M1 - Immunology, Winter 2008

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http://hdl.handle.net/2027.42/64939
The objectives of this lecture are to understand:

1. The Pathogenesis of Autoimmune Diabetes (Type 1A diabetes)
2. The role of T cells in Disease Pathogenesis
3. The role of Cytokines in Disease Pathogenesis
4. The role of Islet Autoantibodies
Diabetes Mellitus

A systemic disease with multiple metabolic abnormalities, chief among which is an elevation in plasma glucose.

In addition to the primary defect in carbohydrate metabolism defects in lipid metabolism are widespread, with elevations in plasma FFA and TG, and, in some circumstances, of ketones.
The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus

I. Type 1 diabetes
   A. Immune mediated
   B. Idiopathic

II. Type 2 diabetes

III. Other specific types
Regulation of Plasma Glucose

\[ \text{Glucose Production} \rightarrow \text{Steady State Plasma Glucose} \rightarrow \text{Glucose Disposal} \rightarrow \text{Peripheral Tissues} \]

- \( \beta \) Cells
- Liver
- Peripheral Tissues
Regulation of Plasma Glucose

Glucose Production

Liver

Steady State Plasma Glucose

Insulin

Glucose Disposal

Peripheral Tissues
Diabetes Mellitus- Type 1

Increased thirst (polydypsia)
Increased urination (polyuria)
Increased appetite (polyphagia)
Weight loss
Fatigue
Rapid, early onset (before age 15)
## Differences Between Type 1 and Type 2 Diabetes

<table>
<thead>
<tr>
<th></th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of onset</strong></td>
<td>Young</td>
<td>Older</td>
</tr>
<tr>
<td><strong>Type of onset</strong></td>
<td>Acute</td>
<td>Insidious</td>
</tr>
<tr>
<td><strong>Genetic background</strong></td>
<td>HLA related</td>
<td>Not HLA related</td>
</tr>
<tr>
<td><strong>Islet cell antibodies</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Insulin secretion</strong></td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Nutritional status</strong></td>
<td>Thin</td>
<td>Obese</td>
</tr>
<tr>
<td><strong>Insulin dependence</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Insulin resistance</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Responsiveness to Orals</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Ketosis proneness</strong></td>
<td>Yes</td>
<td>No</td>
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Relative Proportions of Types 1 & 2 DM

- Type 2: 95%
- Type 1: 5%
Type 1 diabetes (IDDM)

Chronic autoimmune disease with juvenile onset, but may develop in adults as well as elderly (LADA).

Polygenic disease
- Strong MHC linkage
- Non-MHC genes

Autoimmune etiology
- Antibodies to islet autoantigens
- Autoreactive T cells

Immune-modulation alters the course of disease
- Antigen vaccination
- General immunosuppression
Stages in Development of Type 1 Diabetes

- **Genetically at Risk**
- **Multiple Antibody Positive**
- **Loss of First Phase Insulin Response**
- **Beta Cell Mass**
  - Genetic Predisposition
  - Insulitis (Beta Cell Injury)
  - "Pre"-Diabetes
  - Diabetes

Newly Diagnosed Diabetes

G. Eisenbarth, NEJM, 1986
Type 1 diabetes: a chronic inflammatory disease of the islets

Genetic Susceptibility
### Empiric risk of developing Type 1 diabetes

<table>
<thead>
<tr>
<th>Relative Type</th>
<th>Empiric Risk</th>
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</thead>
<tbody>
<tr>
<td>First degree relatives of T1DM probands*</td>
<td>5-7%</td>
</tr>
<tr>
<td>Individuals without relatives with T1DM*</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Children of affected father**</td>
<td>~6%</td>
</tr>
<tr>
<td>Children of affected mother**</td>
<td>~2%</td>
</tr>
</tbody>
</table>

These estimates are for North American Caucasian* and Scandinavian populations**
The Wellcome Trust Case Control Consortium (WTCCC) primary genome-wide association (GWA) scan in T1DM

HLA

Human Leukocyte Antigen

human MHC

cell-surface proteins

important in self vs. nonself distinction

present peptide antigens to T cells

CLASS I: A, B, C

CLASS II: DR, DQ, DP
The Human Leukocyte Antigen Complex (6p21.31)

Class II (1.1 Mb)
- DP
- DQ
- DR

Class III (0.7 Mb)
- B
- C
- A

Class I (2.2 Mb)

Recombination
- Frequent Recombination
- Recombination is Rare
- Recombination is Rare
- Complement and Cytokines
- Class I-like genes and pseudogenes
MHC Haplotype Sharing Increases DR3/4 Sibling Risk

Haplotype Determination:

**Siblings Share**
- **Both** Haplotypes (Family A)
- **One** Haplotype (Family B)
- **No** Haplotype (Family C)

**Siblings Share Both Haplotypes** (Family A)

- Haplotypes: A
  - HLA-A: 12
  - HLA-DRB1: 34
- Haplotypes: B
  - HLA-A: 22
  - HLA-DRB1: 46

**Diabetic Proband**: HLA-A: 1, 29; HLA-DRB1: 3, 4

**DAISY Sibling**: HLA-A: 1, 29; HLA-DRB1: 3, 4

**Siblings Share One Haplotype** (Family B)

- Haplotypes: A
  - HLA-A: 2
  - HLA-DRB1: 4
- Haplotypes: B
  - HLA-A: 68
  - HLA-DRB1: 8

**Diabetic Proband**: HLA-A: 2, 30; HLA-DRB1: 4, 3

**DAISY Sibling**: HLA-A: 2, 30; HLA-DRB1: 4, 3

**Siblings Share No Haplotype** (Family C)

- Haplotypes: A
  - HLA-A: 2
  - HLA-DRB1: 1
- Haplotypes: B
  - HLA-A: 1
  - HLA-DRB1: 3

**Diabetic Proband**: HLA-A: 1, 31; HLA-DRB1: 3, 4

**DAISY Sibling**: HLA-A: 2, 31; HLA-DRB1: 3, 4
MHC haplotype sharing increases risk in DR3/4-DQ8 siblings

% Autoantibody Positive
- Share 2
- Share 0 or 1

% Diabetic
- Share 2
- Share 0 or 1

Age (y)

Source: Aly T et al. PNAS, 2006
Multiple Factors May Drive Progressive Decline of $\beta$-Cell Function

- Hyperglycemia (glucose toxicity)
- $\beta$-cell apoptosis/necrosis
- $\downarrow$ Islet neogenesis
- Islet autoantibodies
- Autoreactive T cells
- (Elevated cytokines IFN$\gamma$, IL-1$\beta$, TNF$\alpha$, etc.)

- Environmental factors

$\uparrow$ Apoptosis/necrosis
$\downarrow$ Islet neogenesis
Environmental Factors
Congenital Rubella Syndrome

- 30% diabetic usually early T1DM, some T2DM
- incubation period 5-20 yrs
- HLA-DR3 or 3/4 in those with diabetes
- other autoimmune diseases (thyroid, AD)
- molecular mimicry with a 52kD autoantigen
- animal model - Syrian hamsters
- No diabetes after postnatal infection or MMR vaccination
Other Environmental factors involved in Type 1 diabetes pathogenesis

- Cocksakie B Virus? Molecular mimicry with he islet autoantigen glutamic acid decarboxylase (GAD)
- Enterovirus?
- Streptozotocine (low doses)?
Loss of self tolerance to self-antigens
Autoantigens in Diabetes

- Insulin
- Glutamic acid decarboxylase (GAD65)
- Islet autoantigen 512aa (ICA512/IA-2)
- Zinc Transporter Znt8
Is there a primary antigen or immune response to multiple antigens required for autoimmunity?

T cells specific for one antigen (insulin)

Insulitis

Epitope and antigen spreading, expansion

Diabetes

OR

T cells specific for multiple antigens

Insulitis

Expansion of T cells

Diabetes

Krishnamurthy et al JCI:116:3258, 2006
Maturing Thymocytes

APC

Autoantigenic Peptide

Thymus Periphery

Normal Thymic Autoantigen Expression

Low Thymic Autoantigen Expression

Potentially Autoreactive T Cells

Self-Tolerant

Autoimmunity
Role of T cells
Pathogenic Cells in Type 1 diabetes

Cell-mediated Immunity

- CD4+ T cells-MHC class II molecules (APC) interaction
- CD8+ T cells-MHC class I molecules (APC) interaction
- NK cells
- Macrophages
- Dendritic cells
INSULITUS. PATIENT DIED FROM DKA
Type 1 diabetes pathogenesis: alteration between pathogenicity (T effector cells) and regulation (regulatory T cells)
Example of regulatory T cell defect: X-linked autoimmunity-immunodeficiency syndrome (XLAAD)

Gene defect: **FOXP3**

• *This genetic defect can lead to Type 1 diabetes in the presence of other autoimmune disorders for abnormalities in regulatory T cell maturation.*
Regulatory T cells (Tregs)

Thymus

Periphery

Naïve CD4^+ 

CD4^+CD25^+ FoxP3^+ 

DC

Naive T cell

TGF-β1

IL-4

IL-12

IL-6

IL-23

IL-17

Th2

Th1

Th17

Treg

FoxP3

TGF-β1 others
Role of cytokines
Differentiation of CD4+ T-cell Subsets

CD4+ T-cell

- IL-4
  - IL-5
    - IL-4
  - TGF-β
    - IL-10
      - IFN-α
        - IL-10
          - IL-2
            - IFN-γ
              - Pro-Inflammation
                - Cell-Mediated Immunity
                  - Autoimmunity
                    - Allograft Rejection
                  - Anti-Inflammatory
                    - Humoral Immunity
Oxidative Stress

IL-β, IFN-γ, TNF-α → Nitric Oxide (NO) production → Oxidative Stress → β-cell death
Role of autoantibodies
Cytoplasmic Islet-cell-antibody staining

Positive reaction

Negative reaction

Source: Diabetes Care, 1988
Islet Cell Autoantibody Assays

GAD65 Autoantibodies

IA-2 Autoantibodies
Immunoprecipitation of *in vitro* transcribed/translated \[^{35}\text{S-Met}\] labeled antigen using patient serum. [CV: inter-assay: 9.5%; intra-assay: 12.4%]

Insulin Autoantibodies (IAA)
New Radioimmunoassay [CV: inter-assay: 19.4%; intra-assay: 8%]

Islet Cell Antibodies (ICA)
Immunoperoxidase staining in rat and human pancreas
Prospective Studies in First Degree Relatives of T1DM Probands

Sibling/offspring cohort
Cumulative risk of developing clinical Type 1 diabetes in relatives of T1DM patients using islet autoantibodies (IAA, GAD65, IA-2, ICA)

Log Rank
P < 0.00001
Objective: To determine whether any immunomodulatory therapy can ameliorate insulin secretion in newly diagnosed T1DM (17-40 yr of age) and to ultimately prevent T1DM onset in first-degree relatives of T1DM probands. First trials in relatives started in 2003.

Criteria for enrolling T1DM patients in TrialNet: ≥ 2Ab to islet antigens.
Conclusions

• Type 1 diabetes mellitus is a polygenic disease. Although at least 19 T1DM-related candidate genes have been identified, polymorphic regions within the HLA complex confers the strongest diabetogenic effect.

• CD4+ and CD8+ T cell responses to islet autoantigens (insulin, GAD65 and IA-2) are pathogenic.

• A defect of Regulatory T cells in suppressing pathogenic autoimmune responses is associated with Type 1 diabetes.

• The proinflammatory cytokines IL-1β, IFN-γ and TNF-α can cause β cell death (increased NO production).

• Gene defects in FOXP3 and AIRE cause multiple autoimmune disease (APECED, APS-I respectively) including Type 1 diabetes

• The presence of multiple autoantibodies to insulin, GAD65, IA-2 are high risk markers of Type 1 diabetes progression.