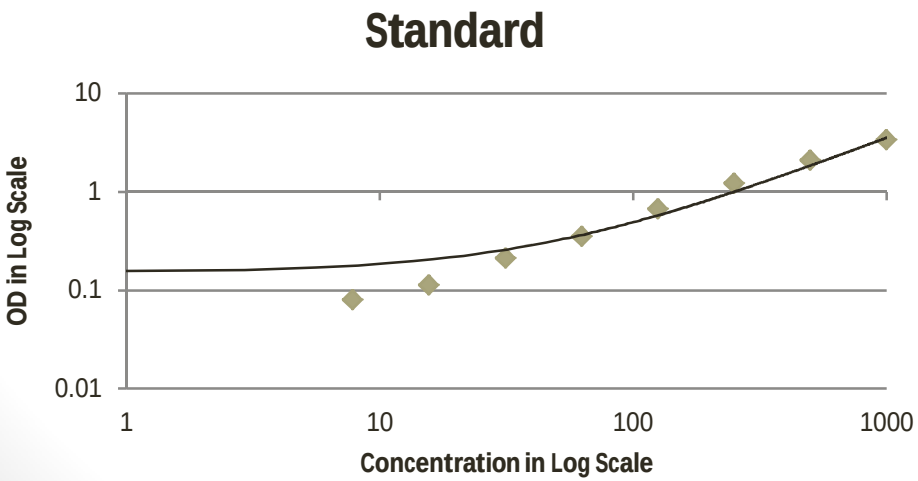


**BDL: Below detectable limit*

**VEH: vehicle*

IL-22 ELISA (1:50 dilution)

Treatment	Concentration Detected (pg/mL)			
	8wk	10wk	12wk	15wk
VEH	BDL	BDL	BDL	BDL
TCDD	BDL	BDL	BDL	BDL



**BDL: Below detectable limit*

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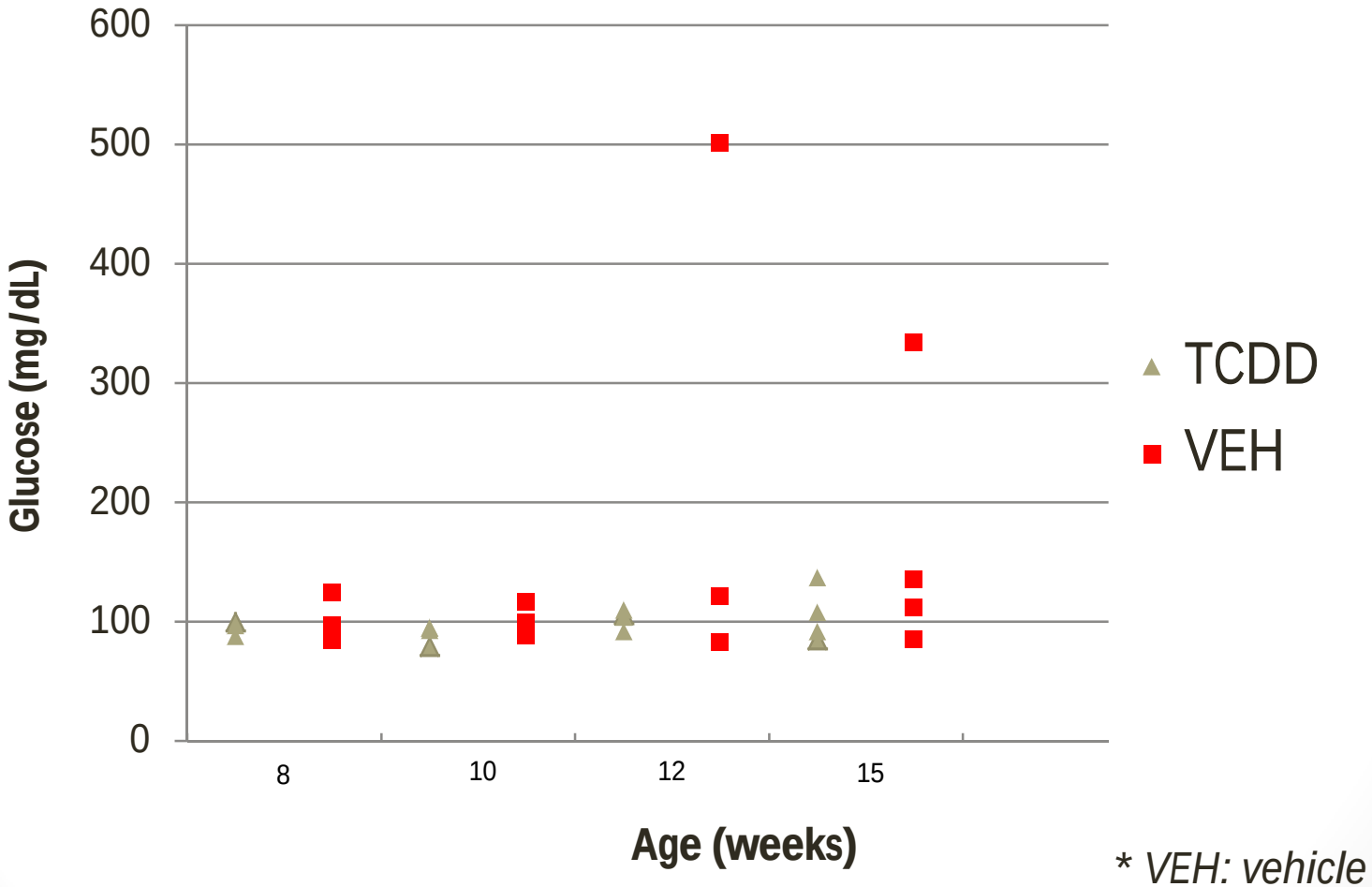
Blood Glucose Level



** 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

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  - Members of Kerkvliet Lab
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- Howard Hughes Medical Institute
- Oregon State University Honors College