Presenter Disclosure Information

In compliance with the accrediting board policies, the American Diabetes Association requires the following disclosure to the participants:

**Martin J. Hessner, Ph.D.**

Disclosed no conflict of interest
The Max McGee Research Center for Juvenile Diabetes

Founded in 1999 and named for our benefactor Max McGee
Wide receiver for the Green Bay Packers from 1954 to 1967
Max and his wife Denise significantly support T1D research because their son has T1D and Max’s brother lived with T1D.

Why Milwaukee?

The diabetes clinic at Children’s Hospital of Wisconsin cares for >1,700 children with diabetes per year
It is one of the largest pediatric treatment programs in the nation.
>90% are T1D patients.

This provides clinical data and samples necessary for quality research
Type 1 Diabetes (T1D) Background

It is a T cell mediated autoimmune disease that targets the insulin producing β cells of the pancreas.

- β cells: anti-insulin (red)
- Leukocytes (T cells): anti CD3 (green)

The cause of this aberrant immune response is not completely understood, but it is a **complex** disease involving both **genetic** and **environmental** factors.
Epidemiology of Type 1 Diabetes

- T1D accounts for 10% of diabetes and affects 1.4 million people in the U.S. & 10-20 million worldwide
- In the U.S. ~30,000 individuals are diagnosed annually with T1D
- T1D can develop at any age; ~50% of patients are diagnosed under age of 20 years (Also Known As: Juvenile Diabetes)
- Overall pediatric incidence in the U.S. (<19 years): 24 / 100,000 / year
- Worldwide incidence of T1D is increasing ~ 2-3% per year = MORE ENVIRONMENTAL PRESSURE
**Genetic Factors**

>40 genes have been identified that convey risk for T1D

Most are related to immune function

HLA (human leukocyte antigen) region conveys the most risk ... >50%

The class II HLA peptides are expressed on macrophages and are important in activating T-cells

~95% of T1D patients have DR3 and/or DR4 class II HLA alleles

HOWEVER ~40% of healthy Caucasians also carry this genotype

Multiple risk loci are needed for T1D progression .... They likely make the immune system hyper-responsive to environmental factors
Viruses have long been considered a trigger for T1D in genetically susceptible individuals. Proof remains elusive:

By serology, T1D patients and “at risk” subjects that have auto-Ab to β-cell antigens show a higher rate of enterovirus infection (coxsackie B1) vs controls.

immuno-staining has detected enteroviral capsid protein at a higher frequency in islets of T1D patients vs controls.
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The microbiome is emerging as an important environmental contact.

The surface area of the G.I. tract is estimated to equal to that of a football field and within the lower G.I. tract are housed trillions of bacteria = microbiome.

G.I. microbiome is comprised of thousands of species and is influenced by diet, antibiotic usage and other variables.

Dysbiosis = an unfavorable biome - may promote intestinal barrier leakage, leading to over-stimulation of the immune system and increased risk of autoimmunity.
Differences in the intestinal microbiome exist between T1D patients and unrelated controls, AND changes in the microbiota occur during T1D progression:

↓ microbial community diversity
↓ phylum Firmicutes (lactobacilli)
↓ reductions in butyrate-producing bacteria (butyrate has important anti-inflammatory properties)

In rodent models of T1D, alterations of the microbiome can be accomplished by:
- dietary alteration (prebiotic)
- feeding of specific bacteria (probiotic)

these manipulations can prevent T1D in rat and mouse models

In human subjects with high genetic risk, probiotic supplementation within the first 27 days of life, was associated with a ~1/3 reduction in the development of anti-β cell autoimmunity during the 1st 10 years of life (JAMA Pediatr. 2016;170(1):20-28)
In genetically susceptible individuals, under the “right” environmental conditions… perhaps dysbiosis + viral infection, T1D is triggered.

Genetic Predisposition

Gene/Environment Interaction

Autoimmunity Develops and beta cell injury begins

Auto-antibodies appear and dysglycemia begins

Onset occurs after a loss of ~80% beta cell mass/function

Followed by “honeymoon”

Long-standing disease

Dependency on insulin replacement

β-cell autoimmunity is induced early in life and progresses over years

Can we predict earlier, before AA development, when β cell mass is high, and a higher likelihood of successful therapeutic intervention?
The Need for BioBanking

We do not know who will develop T1D nor when they will develop it.

β-cell killing occurs for years prior to onset; diagnosis is made when ~80% of the insulin producing function is lost.

Problem: How do we study disease initiation and progression?

Solution: Longitudinally follow families. Most siblings will never develop T1D. However ~6% will, giving us the chance to study events prior to onset.

We have followed nearly 500 families with T1D and collected samples and clinical histories from >3000 individuals.

We have longitudinally captured T1D progression in 12 cases.

Equally important, we have longitudinally followed healthy siblings who have not developed T1D.
Measurement of Autoimmune Activity in T1D is challenging

Relevant tissues (pancreas/PLN) are inaccessible

The β-cells comprise <1% of body mass, so inflammatory mediators (chemokines/cytokines) are generally too dilute in blood samples to measure by traditional methods (e.g. ELISA)

New approaches are needed
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New approaches are needed

We have developed a novel blood test that detects inflammation specific to T1D:

1) that will make it easier to predict who will develop T1D

2) points towards specific inflammatory mechanism/s underlying T1D and possible therapeutic strategies

3) makes it easier to evaluate the effect of drugs being tested in T1D clinical trials
A new approach to detect immune activity

Patient Plasma Sample

Use plasma to stimulate leukocytes from a healthy blood donor

Cells respond to inflammatory factors by turning on genes

This we can SENSITIVELY and COMPREHENSIVELY measure with a microarray

A tool that measures expression of 10,000s of genes simultaneously

The resultant output is called a gene expression profile
What is a gene expression profile?

Humans have ~30,000 genes

Cells under different conditions express different genes

Microarrays tell us which genes have been turned on or off

Study of the gene expression profile allows us to identify disease genes and mechanisms

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Plasma Induced Transcriptional analysis in T1D patients

- T1D plasma induces a unique signature compared to unrelated healthy controls

- It is consistent with exposure to bacterial antigens (e.g. endotoxin)

- It is partially dependent on interleukin-1, a cytokine known to co-stimulate T-cells and cause pancreatic β-cell death in vitro

- The signature partially resolves in long-standing T1D, thus it is associated with active autoimmunity

- It is disease specific

- In longitudinal studies the signature is detected as much as 5 years prior to onset and before the development of AA

Wang et al., JI, 2008, 180: 1929–1937;
Longitudinal Patterns: Progressors: gain inflammation and lose regulation

-2 Fold of Change +2

Non-DR3/4 Progressor

5 years

TTO

Δ↑

Δ↓

0 1 3 3 4 AA Status

Mbnl1
SMAD4
SMURF1
SMCHD1
Cblb
XRCC6
SNRPN
PIGU
POLR2J2
PIAS1
ZBTB11
Esyt2
EEF1G
CLN8
WNK1
PLCL1
LTBP3
SMURF2
STK17A
SKIL
INHBA
IRAK3
PTGS2
EREG
CXCL1
CXCL3
IL1A
CCL4
CXCL2
CCL3
TNFAIP6
IL1B

Regulatory BRAKES

Inflammatory GAS
Longitudinal Patterns: Progressors: gain inflammation and lose regulation
Non–Progressors: gain regulation over time

**Regulatory BRAKES**

- Mbn1
- SMAD4
- SMURF1
- SMCHD1
- Cblb
- XRCC6
- SNRPN
- PIGU
- POLR2J2
- PIAS1
- ZBTB11
- Esyt2
- EEF1G
- CLN8
- WNK1
- PLCL1
- LTBP3
- SMURF2
- STK17A
- SKIL
- SKI
- INHBA
- IRAK3
- PTGS2
- EREG
- CXCL1
- CXCL3
- IL1A
- CCL4
- CXCL2
- CCL3
- TNFAIP6
- IL1B

**Inflammatory GAS**

- CXCL1
- CXCL3
- TNFAIP6
- IL1B

**Fold of Change**

-2 0 1 3 3 3 4

**AA Status**

- 0 0 0 0

**T1D Progressor 5 years**

- Δ↑
- Δ↓
- Δ↓
- Δ↓
- Δ↓

**TTO 5 years**

- Δ↑
- Δ↑
- Δ↑
- Δ↑
- Δ↑

**AA Non-progressor 5 years**

- 0

- 0

- 0

- 0

- 0
Calculation of a composite Inflammatory Index ($I.I_{com}$)

Heat maps can be difficult to interpret and align with other data measures.

A scoring algorithm was developed that was based on gene function.

The algorithm uses regulated probes identified in the cross-sectional study:

$$I.I_{com} = \frac{\text{Average signal intensity of all inflammatory genes}}{\text{Average signal intensity of all regulatory genes}}$$

This allows us to summarize 1000’s of data points to a single value.
T1D progressors exhibit regressions with positive slopes indicative of increasing inflammatory bias
Most auto-antibody negative healthy siblings of T1D patients exhibit plots with negative slopes … indicating decreasing inflammatory bias.

This suggests that inflammation is higher when the subjects are young and management of T1D risk gets better with age .... IS THIS WHY T1D IS JUVENILE???
Most auto-antibody negative healthy siblings of T1D patients exhibit plots with negative slopes indicating decreasing inflammatory bias.

This suggests that inflammation is higher when the subjects are young and management of T1D risk gets better with age. IS THIS WHY T1D IS JUVENILE???

2/12 siblings exhibited plots with positive slopes. Are these future progressors?

Did viral infections destabilize the induction of regulation?
Working Hypothesis:

An elevated inflammatory state, consistent with bacterial antigen exposure, exists in T1D families.

In the presence of high genetic risk, there is temporal induction of regulation.

Diet has changed → gut biome altered → increased intestinal hyper-permeability → heightened systemic inflammation → viral infections can destabilize fledging age-dependent regulatory processes allowing for breaks in tolerance.
Our “DR” Rat model of T1D supports this Hypothesis:

Pathogenesis of diabetes in the DR rat closely resembles human T1D

- MHC (HLA) is largest genetic risk
- Pathogenesis is T cell dependent

DR rats are not spontaneously diabetic but T1D can be induced through viral infection in young but not older rats

Plasma induced signatures and cytokine analyses show maximal inflammatory activity between days 30 and 40

followed by induction of an (IL-10/TGFβ mediated) regulated state

viral induction of T1D only occurs before day 30 when there is high systemic inflammation

Viral infections early in life destabilize induction regulation ... this may parallel what happens in susceptible human subjects.

We have discovered diet alteration can change the biome and normalizes the inflammatory state in young DR rats.
What Have We Learned?

T1D family members, independent of disease progression, have an elevated inflammatory state compared to persons from non-T1D families.

Our rodent studies support that this inflammatory state is genetically controlled but can be environmentally modulated (e.g. diet and microbiome).

In progressors to T1D, increases in inflammation are seen months to years preceding onset.

These changes are captured by plasma induced transcription. We aim to develop this approach as a predictive tool as well as a means to study responses to therapeutic intervention.

In siblings that do not progress to T1D, regulation against the familial inflammatory state increases with age.

This is an important observation that lends insight as to why T1D is often a juvenile onset disease. Can prebiotics or probiotics augment development of this regulated state?
What Have We Learned?

It's about balance.

The increase in T1D incidence has been rapid to have a genetic basis.

Late 20th century lifestyle has likely resulted in a loss of a protective GI biome.

This has increased the proportion of genetically susceptible individuals that will actually progress to clinical onset of T1D.
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**THE DIABETES CLINIC AT CHW**

### External Collaborators

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### Funding Sources

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