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M1 - Immunology, Winter 2008

Fantone, J.; Pietropaolo, M. T.

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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

β Cells

Glucose Disposal

Peripheral Tissues

Liver
Regulation of Plasma Glucose

Steady State Plasma Glucose

Glucose Production

Liver

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>
</tr>
</tbody>
</table>
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

“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>First degree relatives of T1DM probands*</th>
<th>5-7%</th>
</tr>
</thead>
<tbody>
<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)

- Frequent Recombination
- Complement and Cytokines
- Recombination is Rare
- Class I-like genes and pseudogenes

Telomere

Centromere
MHC Haplotype Sharing Increases DR3/4 Sibling Risk

Haplotype Determination:

**Siblings Share Both Haplotypes**
- Family A

**Siblings Share One Haplotype**
- Family B

**Siblings Share No Haplotype**
- Family C

- HLA-A
  - 1
  - 29
  - A
  - C

- HLA-DRB1
  - 3
  - 4

**Diabetic Proband**

**DAISY Sibling**
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)
- Islet Autoantibodies
- Autoreactive T cells
  - (Elevated cytokines IFN_γ, IL-1β, TNF_α, etc.)

- β-cell
  - ↑ Apoptosis/Necrosis
  - ↓ Islet Neogenesis

- 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
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

- Naïve CD4$^+$

- CD4$^+$CD25$^+$ FoxP3$^+$

Periphery

- DC
- Naive T cell
- TGF-β1
- IL-4
- IL-12
- IL-6
- IL-23
- IL-17
- IFN-γ
- IL-4

- Th2
- Th1
- Th17
- Treg
- FoxP3
- TGF-β1 others
Role of cytokines
Differentiation of CD4+ T-cell Subsets

IL-12 → Th1 → IFN-γ → Cell-Mediated Immunity
IL-10 → Tr1 → IL-10 → Anti-Inflammatory
IFN-α → Tn → TGF-β → Suppressor/Regulatory
IL-4 → Th2 → IL-4 → Humoral Immunity

Pro-Inflammation
Autoimmunity
Allograft Rejection
Anti-Inflammatory
IL-β, IFN-γ, TNF-α \rightarrow \text{Oxidative Stress} \rightarrow \text{Nitric Oxide (NO) production} \rightarrow \beta\text{-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-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.