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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***

***Gavin JR et al. Diabetes Care, 20:1183-1197, 1997***

**I. Type 1 diabetes**

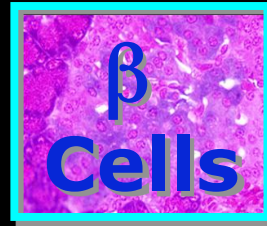
**A. Immune mediated**

**B. Idiopathic**

**II. Type 2 diabetes**

**III. Other specific types**

# Regulation of Plasma Glucose

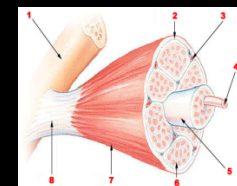
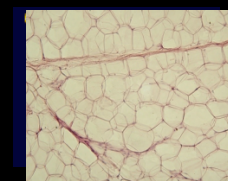
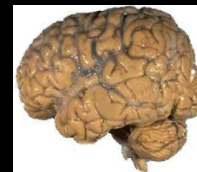


Steady State  
Plasma Glucose

Glucose Production

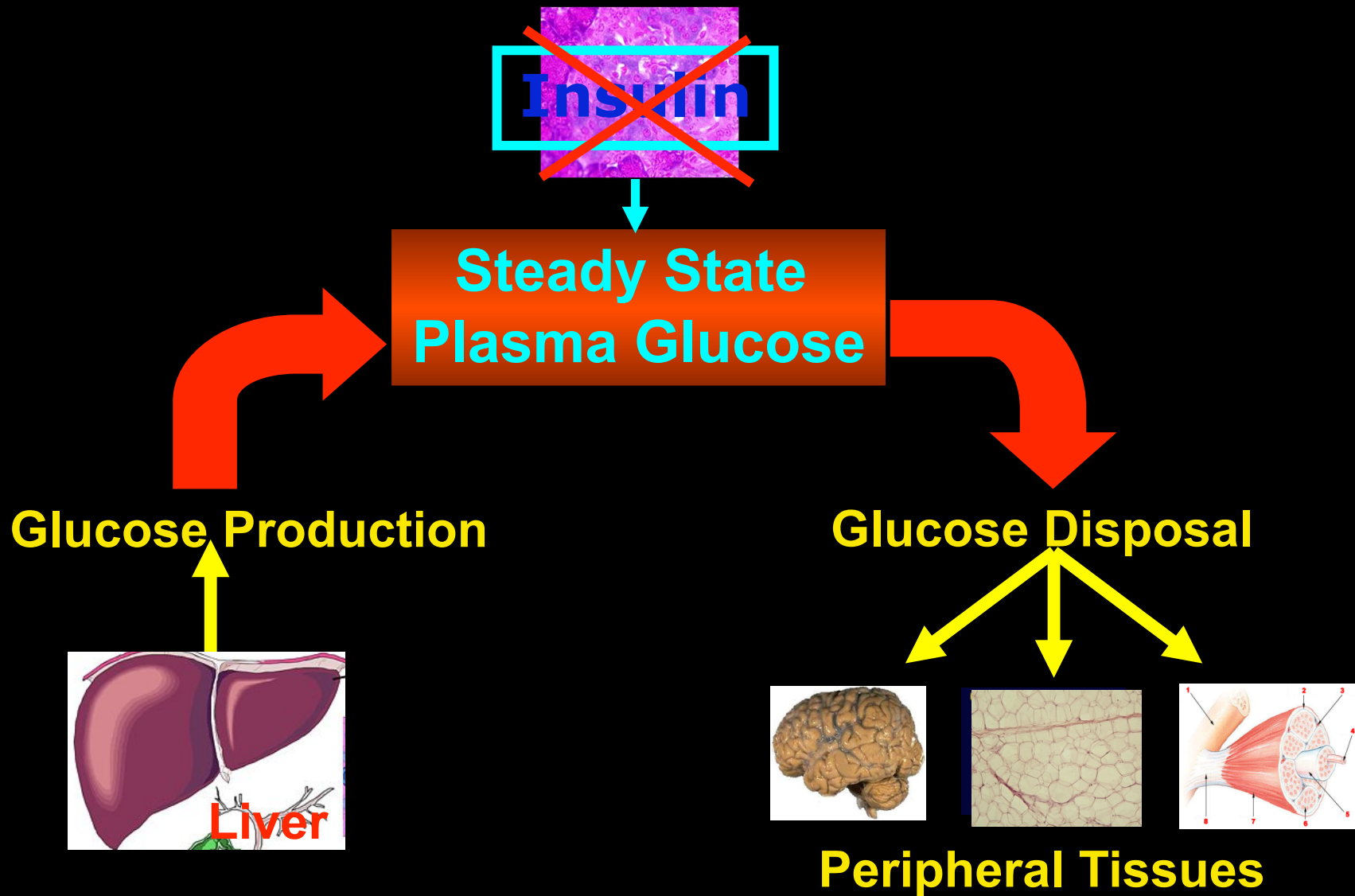


Glucose Disposal



Peripheral Tissues

# Regulation of Plasma Glucose



# Diabetes Mellitus- Type 1

Increased thirst (polydypsia)

Increased urination (polyuria)

Increased appetite (polyphagia)

Weight loss

Fatigue

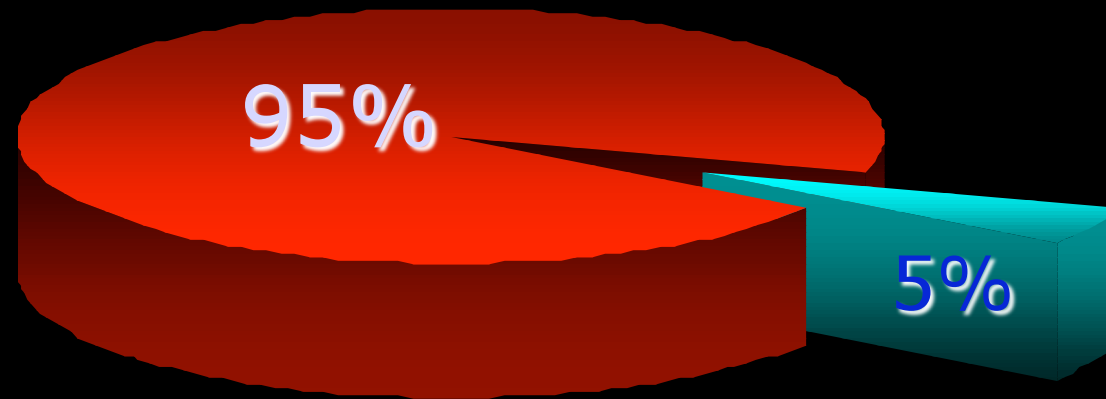
Rapid, early onset (before age 15)

# Differences Between Type 1 and Type 2 Diabetes

	Type 1	Type 2
<b>Age of onset</b>	Young	Older
<b>Type of onset</b>	Acute	Insidious
<b>Genetic background</b>	HLA related	Not HLA related
<b>Islet cell antibodies</b>	Yes	No
<b>Insulin secretion</b>	Absent	Present
<b>Nutritional status</b>	Thin	Obese
<b>Insulin dependence</b>	Yes	No
<b>Insulin resistance</b>	No	Yes
<b>Responsiveness to Orals</b>	No	Yes
<b>Ketosis proneness</b>	Yes	No



# Relative Proportions of Types 1 & 2 DM



■ Type 2

■ 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**

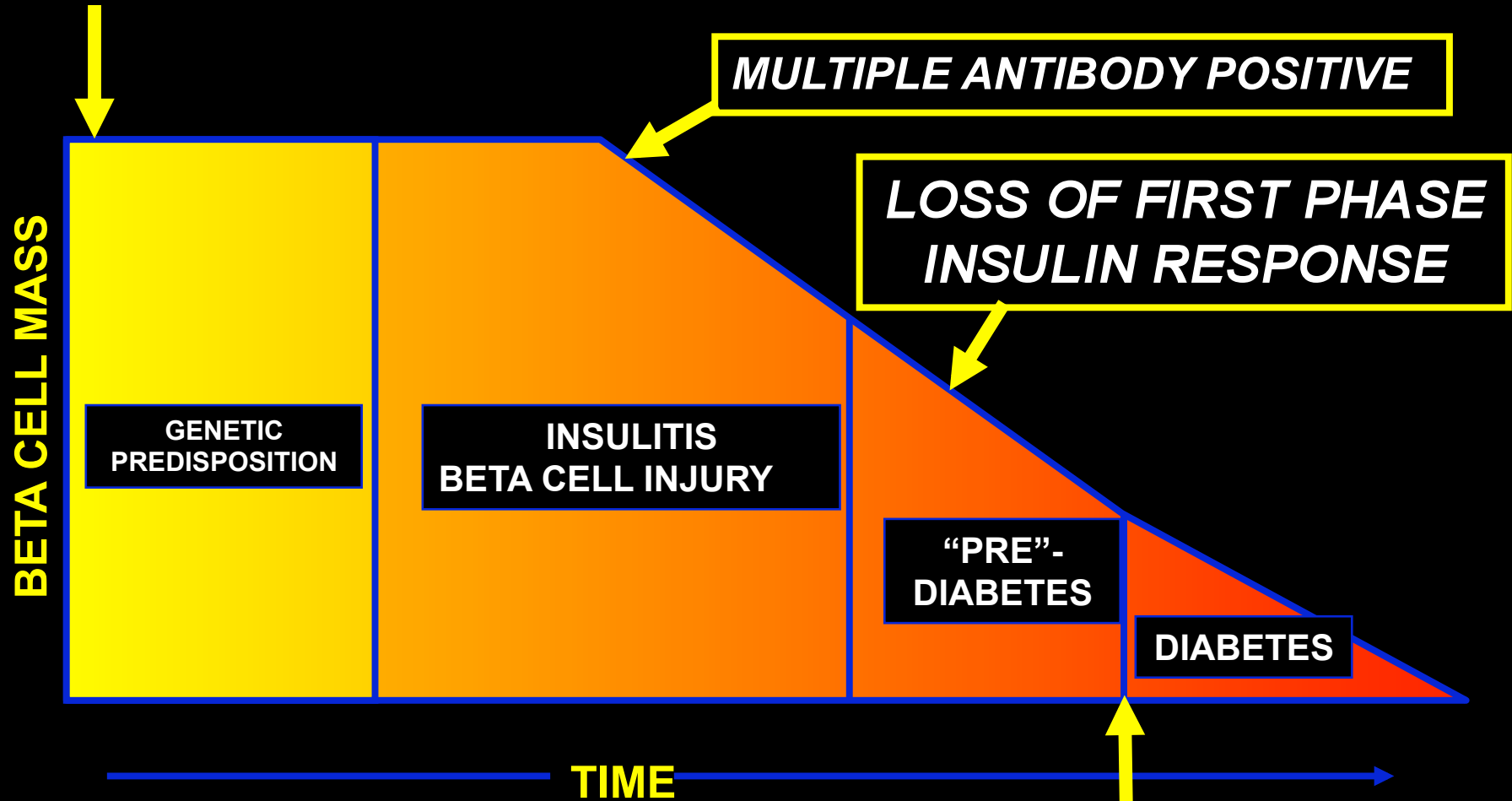
- Antibodies to islet autoantigens
- Autoreactive T cells

## **Immune-modulation alters the course of disease**

- Antigen vaccination
- General immunosuppression

# Stages in Development of Type 1 Diabetes

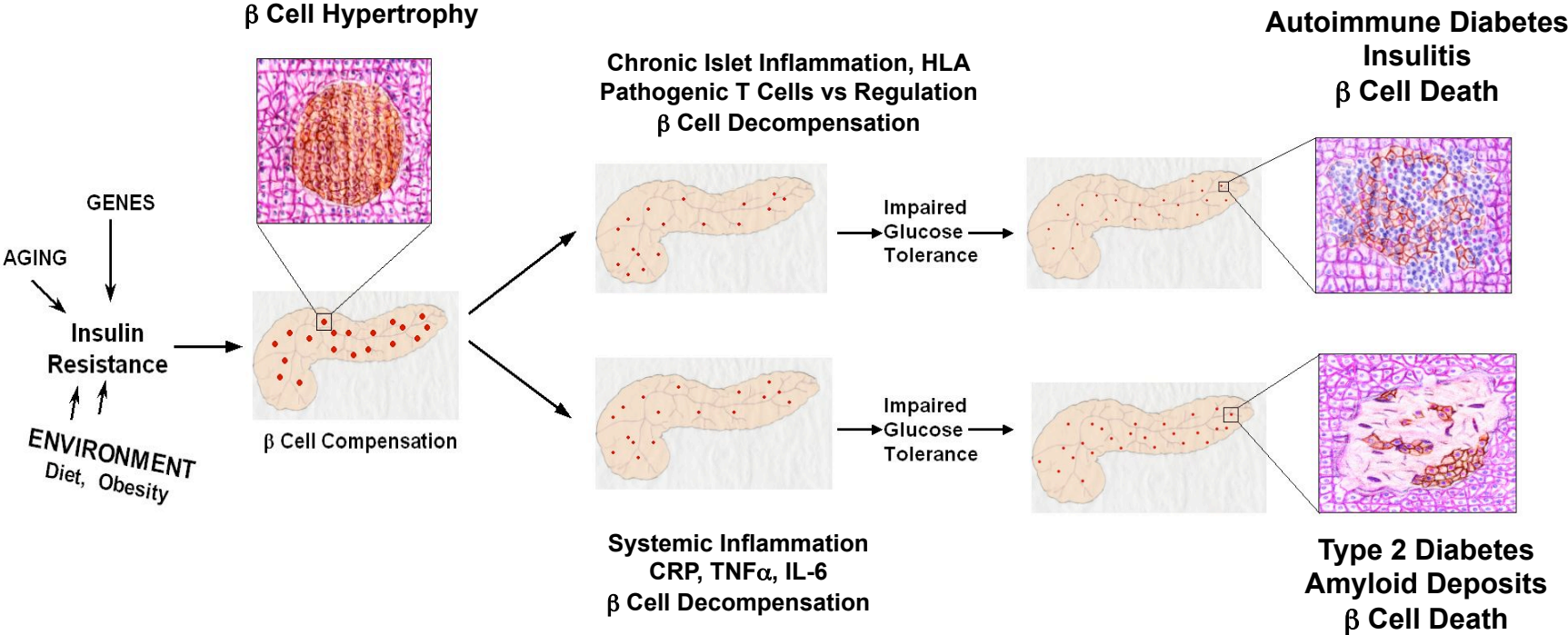
**GENETICALLY AT RISK**



G. Eisenbarth, *NEJM*, 1986

**NEWLY DIAGNOSED DIABETES**

# Type 1 diabetes: a chronic inflammatory disease of the islets



Pietropaolo M et al. *Diabetes* 56:1189-97, 2007

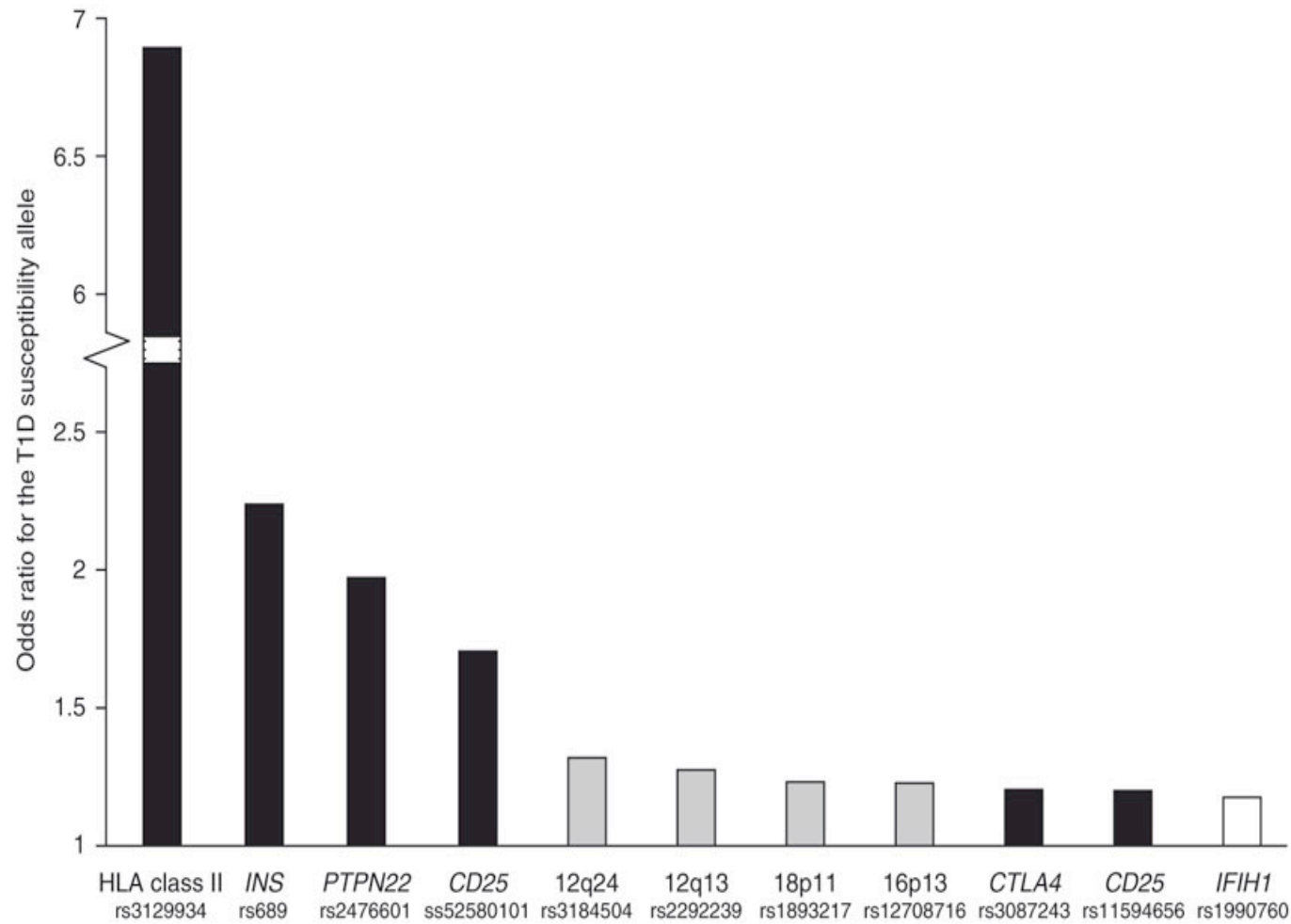
# Genetic Susceptibility

# Empiric risk of developing Type 1 diabetes

	Empiric Risk
First degree relatives of T1DM probands*	5-7%
Individuals without relatives with T1DM*	<1%
Children of affected father**	~6%
Children of affected mother**	~2%

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



Source: Todd JA et al. *Nature Genetics* 39, 857- 864, 2007

# HLA

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Human Leukocyte Antigen

human MHC

cell-surface proteins

important in self vs. nonself distinction

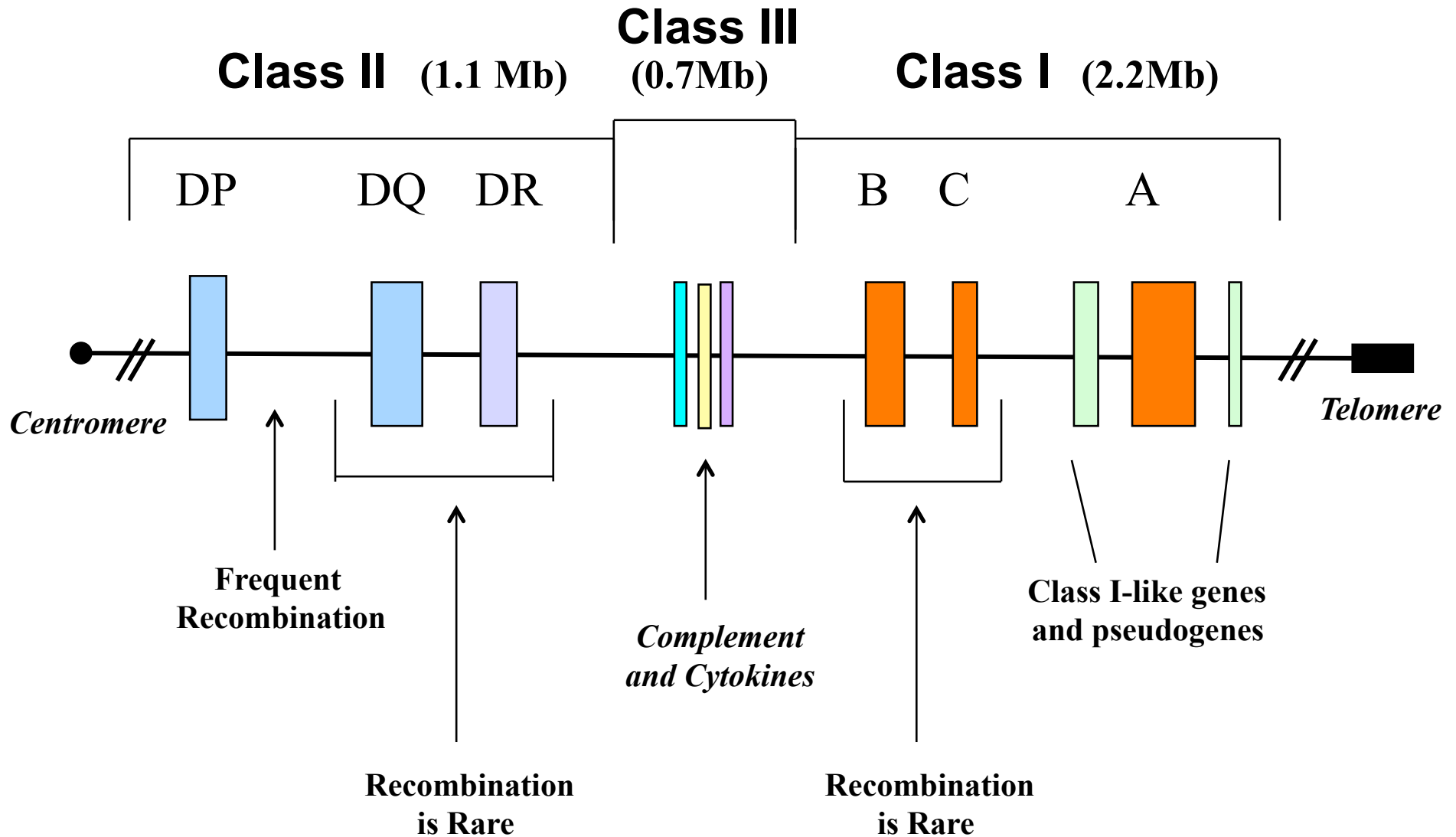
present peptide antigens to T cells

CLASS I: A,B,C

CLASS II: DR,DQ,DP

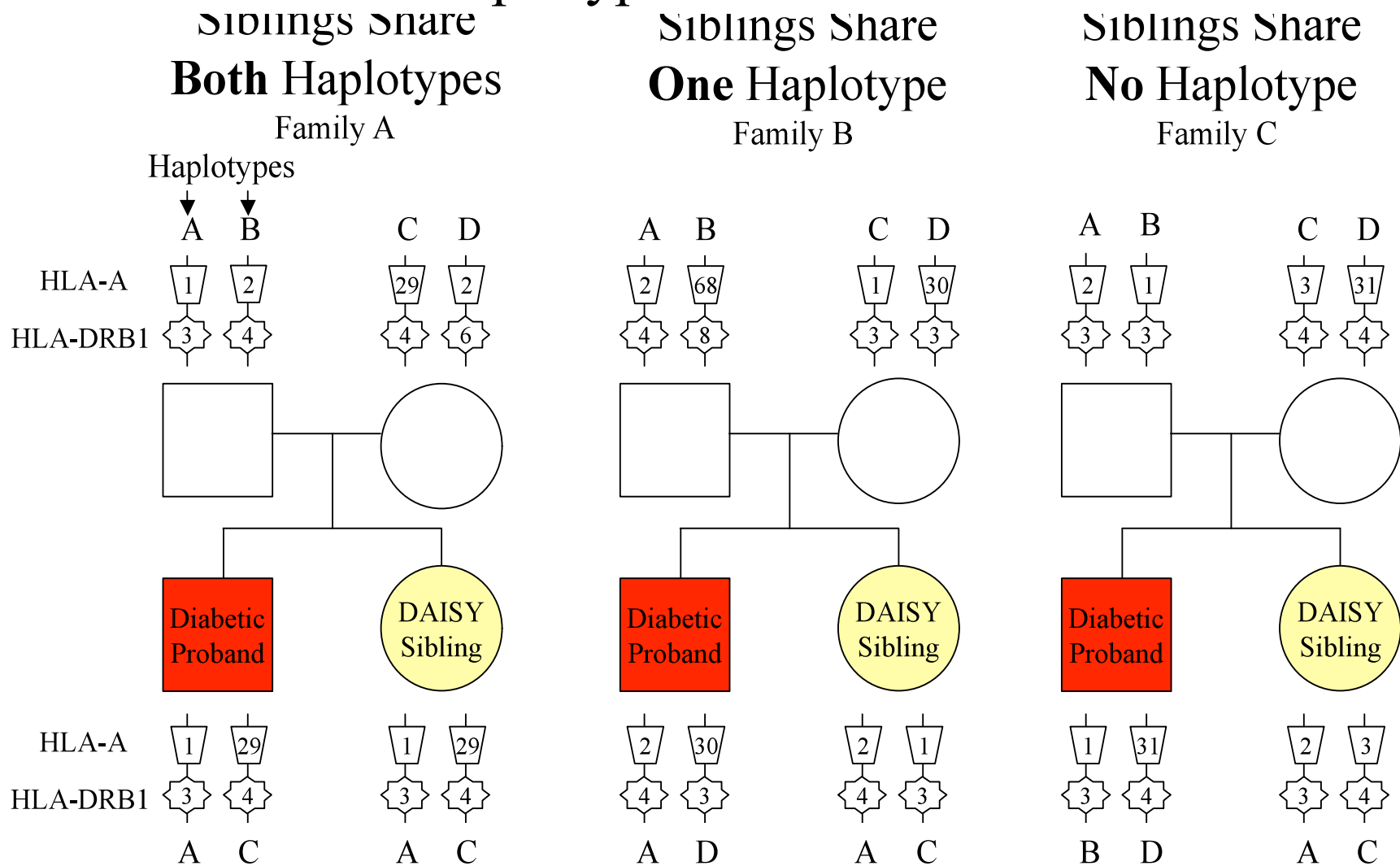


# The Human Leukocyte Antigen Complex (6p21.31)



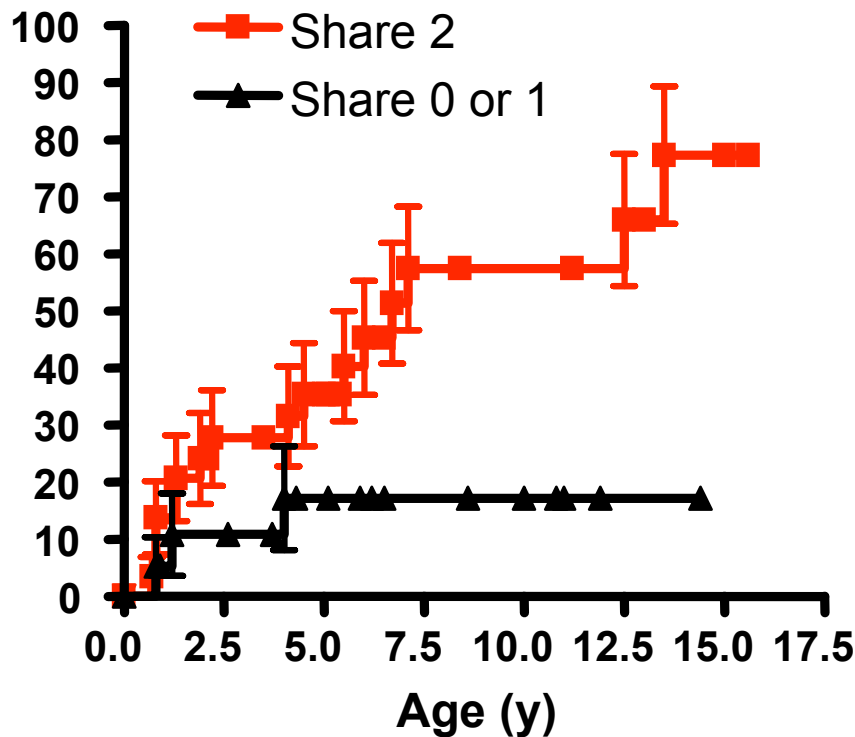
# MHC Haplotype Sharing Increases DR3/4 Sibling Risk

## Haplotype Determination:

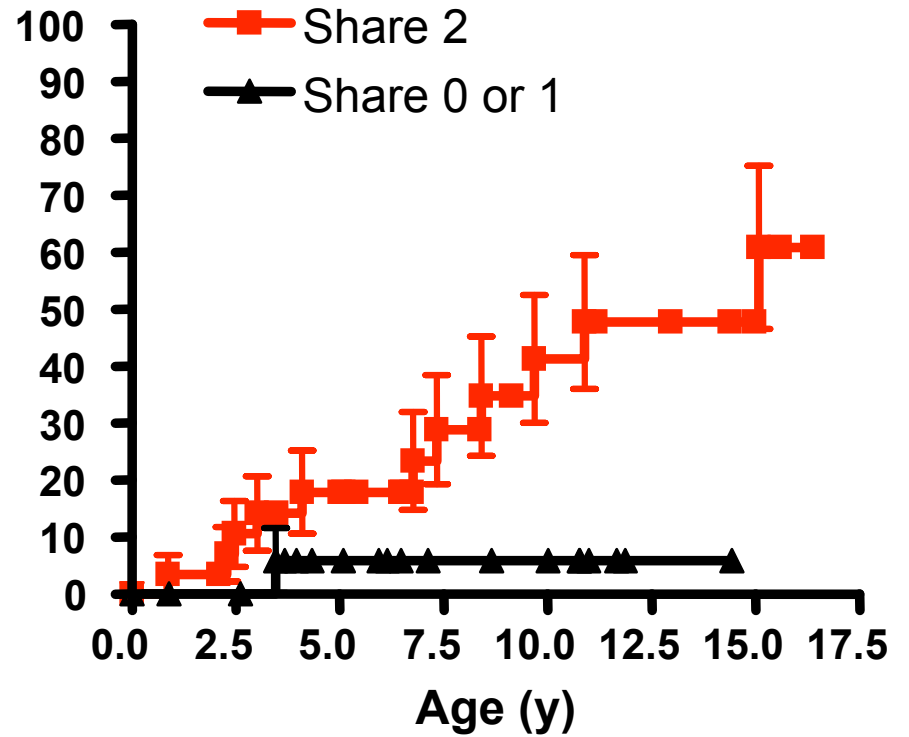


# MHC haplotype sharing increases risk in DR3/4-DQ8 siblings

**% Autoantibody Positive**



**% Diabetic**

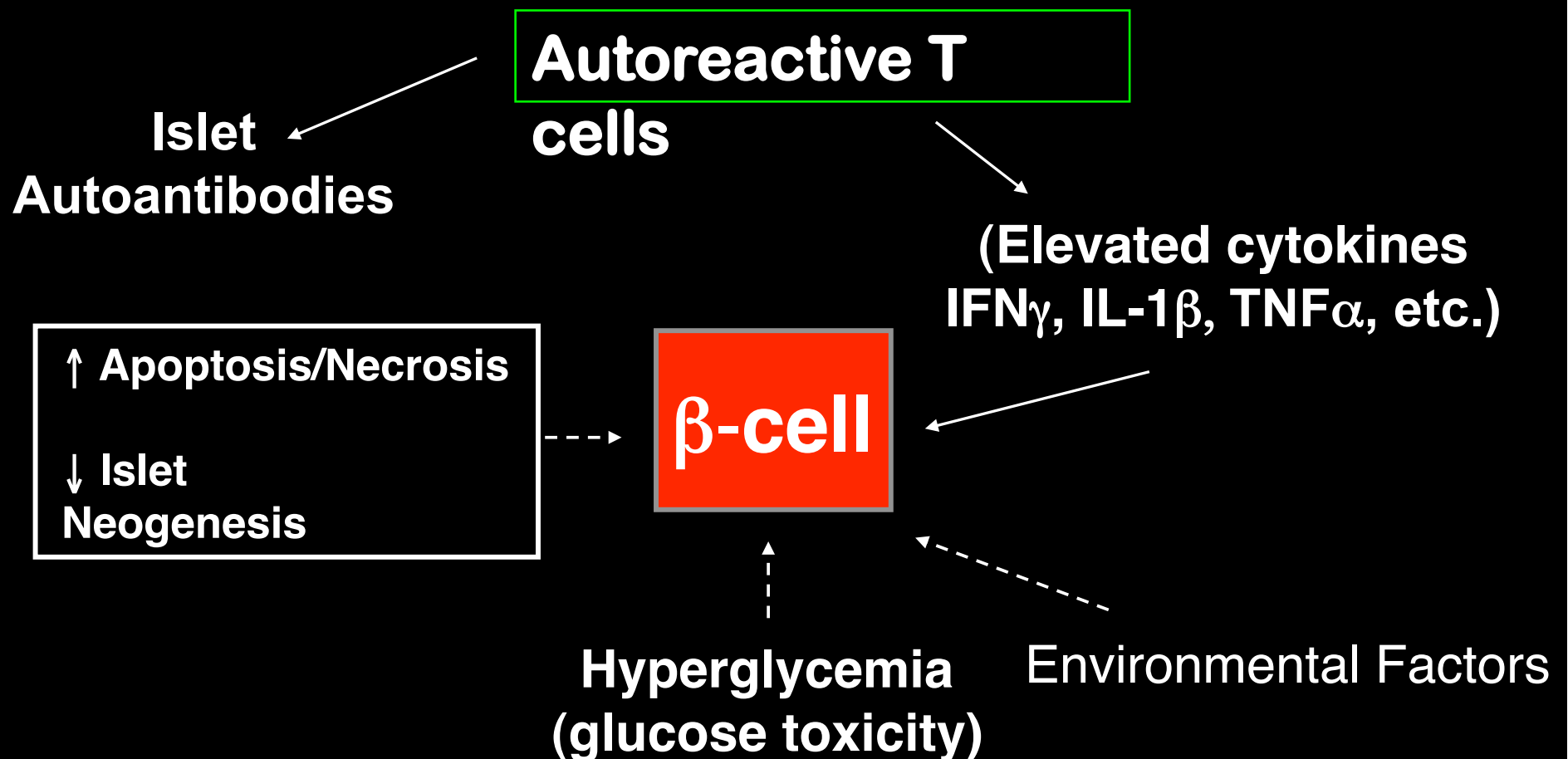


Share 2:	29	20	17	7	6	5	2
Share 0 or 1:	19	16	11	8	6	1	

Share 2:	29	25	22	13	9	7	4	1
Share 0 or 1:	19	18	13	8	7	1		

Source: Aly T et al. PNAS, 2006

# Multiple Factors May Drive Progressive Decline of $\beta$ -Cell Function



# **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) ?**

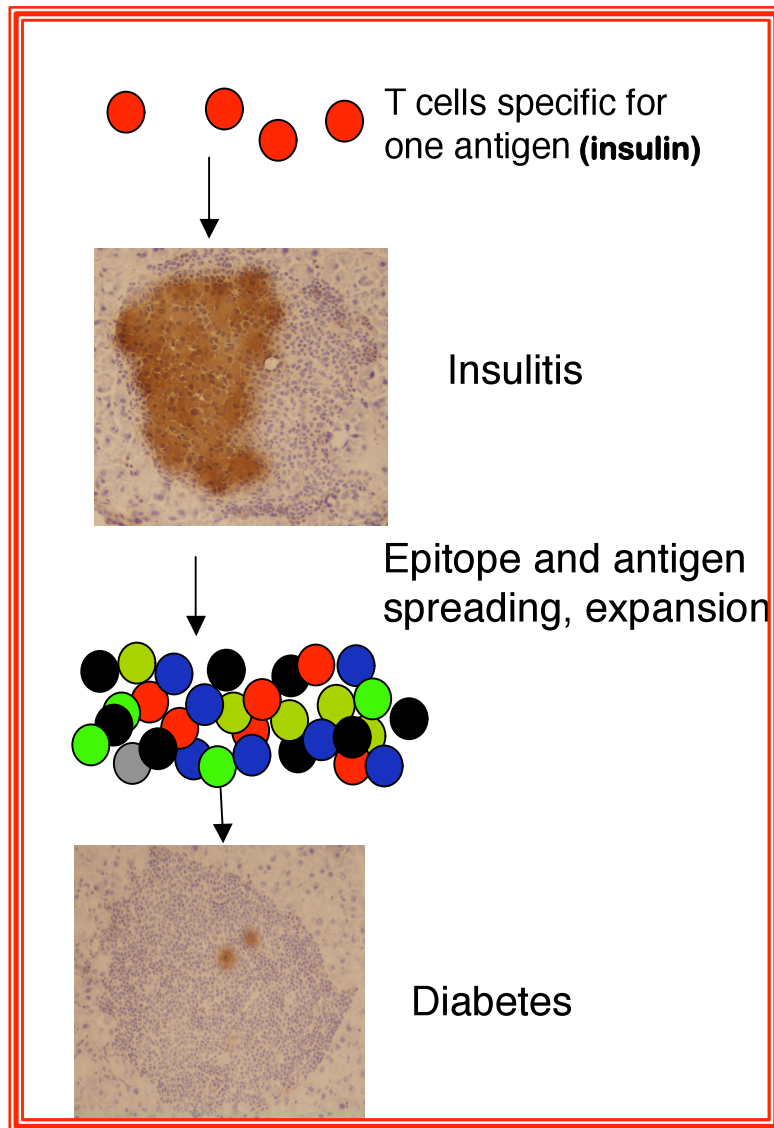
**Loss of self tolerance to self-antigens**



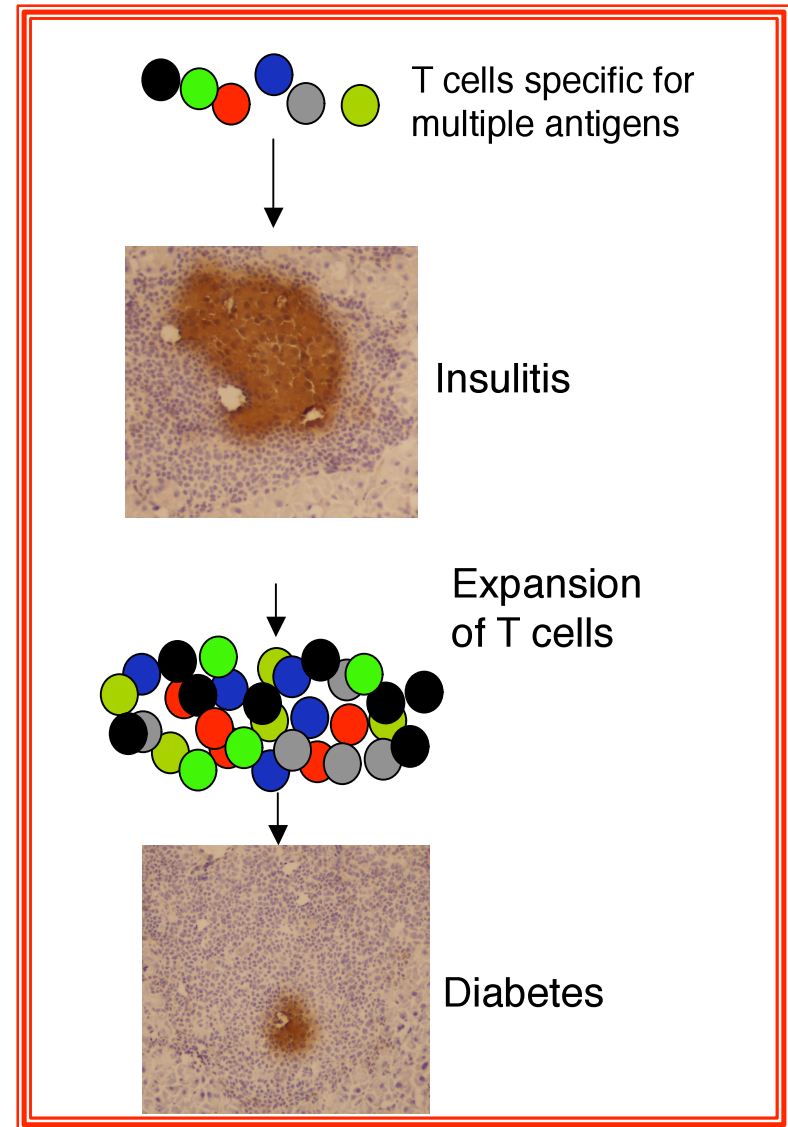
# Autoantigens in Diabetes

- Insulin
- Glutamic acid decarboxylase (GAD65)
- Islet autoantigen 512aa (ICA512/IA-2)
- Zinc Transporter Znt8

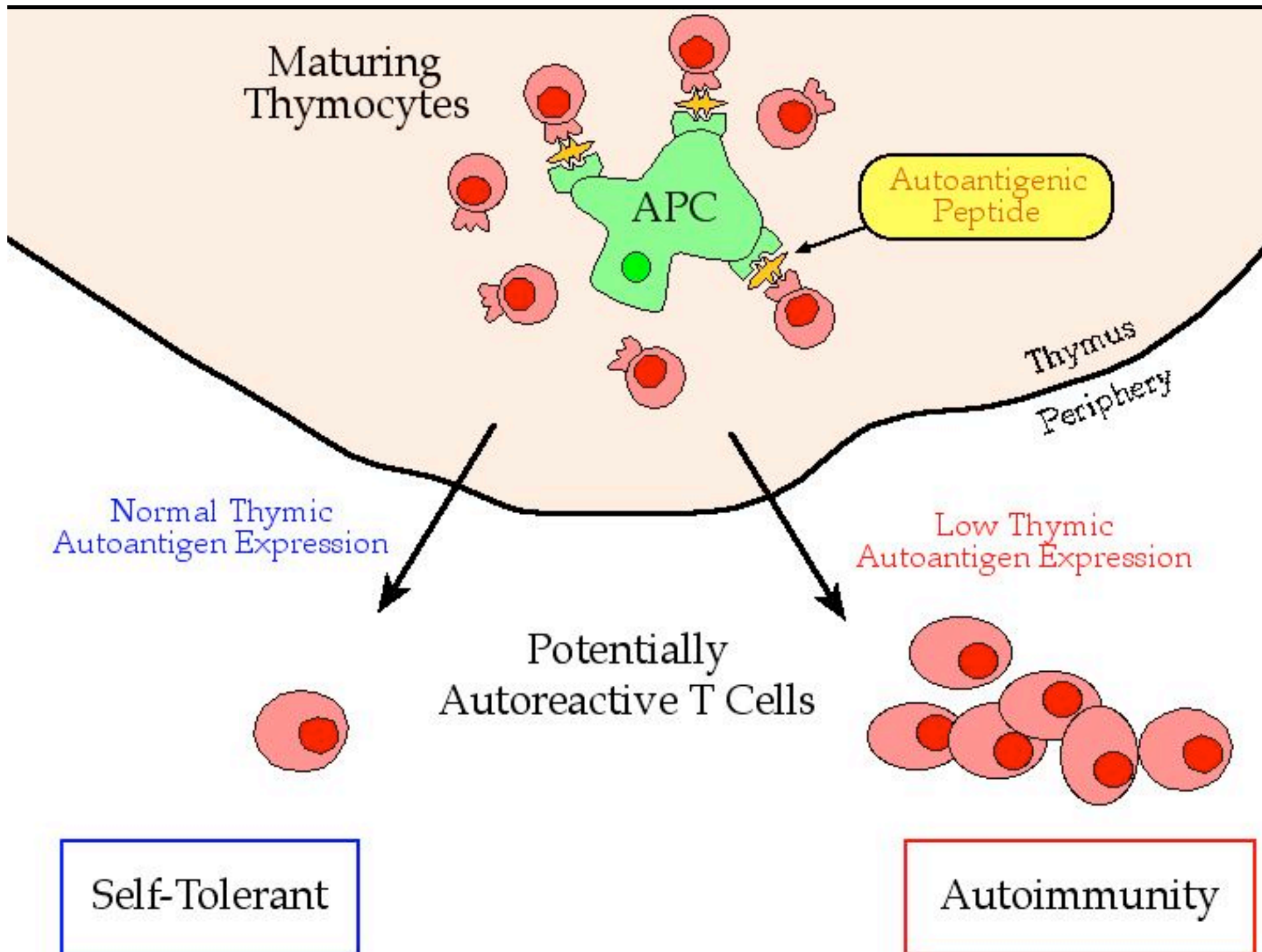
# Is there a primary antigen or immune response to multiple antigens required for autoimmunity?



OR



Krishnamurthy et al JCI:116:3258, 2006



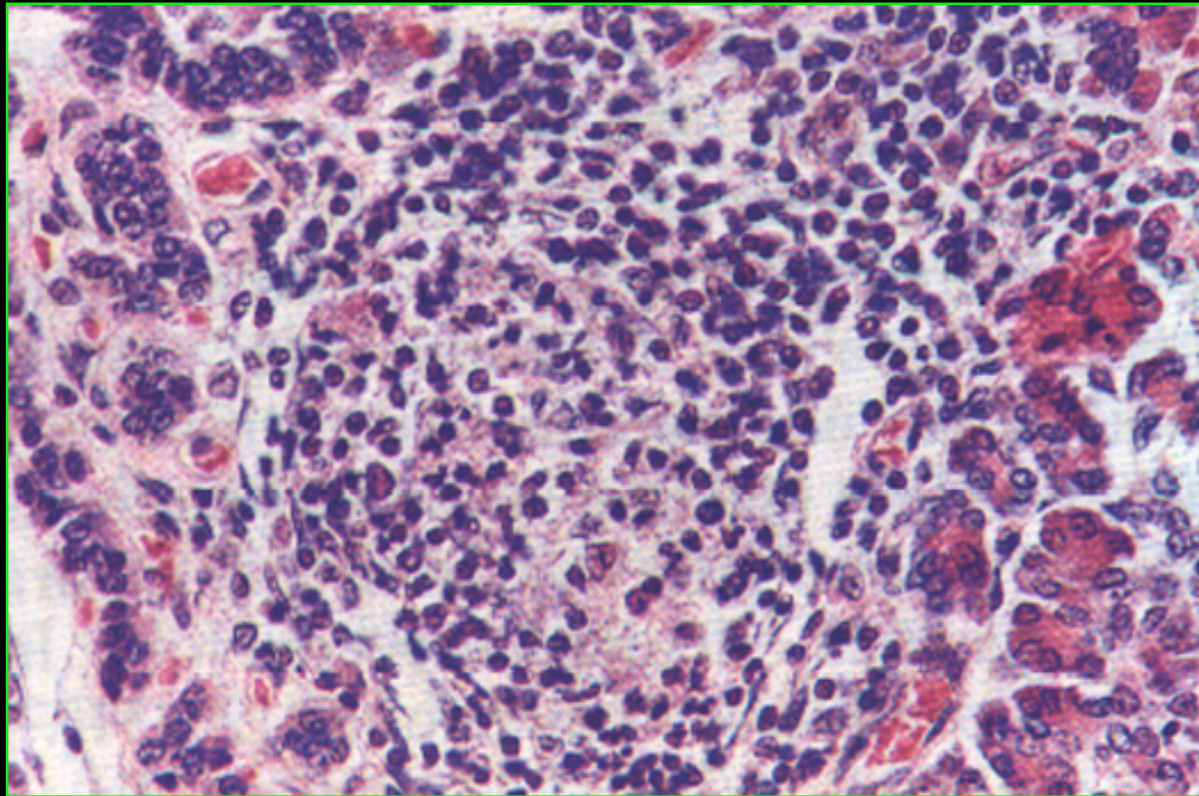
# **Role of T cells**

# Pathogenic Cells in Type 1 diabetes

## Cell-mediated Immunity

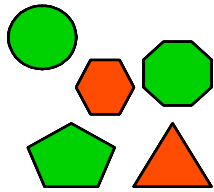
- CD4+ T cells-MHC class II molecules (APC) interaction
- CD8+ T cells-MHC class I molecules (APC) interaction
- NK cells ?
- Macrophages ?
- Dendritic cells ?

**INSULITIS. PATIENT DIED FROM DKA**  
**Conrad, B. et al. *Nature* 371:351 1994**



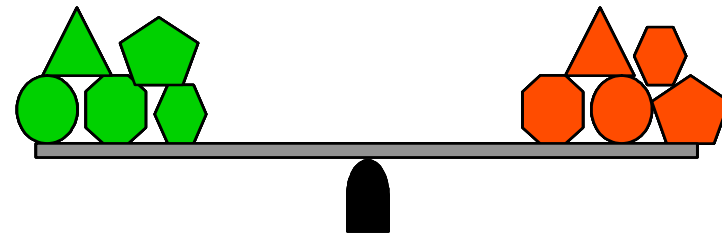
# Type 1 diabetes pathogenesis: alteration between pathogenicity (T effector cells) and regulation (regulatory T cells)

## T1D Development



Contributing Factors

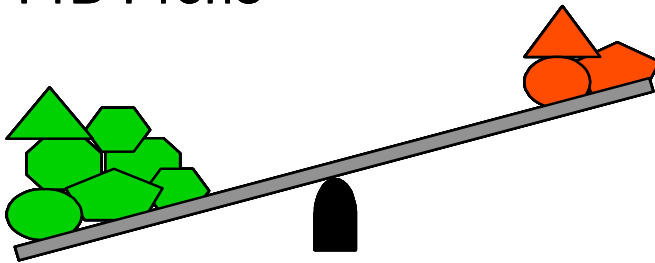
Normal



Pathogenicity

Regulation

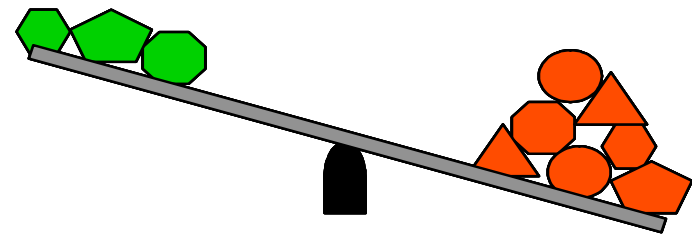
T1D Prone



Pathogenicity

Regulation

T1D Protected



Pathogenicity

Regulation

**Example of regulatory T cell defect:  
X-linked autoimmunity-immunodeficiency  
syndrome (XLAAD)**

**Gene defect: *FOXP3***

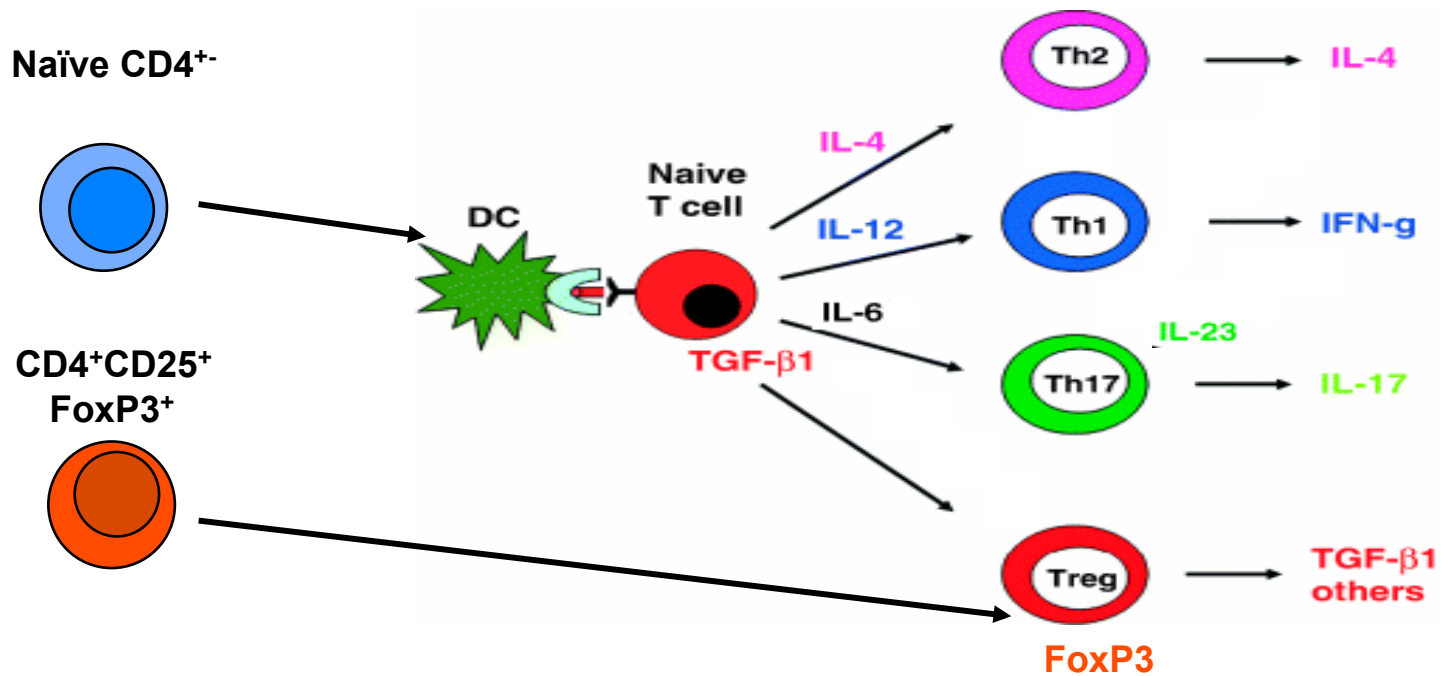
***•This genetic defect can lead to Type 1 diabetes in the presence of other autoimmune disorders for abnormalities in regulatory T cell maturation.***



# *Regulatory T cells (Tregs)*

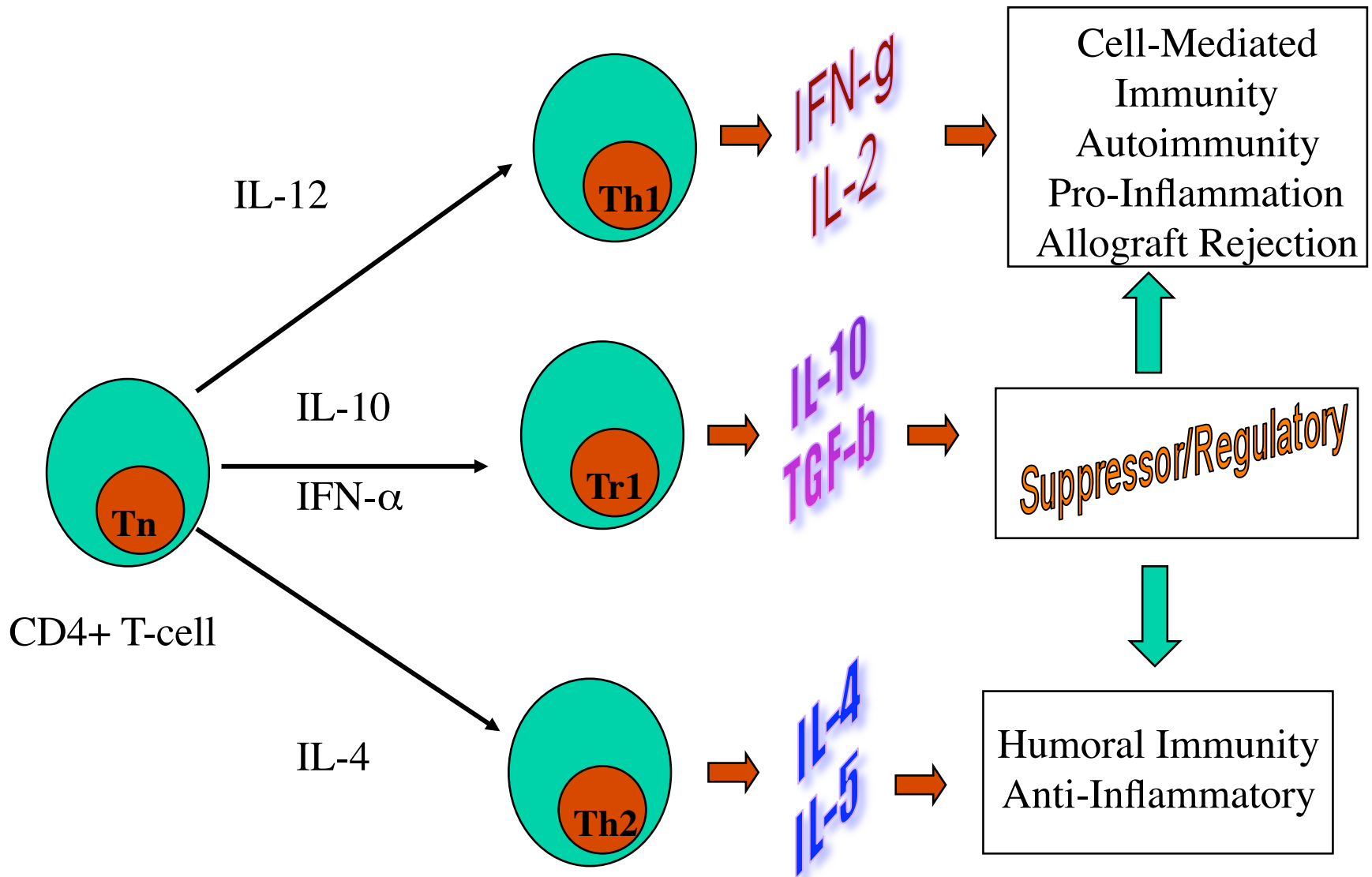
Thymus

Periphery



# **Role of cytokines**

# Differentiation of CD4+ T-cell Subsets



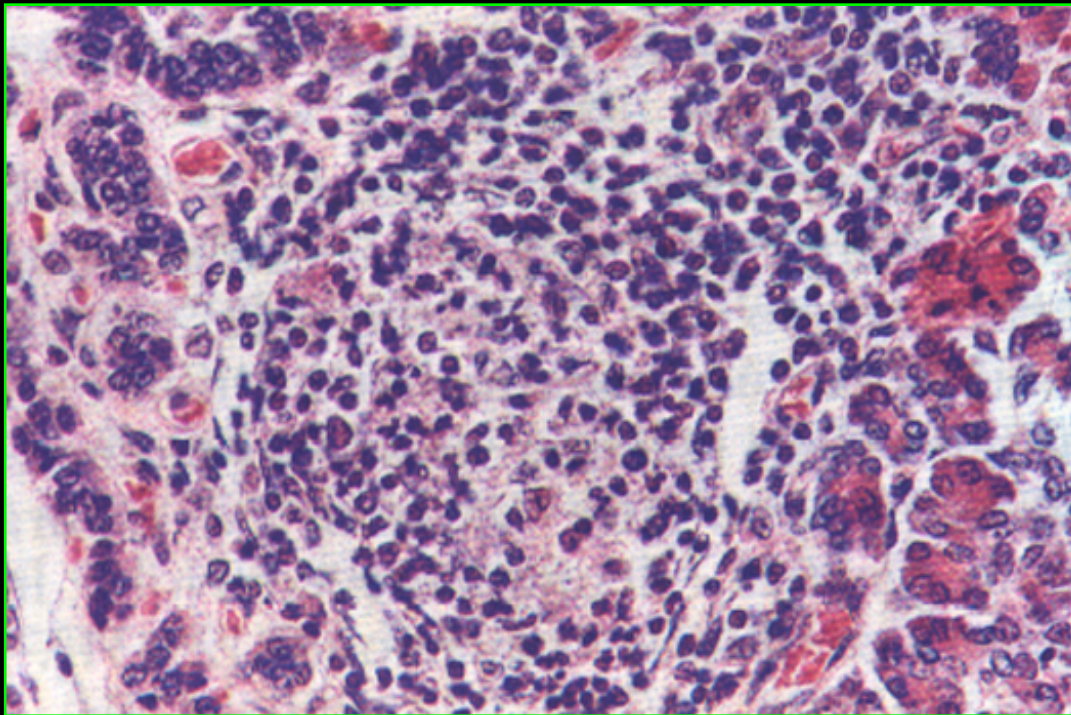
**IL- $\beta$ , IFN- $\gamma$ , TNF- $\alpha$**



**Oxidative Stress**



**Nitric Oxide (NO) production**

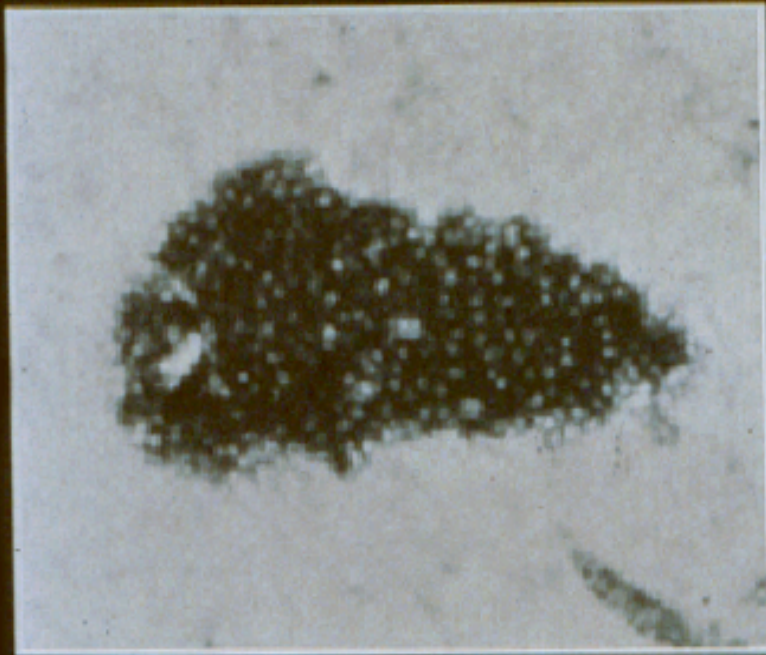


**$\beta$ -cell death**

# **Role of autoantibodies**



## Cytoplasmic Islet-cell-antibody staining



Positive reaction



Negative reaction

Reproduced with permission of the American Diabetes Association, Inc., from Coman PG, Tautkus M, Rabizadeh A, Cahill C, Eisenbarth G: Assay for islet cell antibodies with rat pancreas and peroxidase protein A. *Diabetes Care* 1988;367-368, and from Vardi P, Ziegler AG,

Mathews JH, Dib S, Keller RJ, Ricker AT, Wolzdorf JI, Herskowitz RD, Rabizadeh A, Eisenbarth GS, Soeldner JS: Concentration of insulin autoantibodies at onset of type I diabetes: Inverse log-linear correlation with age. *Diabetes Care* 1988;736-739.

# ***Islet Cell Autoantibody Assays***

## **GAD65 Autoantibodies**

Immunoprecipitation of *in vitro* transcribed/translated [<sup>35</sup>S-Met] labeled antigen using patient serum. [CV: inter-assay: 13.2%; intra-assay: 12.2%]

## **IA-2 Autoantibodies**

Immunoprecipitation of *in vitro* transcribed/translated [<sup>35</sup>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)**

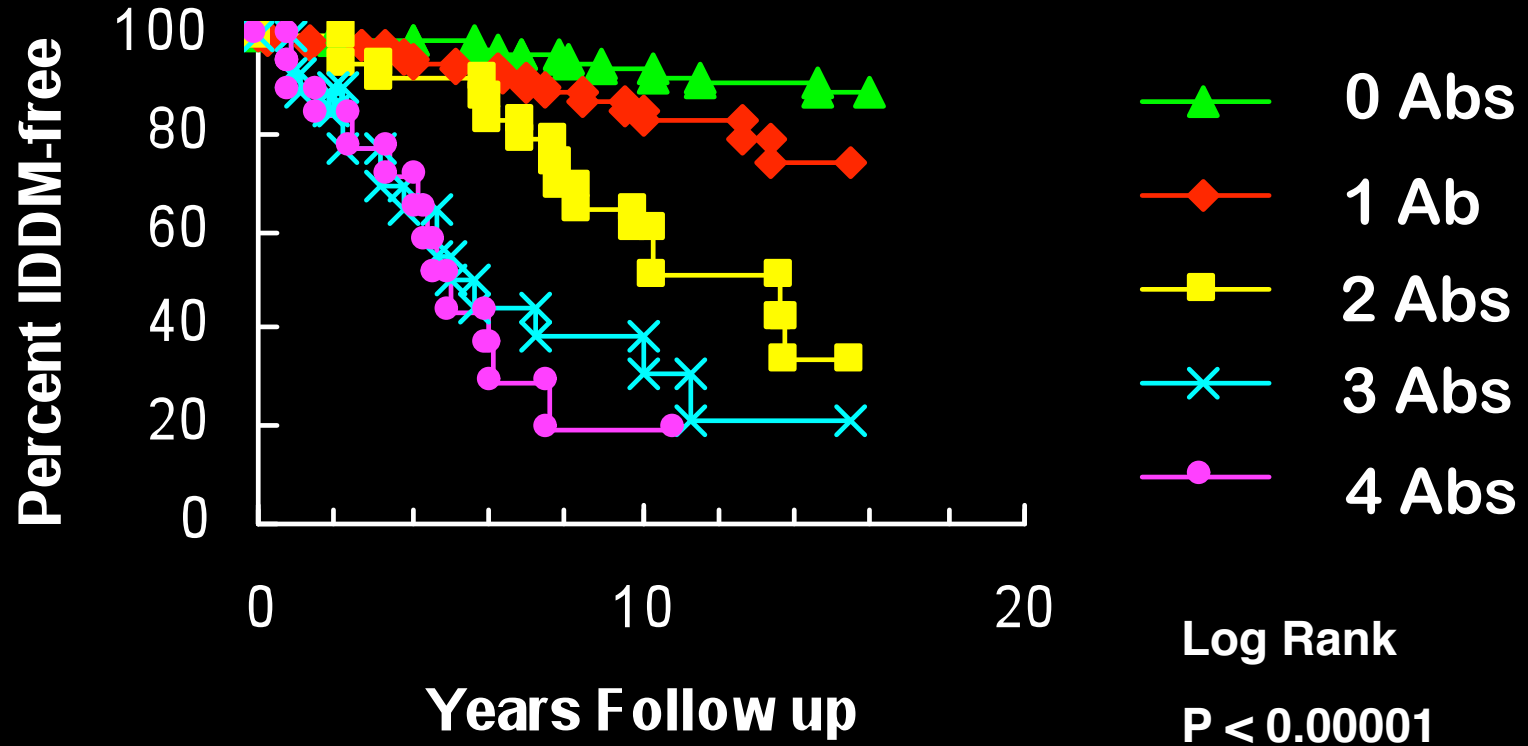
Immunoperoxidase staining in rat and human pancreas

# 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)**



# Type I Diabetes TrialNet

<http://www2.diabetestrialnet.org/>

**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:**  $\geq 2$ Ab 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.**