2008-09

M1 - Immunology, Winter 2008

Fantone, J.; Pietropaolo, M. T.

http://hdl.handle.net/2027.42/64939
Objectives

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

Steady State Plasma Glucose

Glucose Production

Liver

β Cells

Glucose Disposal

Peripheral Tissues
Regulation of Plasma Glucose

Glucose Production

Steady State Plasma Glucose

Insulin

Glucose Disposal

Peripheral Tissues

Liver
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>
</tr>
</tbody>
</table>
Relative Proportions of Types 1 & 2 DM

95% Type 2
5% Type 1
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
- “PRE”-DIABETES
- DIABETES

TIME

NEWLY DIAGNOSED DIABETES

Type 1 diabetes: a chronic inflammatory disease of the islets

Genetic Susceptibility
# Empiric risk of developing Type 1 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Empiric Risk</th>
</tr>
</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)  Class III (0.7Mb)  Class I (2.2Mb)

DP  DQ  DR  B  C  A

Frequent Recombination

Complement and Cytokines

Recombination is Rare

Recombination is Rare

Class I-like genes and pseudogenes

Centromere

Telomere
MHC Haplotype Sharing Increases DR3/4 Sibling Risk

**Haplotype Determination:**

<table>
<thead>
<tr>
<th>Siblings Share</th>
<th>Both Haplotypes</th>
<th>Siblings Share</th>
<th>One Haplotype</th>
<th>Siblings Share</th>
<th>No Haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family A</td>
<td></td>
<td>Family B</td>
<td></td>
<td>Family C</td>
<td></td>
</tr>
</tbody>
</table>

- **HLA-A**
  - Family A: 1, 2, 3, 4
  - Family B: 1, 2, 3, 4
  - Family C: 1, 2, 3, 4

- **HLA-DRB1**
  - Family A: A, B, C, D
  - Family B: A, B, C, D
  - Family C: A, B, C, D

**Haplotypes**

- **HLA-A**
  - Family A: 1, 2, 3, 4
  - Family B: 1, 2, 3, 4
  - Family C: 1, 2, 3, 4

- **HLA-DRB1**
  - Family A: A, B, C, D
  - Family B: A, B, C, D
  - Family C: A, B, C, D

**Diabetic Proband**

- Family A: 1, 2
- Family B: 2, 30
- Family C: 1, 31

**DAISY Sibling**

- Family A: 4, 6
- Family B: 4, 6
- Family C: 3, 4
MHC haplotype sharing increases risk in DR3/4-DQ8 siblings

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

- Hyperglycemia (glucose toxicity)
- β-cells
- Autoreactive T-cells
- Islet Autoantibodies
  - ↑ Apoptosis/Necrosis
  - ↓ Islet Neogenesis
- Elevated cytokines (IFNγ, IL-1β, TNFα, etc.)
- Environmental Factors
- Hyperglycemia (glucose toxicity)
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

Autoantigenic Peptide

APC

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)

T1D Development

Contributing Factors

Normal

Pathogenicity  Regulation

T1D Prone

Pathogenicity  Regulation

T1D Protected

Pathogenicity  Regulation
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⁺

FoxP3

TGF-β1

Th2 → IL-4

Th1 → IFN-γ

Th17 → IL-17

IL-23

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

- **Tn**
  - IL-10
  - IFN-α

- **Tr1**
  - IL-10
  - TGF-β

- **Th1**
  - IL-12
  - IFN-γ
  - IL-2

- **Th2**
  - IL-4
  - IL-5

Cell-Mediated Immunity
Autoimmunity
Pro-Inflammation
Allograft Rejection

Suppressor/Regulatory

Humoral Immunity
Anti-Inflammatory
Oxidative Stress

IL-\( \beta \), IFN-\( \gamma \), TNF-\( \alpha \) → Nitric Oxide (NO) production → Oxidative Stress → \( \beta \)-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)
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-1\(^\beta\), IFN-\(\gamma\) and TNF-\(\alpha\) can cause \(\beta\) 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.