Mechanisms involved in the prevention of type I diabetes onset by *Lactobacillus johnsonii* N6.2

**Graciela L. Lorca**

Patent US 9,474,773
Patent US 9,987,313
Type 1 Diabetes

- Immune-mediated destruction of beta cells in the pancreas.
- 1.25M Americans are living with T1D including about 200,000 youth (less than 20 years old) and over a million adults (20 years old and older)
- 40,000 people are diagnosed each year in the U.S.
- 5 million people in the U.S. are expected to have T1D by 2050

- Between 2001 and 2009 there was a 21% increase in the prevalence of T1D in people under age 20.
- $14B T1D-associated annual healthcare costs in the U.S.

Juvenile Diabetes Research Foundation - JDRF

Atkinson et al., The Lancet 2013
The Perfect “storm”

- **Facet 1 - Altered Microbiota**
- **Facet 2 - Leaky Gut**
- **Facet 3 - Altered Mucosal Immunity**

Type I Diabetes

- Beta Cell Destruction
- Pancreatic Islet Inflammation

- Autoimmune Process (e.g., Induction of Autoreactive T cells, Autoantibodies, etc.)

- Abnormal epithelial activation
- Cytokine Release

- Increased permeability to dietary antigens

ARISA analysis showed different microbiota profiles BB-DP and BB-DR rats

Automated ribosomal intergenic spacer analysis (ARISA) profiles of stool samples obtained from 70 days old BB-DP and BB-DR rats

Roesch, Lorca et al., 2009. The ISME Journal
Feeding design using BB-DP animals

- As the animals developed high blood glucose (over 250 mg/dl in two consecutive days) were sacrificed and the organs harvested.
Administration of *Lactobacillus johnsonii* N6.2 post-weaning prevents the onset of T1D in BB-DP rats

*L. johnsonii* N62 (red line)
*L. reuteri* TD1 (blue line)
PBS feed control (black line)
N=10/group

Valladares et al., 2010. *PLoS ONE* 5: e10507
Prevention of T1D is associated with a reduction in oxidative stress

Valladares et al., PLoS ONE 2010
Kingma et al., J. Nutrition 2011
Reduction in oxidative stress is linked to a lower expression of pro-inflammatory cytokines in Ileum, and a Th17 bias in mesenteric lymph nodes.

Lau et al., J. Immunol. 2011

Valladares et al., PLoS ONE 2010

- Ljo fed
- Control Healthy
- Diabetic
Indoleamine 2,3-dioxygenase (IDO)

- Monomeric 46kDa heme-enzyme
- Catalyzes first step in tryptophan degradation outside the liver
- High levels of expression in the intestinal tract
- Expression further induced by IFNγ
- Ubiquitously expressed
  - High expression in pDCs, macrophages
- Implicated in control of inflammation
- Antimicrobial activity

![Diagram of IDO enzymatic reaction]

L-Tryptophan → N-Formylkynurenine
L. *johnsonii* Decreases Intestinal IDO Expression in BBDP Rats

Valladares et al., 2013. *FASEB J.* 27, 1711–1720
**L. johnsonii N6.2 Produces Elevated Quantities of H$_2$O$_2$ That Inhibit IDO Activity (in vitro)**

![Graph showing the production of H$_2$O$_2$ over time](image1)

![Bar chart comparing catalase activity and H$_2$O$_2$ concentrations](image2)

**L. johnsonii-fed Rats Have Elevated H$_2$O$_2$ in Intestinal Lumen Contents**

![Bar chart showing H$_2$O$_2$ levels in various intestinal sections](image3)

* $p < 0.05$

Valladares et al., 2013. *FASEB J.*
**L. johnsonii** Alters Intestinal and Peripheral Levels of the Alternative Tryptophan Product 5-Hydroxytryptamine (Serotonin)

Valladares et al., 2013. *FASEB J.*
Brain-gut axis: systemic consequences

Valladares et al., 2013. *FASEB J.*
Towards the development of a microbiome-based preventive therapy

Clinical Trial – Phase 1
Healthy subjects: 18-50 yrs

Clinical Trial – Phase 2
T1D: 8-18 yrs

Clinical Trial – Phase 3
Preventive

Proof of concept
Study design: Safety trial

**Group 1:** *L. johnsonii* N6.2
**Group 2:** placebo (dried milk)

- **Baseline**
- **Blood Draw and Stool collection**
- **Blood Draw**
- **Wash-out**

**Daily questioner:** assessment of side effects
**Weekly questioner:** Quality of life and mood

**Blood samples:**
- Hemogram and chemistry profiles
- Immunophenotyping
- Tryptophan-derivatives (Metabolomics)

**Stool samples:**
- *L. johnsonii*: transit/survival
- Microbiome

**L. johnsonii** N6.2 promotes a significant decrease in daily questionnaire syndrome scores

- Gastrointestinal distress syndrome: bloating, flatulence, stomach noises, abdominal cramps.
- Epidermal syndrome: itching, skin rash, skin redness/flushing.
- Cephalic syndrome: headache and dizziness.
- Ear-Nose-Throat syndrome: sore throat, runny eyes, nasal congestion, and blocked ear canal.
- Psychological syndrome: anxiety, depression and stress.
- Emetic syndrome: nausea and vomiting.

**L. johnsonii** N6.2 administration modifies Kynurenine/tryptophan ratios


Evaluation of circulating leukocyte subpopulations

Monocytes and NK cells increase upon *L. johnsonii* N6.2 supplementation.

Modifications on the T cell subset upon *L. johnsonii* N6.2 supplementation

Effect of *L. johnsonii* N6.2 on T effector cells subset

Circulating IgA increased in the *L. johnsonii* N6.2 treated group

No changes observed in IL6, or CD25
Below detection limit: INFα and IL2

The L. johnsonii N6.2 preparation was well tolerated with no risks for healthy subjects.

L. johnsonii N6.2 does not induce changes in the microbiota of healthy subjects

L. johnsonii N6.2 results in significant differences in GSRS ratings
  - Reduction of gastrointestinal distress syndrome
  - Reduction of cephalic syndromes

L. johnsonii N6.2 modulated the concentration of metabolites in the tryptophan pathway

L. johnsonii N6.2 increased the amount of monocytes and NK cells as well as circulating effector Th1 cells and cytotoxic CD8+ T cells.

Towards the development of a microbiome-based preventive therapy

Clinical Trial – Phase 1
Healthy subjects: 18-50 yrs

Clinical Trial – Phase 2
T1D: 8-18 yrs

Clinical Trial – Phase 3
Preventive

Proof of concept
Focus on mechanism

Valladares et al., 2013. FASEB J. 
Texeira et al. 2018. Benef. Microbes 
Graciela Lorca

Guillermo Marcial PhD
Natalie Harrison
Chris Gardner
Dan Cai PhD

Wendy Dahl
Amanda Ford

Claudio Gonzalez
Leandro Dias
Danielle Kling
Ricardo Valladares

Julie Mayer

Michael Haller
Salvador Gezan
Mark Atkinson
Clive Wasserfall
Kieran Mcgrail
Amanda Posgai

Todd Brusko
Daniel Perry PhD

Timothy Garrett
Desmond Schatz
Josef Neu
Eric Triplett