Chapter 10

Humoral Autoimmunity
DIPP Populations Study: Quartile Levels Insulin Autoantibodies (6 month post first IAA) and progression to Diabetes
Parrika et al Diabetologia 2012
Non-Radioactive Electrochemiluminescent Assay for Insulin Autoantibodies

Yu et al Diabetes 61:179, 2012
Major Islet Autoantigens

- ZnT8
- IA-2 (ICA512BDC)
- GAA (GAD_{65})
- IAA
- Insulin autoantibodies

ICA? GAA (GAD_{65}) IA-2 (ICA512BDC)
Rituximab Selectively Suppresses Specific Islet Antibodies
Trialnet-Yu et al Diabetes 2011
Islet autoantibodies can discriminate maturity-onset diabetes of the young (MODY) from Type 1 diabetes. 


GAD and IA-2 measured:

MODY (GCK=227, HNF1A=229, HNF4A=52 patients

5/508 (1%) positive, all GAD only

Type 1 (n=98)

82% Positive
Dietary Intervention in Infancy and Later Signs of Beta-Cell Autoimmunity
Knip et al NEJM 2010

![Graph showing cumulative survival without ≥2 autoantibodies in Casein hydrolysate and Control groups over age (yr)]

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>Casein hydrolysate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>95</td>
</tr>
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</table>
Development of Autoantibodies in the TrialNet Natural History Study
Vehik et al Diabetes Care 2011

Development of autoantibodies in TrialNet

Diagram:
- Cum. Ab+ Seroconversion (%) vs. Annual Rescreen
- Two lines: <10 years and ≥10 years
• DR3 associated with GAD antibody positivity of adults with Type 1 diabetes but absence of IA-2 autoantibodies.
• DR4 associated with IA-2 positivity and younger age of onset as was DR3/4 heterozygotes.
HIGH LEVELS INSULIN AUTOANTIBODIES DAISY STUDY PROGRESSION TO DM
FIRST DOT: AGE ANY ANTIBODY APPEARED/ SECOND: DM AGE
Steck et al Diabetes Care 2011
Age of Islet Autoantibody Appearance and Mean Levels of Insulin, but Not GAD or IA-2 Autoantibodies, Predict Age of Diagnosis of Type 1 Diabetes
Diabetes Autoimmunity Study in the Young

LOW LEVELS INSULIN AUTOANTIBODIES DAISY STUDY PROGRESSION TO DM
FIRST DOT: AGE ANY ANTIBODY APPEARED/ SECOND: DM AGE
MEAN LOG IAA vs Time to DM from age Islet Ab first +

R² = 0.37  P < 0.0001

Steck et al
Diabetes Care 2011
GAD65 Levels with Years to Diabetes  ICA512 Levels with Years to Diabetes

Steck… Diabetes Care 2011
Age of Islet Autoantibody Appearance and Mean Levels of Insulin, but Not GAD or IA-2 Autoantibodies, Predict Age of Diagnosis of Type 1 Diabetes

Diabetes Autoimmunity Study in the Young

Steck et al Diabetes Care 2011

Diabetes Predicted Age =
2.6 - 1.3*\log(\text{mean IAA}) + 0.8*\text{age first Ab+}

P<0.0001
r=0.79
Mean Insulin AutoAb levels by INS genotypes

N = 25
Mean= 0.096
SD = 0.159

13
0.025
0.045

P=0.027

Steck et al
Diabetes Care 2011
Enhancing Prediction Diabetes with ZnT8 Autoantibody Determination

• N=88 Children DAISY prospective study
  + Only one AutoAb (of GAD/IA-2/Insulin)
  >3 Yrs age and >1 year Follow-up
  ZnT8 Antibodies measured first Ab+ sample

• 14% also ZnT8 Antibody Positive (Thus >=2Ab)
  ZnT8+ 37% (7/19) Progressed to Diabetes
  ZnT8-  7% (5/69, P<.003) Progressed

Wenzlau et al PNAS 104:17040-17045, 2007
DAISY subjects with only 1 Ab (GAD/IA-2/Insulin) vs + ZnT8 Autoantibodies

\[
\begin{array}{ccccccc}
\text{D} & 69 & 49 & 32 & 17 & 7 & 2 \\
& 19 & 16 & 11 & 6 & 4 & 1 \\
\end{array}
\]

\[
\text{Non diabetic (%)}
\]

\[
\begin{array}{c}
0.0 \quad 2.5 \quad 5.0 \quad 7.5 \quad 10.0 \quad 12.5 \\
\text{Follow up (yr)}
\end{array}
\]

\[P=0.007\]

Wenzlau PNAS 2007
Receiver Operator Characteristics of the ZnT8 antibody assays
ZnT8 detects autoantibodies in patients who are negative for ICA and gold standard biochemical antibodies.

\[
\begin{align*}
n &= 32 & 215 \pm 32 \text{ cpm} \\
n &= 148 & 20\% + \text{ve} \\
n &= 24 & 0\% + \text{ve}
\end{align*}
\]

J.M. Wenzlau, H.W. Davidson, and J.C. Hutton
Value of 4 antibodies

Sera from 223 newly diabetic individuals were assayed for reactivity to insulin, GAD65, and IA-2 ± ZnT8. Nine individuals were negative for insulin, IA-2, and GAD65 but positive for ZnT8 autoantibodies, increasing the percentage of sera positive for at least 1 autoantibody from 94% to 98%. In addition 26 individuals (11.7%) were re-classified from single to multiple autoantibody positivity on the basis of ZnT8 autoreactivity.

J.M. Wenzlau, H.W. Davidson, and J.C. Hutton
Insulin autoantibodies

ZnT8

ICA

?IA-2
(ICA512BDC)

GAA
(GAD_{65})
mIAA

Wenzlau et al PNAS 104:17041-17045, 2007
**Zinc Transporter (Znt8) Autoantibodies**

Pos = 63%

Follow up analysis (Wenzlau et al PNAS 104:17040, 2007) new onset patients
Znt8 Islet Autoantigen

- ZnT8 cation efflux transporter (Slc30A8)
- Approximately 60% of prediabetic and new onset patients express autoantibodies to C-terminus (amino acids 268-369) with fluid phase radioassay
- Beta cell specific molecular target
- Autoantibodies usually appear post mIAA and GAD65 AA in children followed from birth to type 1 diabetes.

Wenzlau et al PNAS 104:17040-17045, 2007
Stages in Development of Type 1 Diabetes

1. **GENETICALLY AT RISK**
2. **GENETIC PREDISPOSITION**
3. **INSULITIS**
4. **BETA CELL INJURY**
5. **“PRE”-DIABETES**
6. **LOSS OF FIRST PHASE INSULIN RESPONSE**
7. **MULTIPLE ANTIBODY POSITIVE**
8. **NEWLY DIAGNOSED DIABETES**
9. **DIABETES**

J. Skyler
Diabetes Classification

- Type 1A: Immune Mediated
  Type 1B: Insulin deficient, no autoantibodies
- Type 2: No Autoantibodies, Can initially be treated without insulin
- Other Specific forms of Diabetes
- Gestational Diabetes
Cytoplasmic ICA kindly provided by the discoverer Franco Bottazzo
Inhibition of NOD Diabetes in Absence of Transplacental Antibodies (Ab)

- IgM Knockout
- Anti-HEL+KO
- DBA/2 Foster Mother
- SCID Mother

Control  No Maternal Ab
“Biochemical” Autoantibody Assays

- Insulin
- Glutamic Acid Decarboxylase
- ICA512 (IA-2)
ROC: IA-2 Full Length (DASP2002- BDC)
100 Controls, 50 New Onset DM

Percent

IDS Units

-50 150 350 550 750

-50 150 350 550 750

Sensitivity

Specificity
ROC of mIAA (DASP2002-BDC)

ROC = Receiver Operator Curve

Percent vs. Index

Sensitivity
Specificity
New Onset Children with Diabetes Seen at the Barbara Davis Center

Percentage Autoantibody Negative by Ethnicity

- African Am: 60%
- Hispanic: 38%
- Caucasian: 7.8%

$p<.0001$

Babu et al. 2,001
## Caveats of Autoantibody Testing

<table>
<thead>
<tr>
<th>Caveat</th>
<th>Suggestion</th>
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<tbody>
<tr>
<td>Assays Vary sensitivity/specificity</td>
<td>Cytoplasmic ICA not used Biochemical AutoAb in Proficiency: spec&gt;=99th</td>
</tr>
<tr>
<td>Insulin Abs induced by insulin injections</td>
<td>Post &quot;2 weeks&quot; do not use insulin Ab assay</td>
</tr>
<tr>
<td>IAA children; GAD adults</td>
<td>Multiple Abs</td>
</tr>
<tr>
<td>Single Ab Low Risk</td>
<td>Multiple Abs</td>
</tr>
<tr>
<td>Subset No Ab</td>
<td>HLA/Insulin/Autoimmunity</td>
</tr>
<tr>
<td>Abs appear any age</td>
<td>Measurement over time</td>
</tr>
<tr>
<td>Transient Abs possible; &quot;Sera&quot; mistakes</td>
<td>Check more than once</td>
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</table>
Similar “rules” Other Autoimmune Disorders

- Addison’s Disease (DQ8/DQ2 DRB1*0404) 21-hydroxylase Autoantibodies
  30/2,100 type 1 pts (1.5%)
  5/30 Addison’s first Test (1/400 patients)

- Celiac Disease (DQ2 or DQ8) Transglutaminase Autoantibodies
  98/847 type 1 pts (12%)
  15/20 Biopsies Positive/Estimate 1/20 celiac
  1/3 DQ2/DQ2 Transglutaminase +
21-Hydroxylase Autoantibodies

Levels of autoantibodies

Yu et al, JCEM 84:328-335, 1999

Figure 2
Percent 21-OH Autoantibody Positive/ Patients with type 1 DM

Yu et al, JCEM 84:328-335, 1999

BDC
Prevalence of Transglutaminase Autoantibodies by HLA-DR

Prevalence

FPIR in pre-diabetic relatives with initial FPIR > 50mU/L

Melbourne Pre-Diabetes Study (Colman PG & Harrison LC)
## Contrasting Insulin and GAD as Primary Antigen type 1 DM

<table>
<thead>
<tr>
<th>KNOCKOUT/Alter GENE SEQUENCE</th>
<th>INSULIN</th>
<th>GAD</th>
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<tbody>
<tr>
<td>Prevent NOD DM</td>
<td>NO</td>
<td>YES</td>
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<tr>
<td>GAD65 no effect</td>
<td>YES</td>
<td>NO</td>
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<tr>
<th>ANTI-SENSE</th>
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<tr>
<td>N/A</td>
<td>Decrease DM 2/6</td>
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<tr>
<th>Ab LEVEL correlate rate to DM</th>
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<td>NO</td>
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<tr>
<th>High Affinity Ab-NOD</th>
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<tbody>
<tr>
<td>YES</td>
<td>Not Detected</td>
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<table>
<thead>
<tr>
<th>High Affinity Ab-Man</th>
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<tr>
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<th>Early T-Cell NOD</th>
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<table>
<thead>
<tr>
<th>Protection with Ag-NOD</th>
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<th>Induction Diabetes TCR</th>
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<tr>
<th>T-Cell Response Man</th>
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<td>YES</td>
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</table>
Anti-Insulin Abs: Insulin Turnover

Insulin Autoantibodies Versus Age of Diabetes Onset

Diabetes Care 11:736-739, 1988
IAA assay

High Throughput Anti-Insulin Autoantibody Assay

1. Mix \((125)\text{I-insulin and sera}\)
2. Incubate 72 hours at 4°C
3. Add Protein A/G-Sepharose to reaction mix in a 96-well filtration plate
4. Incubate for 45 min at 4°C
5. Wash each well using the vacuum-operated 96-well plate washer
6. Count radioactivity with 96-well plate beta counter
Insulin Autoantibodies: A Chain L13

Insulin Receptor Binding Region

BDC
Mature high-affinity immune responses to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes. Achenbach et al, J.Clin Invest 2004, 114:589
96-Well Plate Micro-IAA Assay for Mouse Serum

Yu et al. PNAS: 97:1701-1706, 2,000
96-Well filtration Plate IAA Radioassay

Yu et al. PNAS: 97:1701-1706, 2,000
RC Curve of mIAA

Yu et al. PNAS: 97:1701-1706, 2000
The Levels of mIAA in Prediabetic Children

Yu et al. PNAS: 97:1701-1706, 2,000
Yu et al. PNAS: 97:1701-1706, 2000
NOD Mice Divided by IAA Appearance

Age Insulin Autoantibodies first Appeared

- 8 wks +
- 12,16 +
- >=20+ or -

P<.001
Rapid induction of Insulin Autoantibodies by Insulin B:9-23 peptide immunization in Normal BALB/c mice

IAA (index)

B:9-23+ IFA

weeks

B:9-23+ IFA
Dynamic Changes GAD65 Autoantibody epitope Specificities...
Schlosser et al, Diabetologia 48:922, 2005

• Analysis competition with recombinant monoclonals to GAD of prediabetic children.
  -- No difference epitopes initial sample
  -- High risk children emergence of antibodies to conformational N-terminus and middle region
  -- For high risk but not low risk children binding to N-terminus and middle region increases
Domains/Splice Variants
ICA512

1. **LUMINAL DOMAIN**

2. **CYTOPLASMIC**

   - **PTP domain**: 577-600
   - **Transmembrane**
   - **Mini**: 930-978

1. **ICA512**

2. **ICA512bdc**

   - **Alternative Splice minus 557 to 629 (73 aa)**

   - **BDC**

   - **Min**: 930-978

   - **Max**: 1576-1979
ICA512 (IA2) Fragments

**ICA512ic**

- **F1**: 1-256, 577-600, 605-979
- **ICA512bdc**: 256-556, 630-979
- **ICA512ic**: 605-979

**Positivity**

- **DM**
  - ICA512ic: 90%
  - ICA512bdc: 98%
  - ICA512ic: (100%)

- **Transient**
  - ICA512ic: 30%
  - ICA512bdc: 10%
  - ICA512ic: 0%

---

Modified from Miao et al. J. Autoimmunity 2002 with F1= Full Length IA-2 BDC
IA-2 mRNA Expression

370 bp, Regular mRNA
151 bp, Alternatively Spliced mRNA

Thymus fetal tissues (21-27 weeks), adult pancreas.
Pugliese et al.
GAD and ICA512 Abs: 71,000 DPT Screening Samples

- Sibling: n=27,128
- Offspring: n=17,063
- Parent: n=15,561

Yu et al, NYAS 2002
GAD and ICA512 Abs:
71,000 DPT Screening Samples

Yu et al, NYAS 2002
GAD and ICA512 Abs: 71,000 DPT Screening Samples

Yu et al, NYAS 2002
DPT-1: Percent GAD or ICA512+
High % Eligible or Diabetic Biochemical Ab+ of Cytoplasmic ICA+ Relatives

Yu et al, Diabetes 50, 2001
Percent of 71,148 DPT Screening Samples
GAD/ICA512/Cytoplasmic ICA+

Yu et al. Diabetes 50: 2001
### DAISY Study Population

#### General population families

- **enrolled** = 108 high risk - \( DR3/3 \)
- 545 moderate risk - \( DR3/x, 3/4 \)
- 553 low risk
- 1,206 All

#### Families with type 1 diabetes

- **screened** = 21,000 patients
- **infants**
  - Diabetic: 96
  - Non-diabetic: 15
  - 607
  - 230
  - 650
  - 415
  - 1,353
  - 660

Rewers et al
DAISY Interviews and Clinical

Interviews: diet, infections, immunizations, allergies, stress

Visits: B, 3m, 6m, 9m, 1y, 15m, 2y, 3y

Clinical Visits: blood sample for GAA, IAA, ICA512, ICA DNA, throat and rectal swabs, saliva sample

Rewers et al
The Age at Autoantibody Conversion in DAISY AAb+ Siblings

Percent BabyDiab (Offspring) Autoantibody Positive at age 5 years
HLA and Insulin Gene VNTR

Progression to Diabetes vs Number of Autoantibodies (GAD, ICA512, Insulin)

Percent not Diabetic

Years of Follow-up

3 Abs  n =  41  
2 Abs  n =  44  
1 Abs  n =  93

Verge et al, Diabetes 45:926-933, 1996
Lack of Progression to DM of ICA+ 0602+ Relatives

Pugliese et al.
Islet Autoantibodies are rapid responders to stimulus  
- rises in GADA immediately post-islet tx

Bosi et al, Diabetes, 2001