The Natural History of Type 1A Diabetes

perspectiva

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ABSTRACT

We can now predict the development of Type 1A (Immune Mediated) diabetes primarily through the determination of four biochemically characterized islet autoantibodies [insulin, GAD65, IA-2 (ICA512) and (Znt8)]. Prediction is possible because beta-cell destruction is chronically progressive and very slow in most, but not all individuals. We can also prevent type 1A diabetes in animal models and a major goal is the prevention of type 1A diabetes in man with multiple clinical trials underway. **(Arq Bras Endocrinol Metab 2008; 52/2:146-155)**

Keywords: Type 1 diabetes; Anti-islet autoimmunity; Autoantibodies; HLA, Genetic

RESUMO

A História do Diabetes Melito Tipo 1.

Atualmente o desenvolvimento do diabetes melito tipo 1 A(imune mediado) pode ser predito através da determinação de quatro auto-anticorpos antiilhotas [antiinsulina, anti-GAD65, anti-IA2 (ICA512) e (anti-Znt8)] caracterizados bioquimicamente. A predição dessa doença é possível devido a destruição das células-beta, não em todos os indivíduos mas na sua maioria, ser crônica e lentamente progressiva. Também é possível prevenir o DM1 A em modelos animais e o objetivo maior é a prevenção dessa doença em humanos, para os quais vários protocolos clínicos estão em andamento. **(Arq Bras Endocrinol Metab 2008;52/2:146-155)**

Descritores: Diabetes do tipo 1; Auto-imunidade antiilhota; Auto-anticorpos; HLA; Genética

INTRODUCTION

During the past decade there has been dramatic progress in understanding the immunogenetics and natural history of type 1A diabetes (1,2). This progress has been driven by the information provided by the genome project and the availability of tools for the interrogation of thousands of single nucleotide polymorphisms (3), the refinement of islet autoantibody fluid phase radioassays (4), detailed molecular studies of animal models of the disorder (5), as well as large studies following thousands of children from birth (6). In this review we put in context a number of recent developments, with the ultimate goal the immunologic prevention of type 1A diabetes. We divide the development of diabetes into six stages beginning with type 1A (immune-mediated) genetic susceptibility and ending with complete or almost complete beta-cell destruction (Figure 1) (7).

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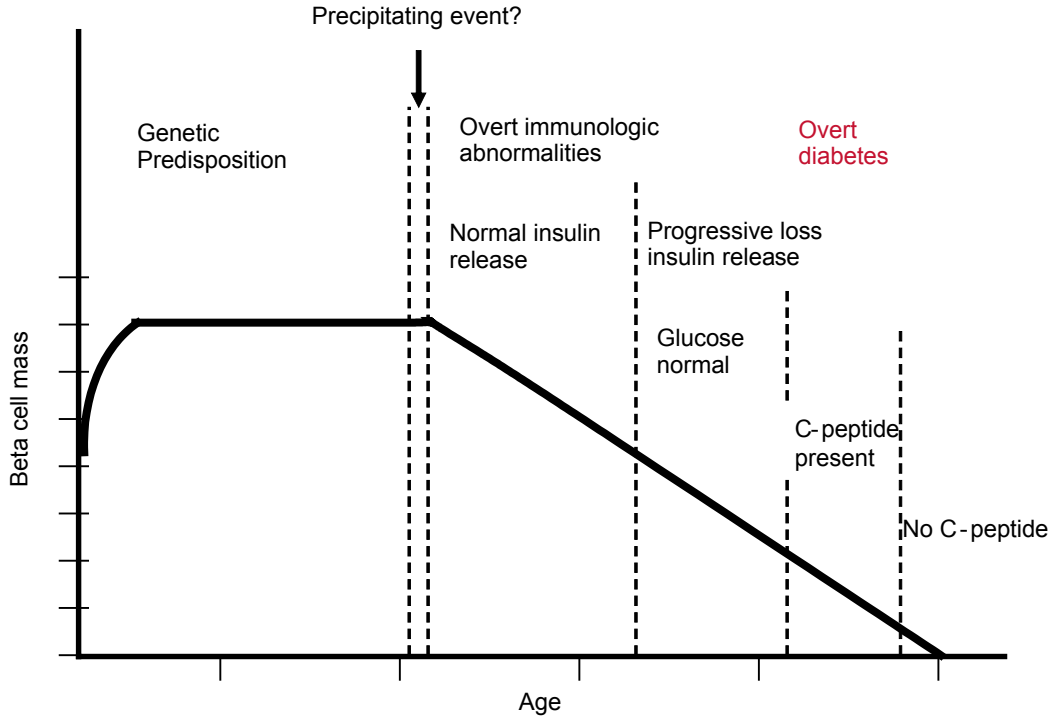


Modified from Eisenbarth GS. Type 1 diabetes mellitus. A chronic immune disease. N Engl J Med. 1986; 314:1360.

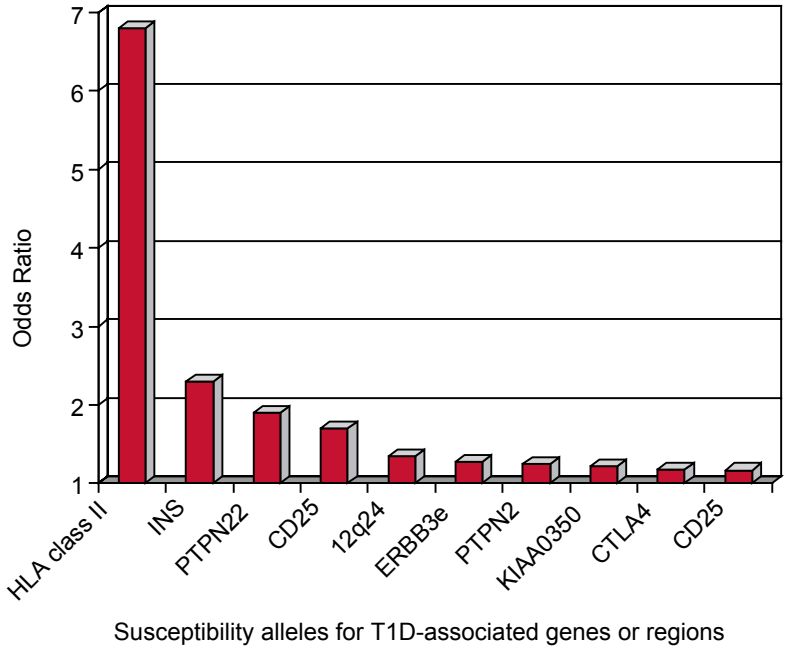
Figure 1. Stages in development of type 1A diabetes (7).

GENETICS OF TYPE 1A DIABETES

Type 1A diabetes can develop in the setting of rare "monogenic" disorders such as the IPEX syndrome (Immune Dysfunction, Polyendocrinopathy, Enteropathy, X-linked) (8) and APS-1 (Autoimmune Polyendocrine Syndrome Type 1) (also known as APECED [Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy]) (9) . The IPEX syndrome develops as a result of mutations of the FoxP3 gene that controls the development of regulatory T cells (10). In the absence of such regulatory T cells, which turn off pathogenic T cells, approximately 80% of IPEX children develop type 1 diabetes. Children with the syndrome can develop diabetes as early as at birth, and often develop diabetes as neonates. These children express GAD65 and insulin autoantibodies which aids in their diagnosis. Children with the severest form of the disorder can benefit from bone marrow transplantation. The APS-1 syndrome develops secondary to mutations of a gene (AIRE: Autoimmune Regulator) that controls expression of "peripheral" antigens in the thymus, such as insulin (11). Over time these children develop multiple disorders, such as mucocutaneous candidiasis, hypoparathyroidism, Addison's disease and type 1A diabetes.

Despite the existence of these monogenic disorders, type 1A diabetes usually develops in a polygenic manner with genes within and linked to HLA (Figure 2) (12). By far HLA DR and DQ alleles are the major determinant of the disease (13), followed by polymorphisms of the insulin gene (14) and thirdly a lymphocyte specific phosphatase (PTPN22) (15). A number of additional genes have been implicated, such as CTLA-4 (16) but their effects on diabetes risk are relatively small and currently play no role in the prediction of diseases (Figure 2) (16).

The highest risk genotype for type 1A diabetes has the HLA alleles DR3/4-DQ8 (DQ8 is DQA1*0301, DQB1*0302) (See www.barbaradaviscenter.org book on Immunology of Diabetes for explanation of HLA nomenclature and Teaching Slides). Children with this genotype comprise 2.4% of newborns in Denver, Colorado and more than 30% of children developing type 1A diabetes. With additional typing for DP alleles we



Modified from Todd et al. Robust associations of four new chromossome regions from genome-wide analyses of type 1 diabetes. Nature Genetics. 2007;39:857-64.

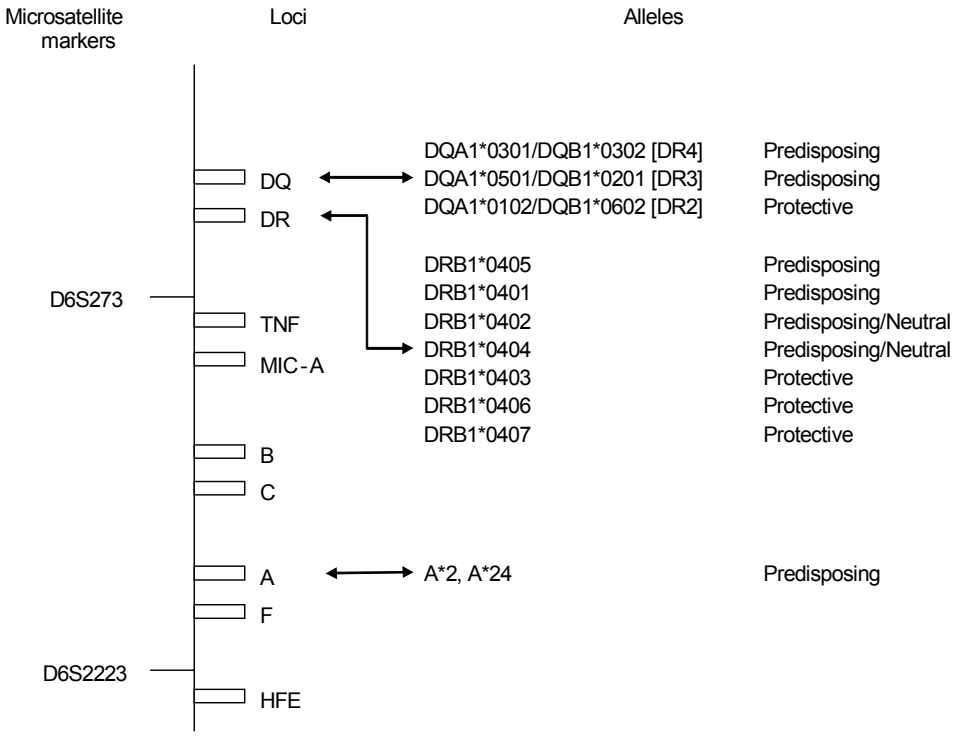
Figure 2. Odds ratios for the susceptibility allele for the ten independent T1D-associated genes or regions according to SNPs marking each gene or region. The HLA class II SNP (rs3129934) was the marker with the highest association with T1D in the MHC region (positions 25–35 Mb on chromosome 6) in the WTCCC study (12).

can now predict risk of 20% for development of anti-islet autoimmunity at birth in the general population and 80% for siblings of patients with this genotype who share identical by descent these alleles (17-19). The improved ability to genetically predict now allows design of trials for the prevention of diabetes prior to the detection of islet autoimmunity, with the caveat that trials in genetically at risk individuals by necessity should utilize very safe agents, given the remaining uncertainty as to subsequent activation of autoimmunity.

The loci in the HLA region encoding DR and DQ molecules display the strongest association for both diabetes susceptibility and protection (Figure 3) (20). The HLA-DQ locus, the locus most strongly associated with diabetes susceptibility, encodes for multiple variants of the molecule, a heterodimer consisting of two chains (α and β) which are involved in immune recognition and antigen presentation to CD4 T cells. Alleles in this locus can either be predisposing or protective, the degree to which is influenced by the DR allele with which they are in linkage disequilibrium. While the DR-3-DQ2 molecules (DQB1*0201) and DR4-DQ8 (DQB1*0302) are associated with susceptibility, the DQB1*0602 allele is associated with dominant protection (19). Two additional haplotypes, which are strongly protective, are DRB*1401, DQA1*0101, DQB1* 0503 and DRB1* 0701, DQA1*0201, DQB1*0303 (21).

Recently Baschal has found that he absence of the reportedly protective alleles DPB1*0402 and/or DRB1*0403 in DR3-DQB1*0201/DR4-DQB1*0302 individuals confers a 55% risk of persistently expressing anti-islet autoantibodies for relatives (children with a parent or sibling with type 1 diabetes) according to survival curve analysis as compared to 0% for those with either protective allele and a 20% risk for developing islet immunity in children without a type 1 diabetes relative (18).

Additional MHC and MHC-linked loci contribute to diabetes risk (22-26).Siblings are known to have a higher diabetes risk than the offspring of a parent with diabetes, even though siblings and offspring share approximately half of their genome with their diabetic pro-



From Steck, et al. Type 1 diabetes mellitus in man: genetic susceptibility and resistance. In Immunology of Diabetes, electronic book available at www.barbaradaviscenter.org.

Figure 3. HLA Region and IDDM Susceptibility. Schematic representation of the HLA region on Chromosome 6 showing microsatellite markers, loci, and alleles associated with IDDM susceptibility. Distances between loci are grossly approximated (20).

band. Siblings can share both HLA haplotypes which are identical to their proband, whereas offspring inherit only one haplotype from their single diabetic parent. Furthermore the same designated haplotype (i.e. the highest risk DR3/DR4-DQ8) can be identical-by-descent from parent of origin between siblings or not. Therefore sharing of multiple genetic polymorphisms of DR, DQ genes and non-DR, DQ genes linked to the MHC region on both copies of chromosome 6 could cause the increase sibling risk, as high as 80% (Figure 4) (27).

"Ancestral" MHC haplotypes extend over 1 million nucleotides so that a series of polymorphisms and gene loci are remarkably conserved in almost total linkage disequilibrium (28-30). This has been confirmed by throughput SNA analysis and extensive resequencing of the MHC region.

AUTOIMMUNITY

The islet beta-cell zinc cation efflux transporter Znt8 (Scl30A8) is a major newly defined (31). This transporter was discovered as an autoantigen because it is specifically expressed in islet beta–cells, where it is associated with the regulated pathway of insulin secretion. Znt8 facilitates the transportation of Zn^{2+} from the cytoplasm into the insulin secretory granule and the concentration of Zn^{2+} within the granule lumen where the zinc cation binds to insulin hexamers. Fluid phase radioassays have already been validated for autoantibodies to this autoantigen in the most recent CDC affiliated DASP workshop and approximately 60% of new onset patients have autoantibodies reacting with the zinc transporter. These radioassays follow the earlier development of radioassays for autoantibodies reacting with insulin, GAD65 (Glutamic Acid Decarboxylase) and IA-2 (Insulinoma Associated). Insulin autoantibodies develop within weeks of the starting of subcutaneous injection of insulin, and, thus, after insulin therapy measurement of insulin autoantibodies is not useful. Assays for each of the above 3 autoantibodies can be set at the 99th percentile of controls and approximately 90% of children with new onset diabetes express either one or the other autoantibody (Figure 5).

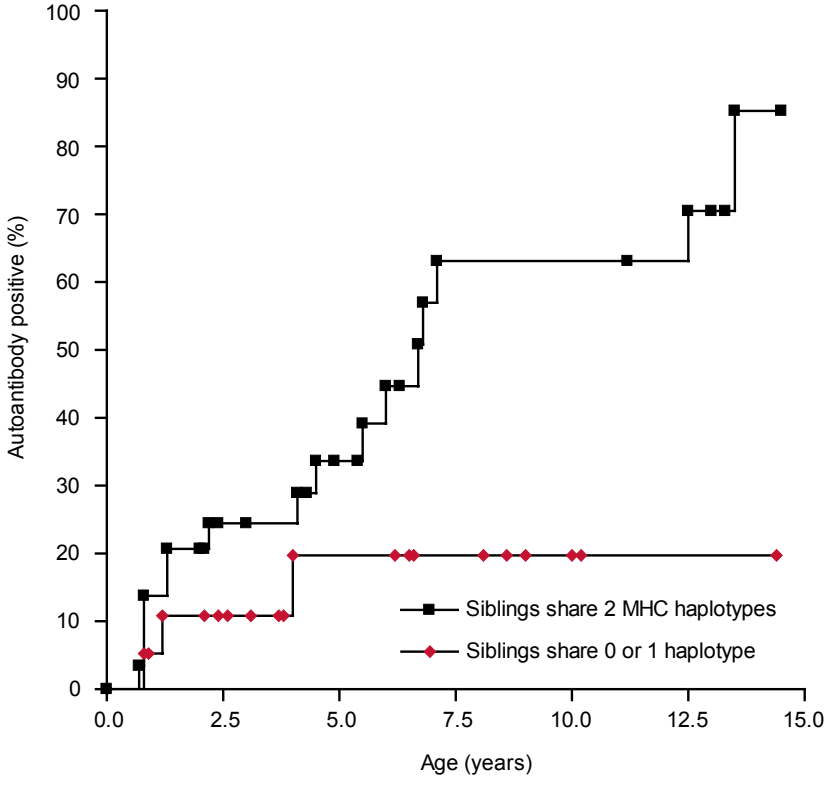


Figure 4. Extreme risk for diabetes autoimmunity. Life table analysis of DR3/4-DQ2/8 siblings of patients with type 1 diabetes in the DAISY study followed from birth for the development of anti-islet autoantibodies. These relatives with the highest risk DR3/4-DQ2/8 HLA genotype were subdivided by the number of HLA haplotypes inherited identical by descent to their proband diabetic sibling. High risk cohort are DR3/4-DQ8 siblings that share both MHC haplotypes identical-by-descent with their proband, N=29. Low risk cohort are DR3/4-DQ8 siblings that do not share both MHC haplotypes identical-by-descent with their proband, N=19 (27).

Aly et al. Extreme genetic risk for type 1A diabetes. PNAS 2006. 103:14074.

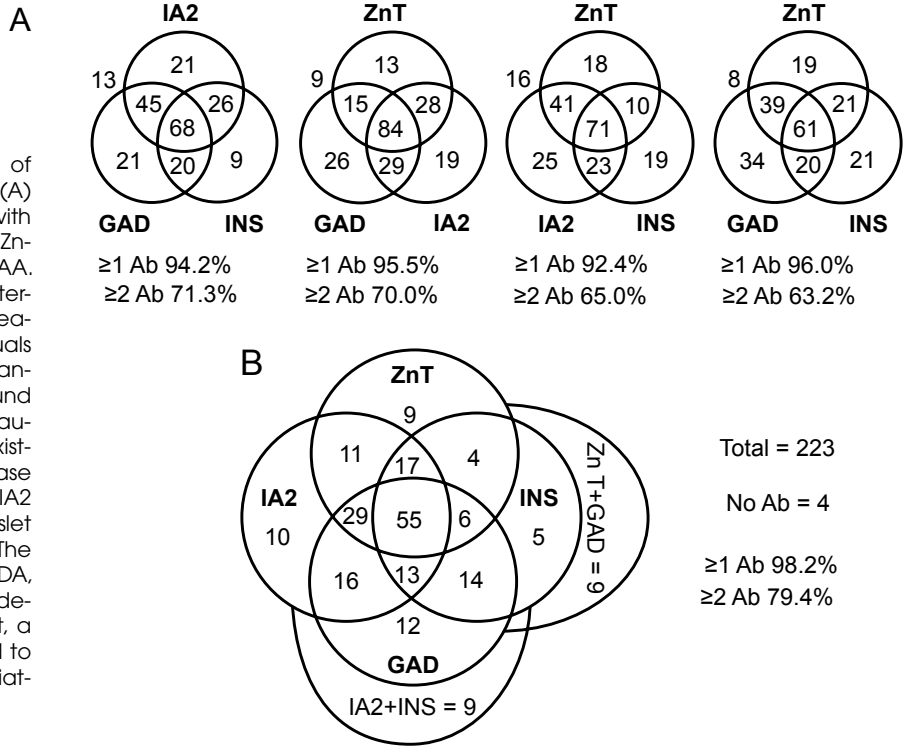


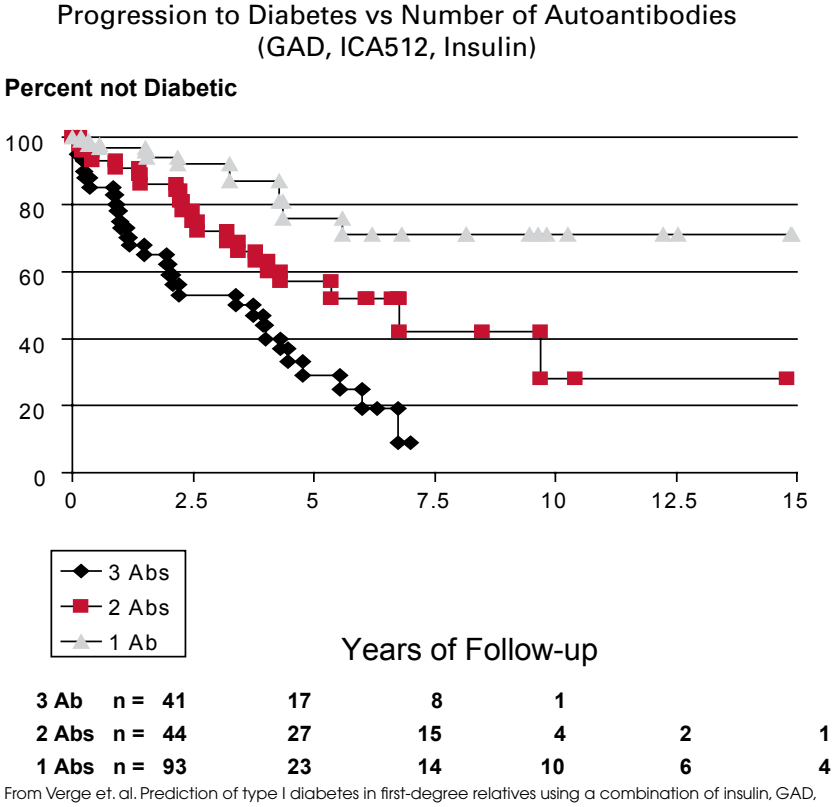
Figure 5. Overlapping prevalence of ZnT8A, GADA, IA2A, and IAA at onset. (A) Seropositive individuals evaluated with three-autoantibody standard or with ZnT8A substituted for GADA, IA2A, or IAA. The ZnT8A assay incorporates both C-terminal and N/C assays in the one measurement. (B) Seropositive individuals evaluated with four-autoantibody standard. ZnT8 antibodies (ZnTA) were found in 26% of T1D subjects classified as autoantibody-negative on the basis of existing markers (glutamate decarboxylase (GADA), protein tyrosine phosphatase IA2 (IA2A), antibodies to insulin (IAA), and islet cytoplasmic autoantibodies (ICA)). The combined measurement of ZnT8A, GADA, IA2A, and IAA raised autoimmunity detection rates to 98% at disease onset, a level that approaches that needed to detect prediabetes in a general pediatric population (32).

Modified from Wezlau JL. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. PNAS. 2007; 104:17040-5.

A higher threshold of specificity is usually required for the prediction of type 1A diabetes (Figure 6) (32) and the presence of ≥2 of four biochemical autoantibodies indicates high risk (33) exceeding 90% with long term (decades) follow-up. Modified ELISA assays that utilize binding of GAD65 to antigen captured by plate bound anti-GAD antibodies can perform as well as the fluid phase radioassays and kits for such assays are now available (4). Despite excellent assays, a subset of children with new onset diabetes are still negative for all anti-islet autoantibodies (33).

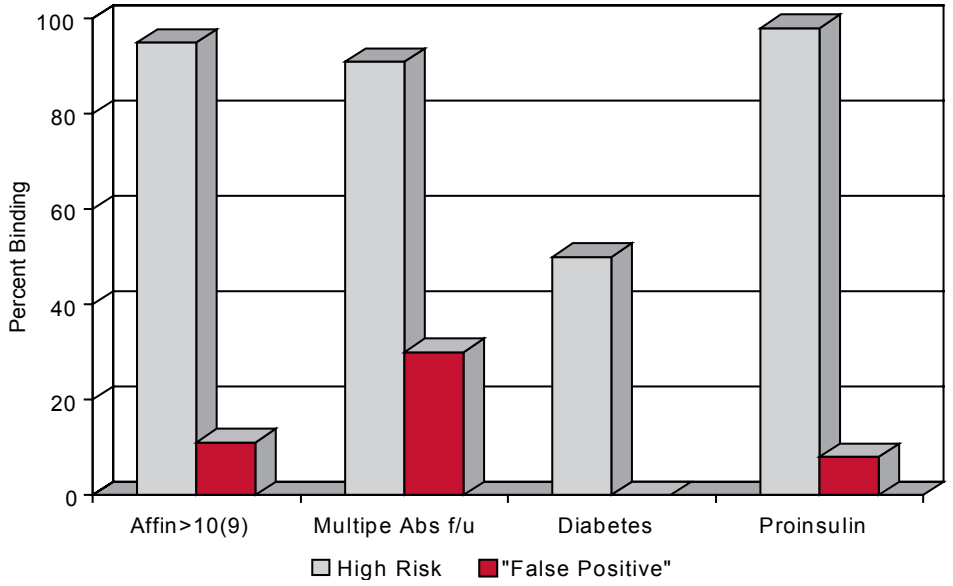
Given genetic susceptibility, the first islet autoantibody to appear during the first five years of life is usually autoantibodies to insulin (30). Subsequently GAD65 autoantibodies may be the first to appear and insulin autoantibodies become less common, such that if onset of diabetes is after age 12 the majority of children do not express insulin autoantibodies (34). GAD65 autoantibodies are the most common in adults with Latent Autoimmune Diabetes of Adults (LADA) (35). We believe LADA is type 1A diabetes developing in an adult, diagnosed prior to development of ketoacidosis and a severe insulin deficiency.

The insulin antibody affinity and epitope specificity for insulin autoantibodies can predict which children progress to diabetes. Children with high genetic risk who develop insulin antibodies (IAAs) early in life may subsequently develop multiple antibodies and eventually diabetes (36). Using a competitive radiobinding-assay Achenbach et al measured IAA affinity in sequential IAA-positive samples from children who are followed by birth in the BABYDIAB cohort in Europe. All high-affinity IAAs required conservation of human insulin A chain residues 8-13 and were reactive with proinsulin. High affinity was associated with HLA DRB1*04, young age of IAA appearance, and subsequent progression to multiple islet autoantibodies or type 1 diabetes and thus identifying children at high risk (Figure 7). Of note the data were consistent with the early and sustained presentation of proinsulin in the context of the highest risk allele HLA DR4. The same group followed autoantibodies to GAD (GADAs) for heterogeneity in affinity and epitope recognition in the BABYDIAB cohort of children (37). Affinity was higher in multiple islet autoantibody-positive children and in children who carried the HLA DR3 haplotype.



From Verge et. al. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes. 1996;45:926-33.

Figure 6. Progression to type 1 diabetes of relatives of patients with type 1 diabetes subdivided by number of "biochemical" anti-islet autoantibodies for GAD, ICA512 (IA-2) and insulin (31).



Modified from Achenbach et al. Mature high-affinity immne resposes to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes. J Clin Invest. 2004;114:589.

Figure 7. Mature high-affinity immune responses to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes (30).

At present we know relatively little concerning the pancreatic pathology of the prediabetic process in man, but a recent collaborative study, Network for Pancreatic Organ Donors with Diabetes (nPOD), sponsored by the JDRF is seeking to address this lack. In particular pancreases from cadaveric donors expressing islet autoantibodies have been analyzed and their histology made available on a web page (www.jdrfnPOD.org). Initial studies suggest that individuals expressing multiple islet autoantibodies are likely to have insulitis (38,39), but it is likely that there will be several different pathologic processes leading to beta-cell destruction. Insulitis is not uniform and the same pancreas will have normal islets, pseudoatrophic islets (islets lacking all insulin producing cells) that have no insulitis, and islets with insulitis (40). The insulitis of man, concordant with the chronic nature of the development of type 1A diabetes, is usually relatively mild in terms of number of islet involved at any given time. A major question is whether islet insulin-producing cells remain in patients with longstanding type 1A diabetes. Current evidence suggests that for most individuals less than 1% of islet beta-cell mass remains, but there are individuals with long-term type 1 diabetes with remaining islet beta-cells (41).

Though islet autoantibodies are measured to aid the diagnosis and prediction of type 1A diabetes the disease is most likely T-cell mediated. (Even T-cell mediated disorders can be dependent upon B lymphocytes, particularly for antigen presentation, and a trial of anti-CD20 antibodies in new onset patients by TrialNet is now fully enrolled.) The measurement of T cells targeting islet autoantigens, and in particular T cell assays with the ability to distinguish patients with type 1 diabetes from controls, has been particularly difficult. Recent assays measuring response of memory T cells in contrast to naïve T cells (naïve T cells of controls respond to islet autoantigens) holds promise for the development of predictive T cell assays (42,43).

METABOLIC PROGRESSION

Given the recognition of the chronic nature of the development of type 1A diabetes and the existence of excellent islet autoantibody assays, it has been possible to define multiple metabolic parameters that detect abnormalities prior to the onset of diabetes. Loss of first phase insulin secretion following intravenous glucose develops in most individuals months to years prior to the onset of diabetes (44). Similarly, abnormalities on oral glucose tolerance testing, especially at the two-hour time point, usually precede diabetes (45,46). In children followed to the onset of diabetes a chronic rise

of HbA1c within the normal range usually precedes the diagnosis (46). It is likely that most patients that present with type 1A diabetes have had hyperglycemia for months prior to diagnosis.

OVERT DIABETES

It is clear that beta-cells of the mouse can regenerate with recovery from acute destruction and severe hyperglycemia (47). Islet beta-cells appear to arise primarily from replication of existing beta-cells in the mouse. (48) As NOD mice progress to diabetes beta-cell replication increases, apparently slowing progression to diabetes, with diabetes occurring when approximately 20% of beta-cells have been destroyed. With immunotherapy at the onset of overt diabetes recovery of beta-cell function can be demonstrated in the NOD mouse (49). Evidence for beta-cell replication has been presented for man at onset of diabetes (50) and functioning beta-cells can remain in long-term patients. Unfortunately beta-cell mass appears to be severely compromised and potential for replication in man is unknown (51). Patients with more than fifty years of type 1 diabetes are being studied at the Joslin Diabetes Center (50-year Medalist Study) with a subset of patients still expressing limited amounts of C-peptide (52). Understanding the limits of islet beta-cell replication in man and the pathway from islet stem cell to mature islet beta-cell is an important avenue to achieve beta-cell replacement therapies(53).

CONCLUSION

At present we still lack the tools for *in vivo* imaging of either beta-cell mass or insulitis in man. In animal models a number of techniques have shown promise (54,55), but to date we either lack sufficient data in man to assess utility or the studies have been inconclusive. It is likely that methods that have been utilized to image insulitis in the NOD mouse model (vascular leakage imaged with iron nanoparticles) will be difficult to apply to man if our current understanding of histology of new onset pancreas is accurate. There is relatively little insulitis in man and the insulitis is non-synchronous. With the lack of ability to image the pancreas, our understanding of the natural history of the disease comes primarily from indirect measurements of insulin secretion and the ability to detect anti-islet autoimmunity through measurement of autoantibodies and autoreactive T-cells. It is estimated that approximately 1/300 individuals in the United States express ≥2 biochemical islet autoantibodies (39). Programs such as nPOD (Pancreas of Diabetics) hopefully will provide additional understanding of the pathology of the disorder at all stages, including the "prediabetic" phase, and this will provide histologic data to help guide development of imaging modalities. In the absence of firm knowledge that would be provided with imaging modalities, we believe that type 1A diabetes develops for most individuals as a result of chronic progressive beta-cell destruction and that the process once initiated (e.g. expression of ≥2 biochemical autoantibodies) very few individuals escape from almost complete beta- cell destruction. We believe a concentred effort to find individuals who escape progression to diabetes should be undertaken to both better define the natural history of the disease and to search for factors that might naturally abrogate the pathologic process leading to type 1 diabetes.

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