Targeting the Trimolecular Complex for Immune Intervention

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Take Home Points

• Type 1 diabetes is an immunologic disease.
• It is a predictable disease increasing in incidence.
• Maintaining beta cell function at the time of diagnosis is a goal.
• Safe & specific immune therapies are needed for prevention and ultimately a cure.
T1D incidence is rising 3-5% per year

Due to environmental cause(s)?

Incidence /100,000/ yr in children aged 0-14

Courtesy of Marian Rewers
Type 1 Diabetes is Predictable (>2 positive islet autoantibodies)
Type 1 Diabetes

- Immune mediated
- Increasing Incidence
- Predictable
A New Molecular Target for Type 1 Diabetes
Genome-wide Associations in Type 1 Diabetes

Concannon et al
NEJM 2008
The Major Histocompatibility Complex

Human
Chromosome 6

Class II

DP
DQ
DR

Class III

B
C
A

Class I

Allele:
DRB1*0401

Haplotype:
DRB1*0401
DQB1*0302

Genotype:
DRB1*0401
DQB1*0302
DRB1*0301
DQB1*0201
T cell Recognition of Antigen on an APC

- Antigen
- Endocytosis
- APC
- MHC II
- Peptide
- CD4+ T cell
- T Cell Receptor
- Trimolecular Complex
The Trimolecular Complex

TCR

MHC

PEPTIDE

INS B:9-23

AA Peptide Side Chains

MHC

Peptide
Approach 1: Using small drug-like molecules to prevent diabetes
Screening Small ‘Drug-like’ Molecules with a Supercomputer

Test high-scoring molecules

Library of small molecules

Target protein

Hits

Test all molecules

Methyldopa (Aldomet)

- Clinically well-established drug used to treat hypertension & pregnancy induced hypertension for the last 50 years.
- Pregnancy class B.
- Blocks DQ8 antigen presentation.
Methyldopa Blocks Insulin Presentation

![Graph showing the effect of Methyldopa concentration on T cell response in IL-2 (pg/ml).](image)

- **Background**
- **Insulin**
- **$10^{-2}$**
- **$10^{-1}$**
- **$10^{-10}$**
- **$10^{-9}$**
- **$10^{-8}$**
- **$10^{-7}$**
- **$10^{-6}$**
- **$10^{-5}$**
- **$10^{-4}$**
- **$10^{-3}$**

**Methyldopa Concentration (Molar)**

**T cell response in IL-2 (pg/ml)**

- Background: 0
- Insulin: 200
- $10^{-2}$: 150
- $10^{-1}$: 100
- $10^{-10}$: 50
- $10^{-9}$: 25
- $10^{-8}$: 12.5
- $10^{-7}$: 6.25
- $10^{-6}$: 3.125
- $10^{-5}$: 1.5625
- $10^{-4}$: 0.78125
- $10^{-3}$: 0.390625

The graph shows a decrease in T cell response in IL-2 (pg/ml) as Methyldopa concentration increases.
Methyldopa Blocks Insulin Presentation in Mice

DQ8 Mice → Splenocytes or Blood → Culture with insulin & T cell → Readout

![Diagram showing the process of methyldopa blocking insulin presentation in mice.]

- **Drug**
- **DQ8 Mice**
- **Splenocytes or Blood**
- **Culture with insulin & T cell**
- **Readout**

![Bar chart showing DQ8 presentation (arbitrary units) for different insulin concentrations.]

- **Control**
- **Methyldopa**

**Insulin Concentration**
- 165μM
- 82.5μM
- 8.25μM
Phase 1b Clinical Trial: Methyldopa in Recent Onset Type 1 Diabetes

• Inclusion Criteria:
  – Ages 18-46
  – Type 1 diabetes < 2 years duration
  – HLA-DQ8 present
  – Residual insulin production (C-peptide > 0.1ng/mL)

• Open label, single-center, n=20 participants

• Phase 1b dose escalation study (NCT01883804):

<table>
<thead>
<tr>
<th>Baseline</th>
<th>500mg BID</th>
<th>500mg TID</th>
<th>1000mg TID</th>
<th>Stop Therapy, Follow up</th>
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<td>0</td>
<td>1</td>
<td>3</td>
<td>6</td>
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</table>

Primary Outcome: Antigen Presentation Assay

- HLA-DQ8 T1D Patients
- Blood
- Culture with antigen
- Engineered T cells
- Readout for each T cell
Targeting Specific MHC Class II Peptide Presentation

Autoreactive T cell

Autoimmune Response

No activation

Normal Immune Response

Activation
Second Approach

Why do people not develop type 1 diabetes?

How do protective HLA alleles provide protection from diabetes?
Approach 2: Insulin vaccine to prevent diabetes

Goal:
Insulin

Back to the Hotdog Stand

Non-Diabetic

Diabetic
A novel insulin B chain peptide
New-Onset T1D patients respond to the insulin peptide

![Graph showing IFN-γ Elispots per 10^6 PBMCs for different conditions and genotypes.](image-url)
Non-diabetic Controls respond to the insulin peptide
Novel Insulin B chain peptide induces protective responses in mice & humans
Develop a vaccine to return the insulin immune response back to normal

Goal:
Summary

• The trimolecular complex plays a pivotal role in T1D development.

• HLA-DQ8 confers genetic risk for T1D and is remarkably amenable to small molecule targeting.

• Understanding the molecular basis for insulin specific T cell activation may lead to safe & specific prevention therapies for T1D.
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