Type 1 Diabetes—Pathogenesis, Prediction, and Prevention

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Abstract

Type 1 diabetes affects over 1.4 million people in the US, with a rising incidence in many western nations. It is clear that there is a strong hereditary component and that autoimmunity plays a large role in disease pathogenesis. In the last two decades novel technologies have been developed to study the genetics, biochemistry, and molecular pathology of type 1 diabetes. These, in turn, have allowed for early recognition of disease as well as the potential for prevention trials and early insulin treatment. This article highlights the prediction of type 1 diabetes risk and developing immunotherapeutic concepts.

Keywords

Autoimmune polyendocrine syndrome (APS), autoantibodies, autoimmunity, environmental risk, genome-wide association study, human leukocyte antigen (HLA), insulin, major histocompatability complex (MHC), pancreatic beta cells, trimolecular complex

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Type 1 diabetes has become one of the most studied polygenic disorders. It effects over 1.4 million people in the US, with a rising incidence in many western nations.[1,2] It is clear that there is a strong hereditary component in the development of disease, with siblings at higher risk than offspring and both at higher risk than the general population. It is also clear that autoimmunity plays a large role in disease pathogenesis. Development is insidious and chronic, but the initial presentation is often acute (hyperglycemia, ketoacidosis, and cerebral edema) and can be deadly as it is often unexpected.[3]

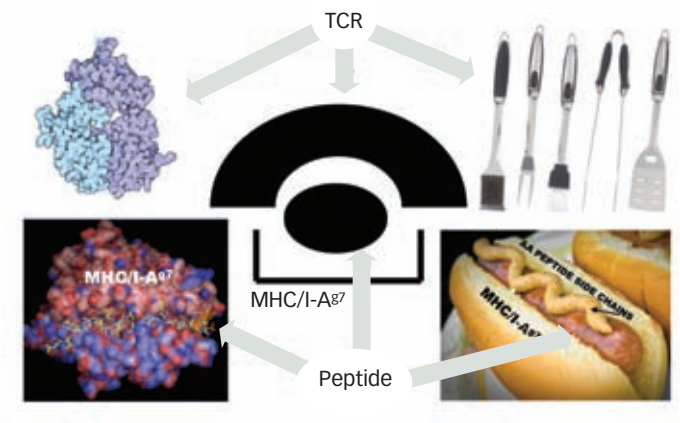
In the last two decades novel technologies have been developed to study the genetics, biochemistry, and molecular pathology of type 1 diabetes. These, in turn, have allowed for early recognition of disease, as well as the potential for prevention trials and early insulin treatment. In this article we will highlight the prediction of type 1 diabetes risk and developing immunotherapeutic concepts.

Immunology and Pathophysiology of Type 1 Diabetes

An overwhelming amount of evidence in the last several decades points to type 1 diabetes being an autoimmune, specifically T-cell-mediated, disease. Through study of multiple animal models, including the non-obese diabetic (NOD) mouse (which has many similarities to human type 1 diabetes), the pathogenesis of autoimmune beta-cell destruction is becoming clearer. Central to T-cell response, including autoimmune responses, are components of the trimolecular complex. For CD4-positive T cells this complex consists of the T-cell receptor (TCR), an antigenic peptide, and a human leukocyte antigen (HLA) molecule on antigen-presenting cells (APCs) (e.g. the NOD mouse I-Ag7, homologous to HLA class II DQ of humans).

Figure 1 illustrates the trimolecular complex, which can be likened to a hotdog (the peptide), bun (class II or class I molecules of the major histocompatability complex or MHC), and barbecue instruments (the TCR). The peptide sequence being presented to the TCR sits in the groove of class II or class I molecules on the surface of an APC. The TCR is then able to recognize it, bind to it (with varying affinity dependent on molecular shape and charge), and mount an immune response. The TCR is crucial for T-cell selection in the thymus as well as immune targeting of peptides by mature T-lymphocytes. The receptor structure includes variable alpha and beta chains, both with germline-encoded V and J sequences. These segment sequences are 'randomly' combined to form literally billions of different TCRs that recognize specific antigenic sequences.[4,5] When an antigenic peptide is presented by thymic

Figure 1: The Trimolecular Complex



MHC = major histocompatibility complex; TCR = T-cell receptor.

epithelium (via major histocompatibility [MHC] class I and II molecules), the TCR binds to it. In the absence of any TCR engagement in the thymus, T cells will die by 'neglect.' If recognition in the developing thymus is modest (due to weak binding related to variations in the presenting molecule, the antigenic peptide, and the TCR binding sequence), T-cells fail to be 'deleted.' They then leave the thymus and enter the peripheral circulation. A subset of autoreactive T-cells fail to be deleted in the thymus and can react with self-antigens in the periphery. Self-antigen reactivity can occur by several mechanisms, including modification of self-molecules in the periphery but not the thymus (e.g. citrinylated peptides), failure of the thymus to express certain peripheral antigens in concentrations that are sufficient to delete all self-reactive T cells, and innate immune activation of self-reactive T-cells in the periphery. These peripherally activated, autoreactive T cells can then trigger a cascade of events leading to a large-scale immune response that ultimately ends in tissue destruction.

Insulin peptide sequences are now thought to be central to the development of autoimmunity in the NOD mouse.[6–14] The insulin B:9–23 peptide sequence may be of particular importance in loss of tolerance leading to diabetes.[9,14] In the mouse there are two insulin genes (insulin 1 and 2) that form nearly identical preproinsulin molecules. Insulin 1 and 2 are both expressed in pancreatic islet beta cells, but only insulin 2 is expressed in the thymus. Ideally, insulin-reactive T cells would be deleted in the thymus. Knocking out insulin 2 accelerates the development of type 1 diabetes, while knocking out insulin 1 prevents the majority of type 1 diabetes development.[15] Thus, it is likely that attenuated expression of insulin 2 in the thymus of knockout mice enhances autoimmunity by decreasing negative selection in the thymus, while eliminating Insulin 1 in the periphery may remove an important islet target peptide. Of note, knocking out both insulin genes and providing mice with a single mutated insulin gene (replacing beta-chain 16 tyrosine with alanine) prevents all diabetes of NOD mice.[13,16–18]

A specific V alpha segment of the TCR, TRAV5D-4*04, appears to play a unique role in targeting the B:9–23 sequence of the insulin molecule. Experiments involving variation of this specific alpha chain sequence but conservation of other elements in the beta and alpha construct of the TCR have led to the hypothesis that this V-alpha segment is important in enhancing diabetes susceptibility.[19–21] The final component in the trimolecular complex in the NOD mouse is I-Ag7, homologous to the DQ8 HLA class II molecule in humans. HLA class II molecules play a major role in the development of autoimmunity (see below). Human DR3–DQ2 and DR4–DQ8 haplotypes, which are closely associated with type 1 diabetes risk, have similar polymorphisms to I-Ag7.[22,23] These polymorphisms alter the peptides bound and presented to TCRs, and thus alter self-antigen recognition as described above.

Although in the NOD mouse model there are convincing data supporting the hypothesis that insulin is the primary autoantigen, studies in humans are not definitive. In particular, although insulin autoimmunity is prominent and polymorphisms of the insulin gene influence diabetes risk, there are multiple islet autoantigens targeted in humans. Autoantibodies to IA-2, glutamic acid decarboxylase (GAD), and the newly discovered autoantigen ZnT8 (discussed in more detail below) are important markers of disease risk. Furthermore, loss of tolerance and development of autoimmunity clearly depend on more than the trimolecular complex recognition of insulin. Environmental factors and polymorphisms of non-MHC genes involving maintenance of tolerance can play a distinct role in disease development (see below). Inability to maintain tolerance is a key aspect of the NOD mouse model and humans.

Disease Prediction in Type 1 Diabetes

Genetic Markers

Approximately one in 300 individuals in the general population in the US develop type 1 diabetes, while approximately one in 20 first-degree relatives of patients with type 1 diabetes (offspring or sibling) develop diabetes.[24,25] More than 60% of monozygotic twins with a twin-mate having type 1 diabetes will develop diabetes and more than 70% develop anti-islet antibodies.[26] Dizygotic twin risk is much lower and similar to that of siblings (again, one in 20 or 5%).[27]

Environmental factors play a role in the development of type 1 diabetes. This is evident by the lack of 100% concordance in twin studies, the increasing incidence of type 1 diabetes worldwide (at a rate too fast to be explained by genetic changes alone), potential disease links to medications, temporal associations with environmental factors (e.g. diet and viral infections), and variability of disease penetrance in mouse models with different environmental exposures.[28–34] One recent study followed monozygotic and dizygotic twins for 10 years and reported that 88% of phenotypic variance was due to genetics, while 12% could be attributed to the environment.[35] More research is warranted in this field, and studies such as The Environmental Determinates of Diabetes in the Young (TEDDY) are under way to help explore these issues.

Regarding genetic susceptibility to diabetes, there are well-known single-gene causes of autoimmune diabetes. They include autoimmune polyendocrine syndrome type 1 (APS1) caused by mutations in the *AIRE* gene and immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome caused by mutations in the *FOXP3* gene. Both of these syndromes are well studied and have contributed to current understanding of diabetes pathophysiology. The *FOXP3* gene in particular is essential for the development of regulatory T cells, and

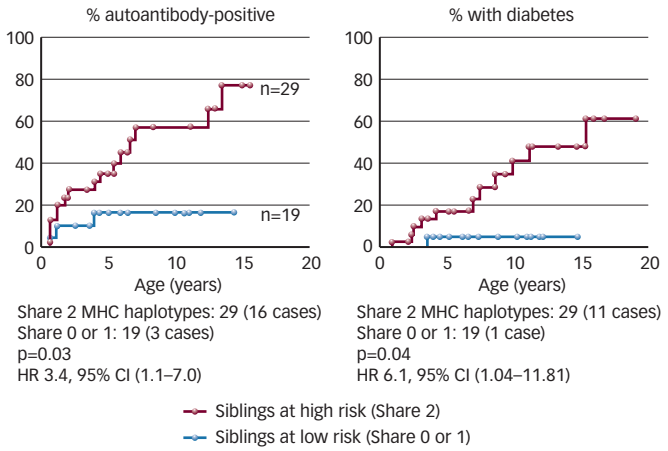
approximately 80% of children with *FOXP3* mutations develop type 1 diabetes with onset as early as the first days of life.[36] The *AIRE* gene controls expression of peripheral antigens such as insulin in the thymus and it is hypothesized that lack of multiple peripheral antigens in the thymus of individuals with mutations of the AIRE gene contribute to their widespread autoimmunity.[37] Other diseases contributing to the knowledge of diabetes pathogenesis include autoimmune diseases known to be associated with type 1 diabetes, e.g. Addison's disease, celiac disease, pernicious anemia, and thyroiditis.[38] These polygenic conditions reflect the immunogenetics behind common forms of autoimmune diabetes. Approximately one-third of children with new-onset type 1 diabetes have associated organ-specific autoimmunity (e.g. thyroid peroxidase, transglutaminase, 21-hydroxylase autoantibodies).

The MHC class II region on chromosome 6 has long been linked to diabetes susceptibility. Effects of high-risk alleles in this region are consistent across different ethnicities despite large differences in allele frequencies.[39] There is also significant homology between species, with defined genes and regions of risk in man, NOD mice, and susceptible rat strains.[23,40–43] The extremely high-risk genotype DR3/4–DQ2/DQ8 (DR3–DQA1*501–DQB1*201; DR4–DQA1*301–DQB1*302) occurs in 2.4% of Denver newborns, and 30–40% of all type 1 diabetes patients carry this heterozygous genotype. Children with this genotype have an absolute risk of one in 15 versus one in 300 in the general population.[24,25] In long-term follow-up studies of 30,000 newborns (selected for either high-risk HLA or a first-degree relative with type 1 diabetes), we find that 41% of DR3/4 siblings, as well as 16% of offspring of type 1 diabetes patients, expressed islet autoantibodies by seven years of age. Furthermore, in siblings identical by descent for both DR3/4 haplotypes, 63% had positive autoantibodies by seven years of age and 85% were positive by 15 years of age (see *Figure 2*). This is in contrast to only 20% developing autoantibodies in DR3/4 siblings sharing no or one haplotype identical by descent.[44] Within the general population, the DR3/4 genotype combined with analysis of DP alleles (absence of protective DPB1*0402) and DR4 (absence of DRB1*0403) confers a 20% risk of developing islet autoimmunity.[45]

While 2.4% of the population of Denver carry the DR3/4 heterozygous genotype, over 30% of patients with diabetes have this genotype.[27] Interestingly, either DR3 or DR4 haplotypes in homozygous form (DR3/DR3 or DR4/DR4) are lower-risk compared with the DR3/4 genotype. The mechanism for the increased heterozygote risk is not completely delineated, but it has been hypothesized that the DQA1*0501 allele of DR3 haplotype and the DQB1*0302 allele of DR4 haplotype combine, creating a 'chimeric' molecule (DQA1*0501, DQB1*0302) for antigen presentation that increases the risk of diabetes.[40] There are also MHC class II alleles that are protective (see *Table 1*): DQB1*0602 is present in 20% of the population but only 1% of children with type 1 diabetes. Other protective alleles include DRB1*0403 (even when DQB1*0302 is present) and DRB1*1401.[40,46] Even within DRB1*04 alleles there are variations conferring greater and lesser risk.[47,48]

MHC class I loci (HLA-A, B, and C) play a lesser role in diabetes susceptibility. A24 is associated with a younger age at presentation and A30 is associated with higher risk; A1 is lower-risk than other HLA-A alleles when associated with the DR3–B8 haplotype.[49] HLA-B18, B39, B44, and B8 are all associated as well, with HLA-B39 conferring higher risk in three different populations and HLA-B8 being lower-risk when linked with the DR3 allele in the well-known extended haplotype with DR3, HLA-B8, and HLA-A1.[50,51] HLA-C3, C8, and C16 have been reported to increase susceptibility.[51] Extensive long-range linkage dysequilibrium between alleles of genes of the MHC make it difficult to pinpoint specific genes contributing to risk. One of the most common extended haplotypes consists of DR3–B8–A1 alleles, termed the 8.1 haplotype (containing DRB1*0303–DQA1*0501–DQB1*0201–HLA-B8–HLA-A1). This is the most common extended haplotype in the Caucasian population, with over 99% identity across the MHC by single nucleotide polymorphism (SNP) analysis. Interestingly, it is increased in type 1 diabetes individuals (18%, versus 9% of Caucasian controls)[52] due presumably to the DR3 and DQ2 alleles and not as much to the HLA-B8 and HLA-A1 portion of the haplotype. The DR3–B8–A1 haplotype confers less risk than other DR3 haplotypes. Higher risk was found in the less common DR3–B18–A30 haplotype (Basque haplotype) as well as other non-B8 DR3-positive individuals.[53,54] This would point to susceptibility loci telomeric to class II alleles. For several years, interest has turned to regions outside the MHC region for susceptibility to type 1 diabetes. Because there is a known correlation between development of diabetes and anti-insulin antibodies

Figure 2: Genetic Risk of Siblings of Patents with Type 1 Diabetes



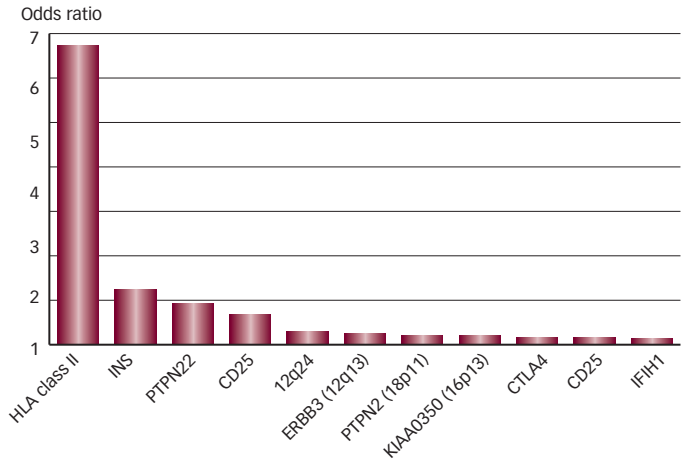
DR3/4 (a high-risk genotype in type 1 diabetes) confers extreme genetic risk for siblings of patients with type 1 diabetes if they have inherited both HLA-DR3 and DR4 identical by descent. By contrast, the DR3/4 genotype of siblings confers much lower risk if either the DR3 or the DR4 haplotype was not the same haplotype found in the proband sibling.[40,44]
HR = hazard ratio; MHC = major histocompatibility complex; CI = confidence interval.

Table 1: DRB1*04 Subtypes Conferring Varying Degrees of Risk in Patients with Diabetes

HLA-DRB1*04	HLA-DQB1	Odds Ratio (OR)
0405	0302	11.4
0401	0302	8.4
0402	0302	3.6
0404	0302	1.6
0403	0302	0.27
0401	0301	0.35

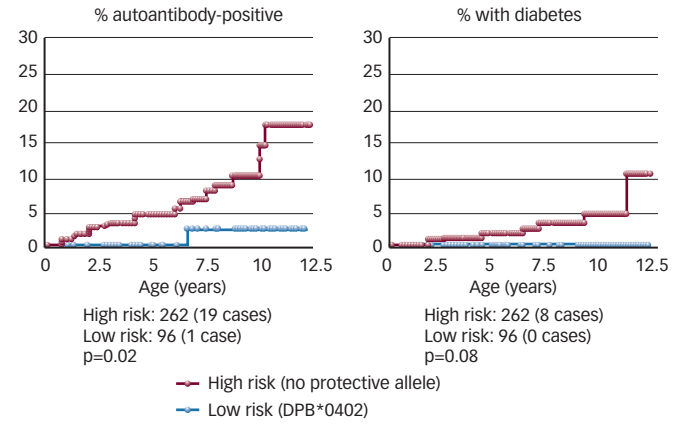
*Note the extreme risk of DRB1*0405 and DRB1*0401 allele and the protective nature of DRB1*0403.[40]*

Figure 3: Non-human Leukocyte Antigen Loci Found to Have an Association with Diabetes



Over 40 non-human leukocyte antigen (HLA) loci have been found to have an association with diabetes via genome-wide association. Here, some of the highest are listed. Note that the scale starts with an odds ratio of 1. The HLA region is by far the most important, with an odds ratio of over 6.5, while most others do not exceed an odds ratio of 2.[68]

Figure 4: Results of the DAISY Study



(see below), the insulin gene has been of particular interest. There is a variable number tandem repeat sequence (VNTR) at the 5´ end of the insulin gene that has been known for over two decades to be associated with risk in type 1 diabetes. Longer repeats are protective and are associated with increased insulin expression in the thymus.[55,56] Differences in expression of the insulin 2 versus insulin 1 gene in NOD mouse thymus presumably relate to the same mechanism (see above).

PTPN22 is located on 1p13 and encodes for protein tyrosine phosphatase non-receptor type 22 (PTPN22)/lymphoid phosphatase (LyP). Position 1858 contains a non-synonymous SNP that changes arginine to tryptophan at position 620. This polymorphism results in a gain of function that increases inhibition of TCR signaling. Many groups have confirmed its presence in type 1 diabetes patients in many different populations, with an odds ratio of 3.4 in its homozygous form.[57–59] It is hypothesized that this SNP decreases T-cell signaling, thereby decreasing negative selection in the thymus. This risk allele is therefore associated with many autoimmune diseases.[60] Genome-wide association studies have also been performed on type 1 diabetes patients in hopes of finding other loci of interest. High-density SNP analysis (over 300,000 per individual) and follow-up meta-analyses have added to the list of regions involved in type 1 diabetes. Several signals of interest include confirmation of the *MHC*, *PTPN22*, cytotoxic T-lymphocyte antigen 4 (*CTLA4*), and insulin gene loci. Other genes of interest include those encoding CD25/interleukin-2 receptor alpha (IL2RA) and interferon-induced helicase C domain-containing protein 1 (IFIH1); the strongest signal from the latter three genes was in *CD25/IL2RA*, with more than one SNP associated with risk. *CTLA4*, despite an odds ratio of 1.1–1.2, has been implicated in multiple studies and is known to be strongly involved in T-cell signaling.[61–67] More than 40 loci are now firmly associated with risk of type 1 diabetes, with the strongest signals by far associated with the MHC (see *Figure 3*).[68]

Serological Markers

Autoantibody development and insulitis are the end result of loss of self-tolerance and a component of the heightened immune response that results in destruction of beta cells in the pancreatic islets. Numerous antibodies are generated from a very early age in type 1 diabetes-susceptible patients. The most important autoantibodies include (in typical order of appearance chronologically) anti-insulin, anti-GAD65, anti-IA-2, and anti-ZnT8 antibodies.[69–71] Presence of multiple islet autoantibodies is the most important predictor of progression to disease in type 1 diabetes.[72] Development of autoantibodies can begin as early as four to 12 months of age, with earlier development correlating with greater risk of progression to overt disease (type 1 diabetes).[73] For some patients with pre-diabetes, autoantibodies do not appear before 50 years of age. Insulin autoantibodies, often the first to appear, can be a predictor of severity as their levels are inversely related to age at disease onset.[74] Furthermore, if two or more of the above antibodies are elevated (with each assay set at positivity ≥99 percentile of normal populations), both relatives and individuals in the general population will 'inevitably' develop overt disease (>90%) versus individuals with only one antibody (20%).[44,75,76]

In the DAISY Study, the development of autoantibodies in DR3/4 general population individuals is influenced by DP alleles (see *Figure 4*). The rate of progression to diabetes increases in direct proportion with autoantibody positivity, with autoantibody development often preceding disease onset.[77] Individuals can express autoantibodies for decades prior to hyperglycemia. Despite a percentage of false or transiently positive individuals, people deemed 'high-risk' via genotyping can be followed with a reasonable prediction of disease progression such that prevention trials are under way.[78] These individuals, found in studies such as DAISY, can also then be followed with glucose tolerance testing and glycated hemoglobin (HbA_{1C}) to diagnose hyperglycemia early, and often they can be started on insulin therapy without hospitalization or the development of ketoacidosis.[79,80]

Disease Modification in Type 1 Diabetes

For the last few decades standards of management for type 1 diabetes have centered on glucose monitoring and insulin replacement therapy. While the technology has greatly improved with regard to insulin pumps and continuous glucose monitoring to simulate as best as possible physiological pancreatic beta-cell function, it is by no means a perfect

solution to the disease. Currently, efforts are under way to prevent beta-cell loss or replace lost cells via beta-cell regeneration or transplantation of islets. Subjects who undergo islet cell transplant often initially develop insulin independence and markedly improved glucose levels. However, these benefits are short-lived with a significant number losing insulin independence over long-term (two- and five-year) follow-up. There remains some benefit with improved blood glucose control and prevention of severe hypoglycemia, however.[81] Toxicity of immunosuppressive regimens and failure of islet grafts with time suggest that for most patients complications of therapy outweigh the benefits, and for now islet transplantation is still in development.[82]

It was reported more than 20 years ago that horse anti-thymocyte globulin or cyclosporine therapy prolongs the honeymoon phase in new-onset diabetic patients.[83,84] Since the initial publications, many therapies have been studied to modulate the immune system both generally as well as through targeting specific antigens. General immunosupression is a poor option for treatment and prevention of diabetes, given the very high cost–benefit ratio. Trials using oral insulin (through the National Institutes of Health [NIH] Diabetes Prevention Trial) to stimulate self-tolerance in the subgroup of individuals with high levels of anti-insulin antibodies showed some promise only in individuals with high levels of insulin autoantibodies by delaying the onset of diabetes. In several well-powered studies, however, the onset of disease could not be prevented overall.[69,85–89] Other promising therapies include vaccination with GAD65 (a known target of anti-islet antibodies) and monoclonal antibody therapy with anti-CD3 and anti-CD20.[89–91] Again, long-term arrest of disease progression has not been found with these therapies. While antigen-specific therapies are safer than broad immunomodulation, there is less evidence for efficacy. Still, there is optimism and ongoing research devoted to this area. Several phase III trials are either under way or planned with goals to delay loss of beta cells after onset of hyperglycemia or in autoantibody-positive high-risk individuals. In North America, individuals can be screened for islet autoantibodies and considered for participation in NIH-sponsored trials (either new onset or pre-diabetic) by contacting Trialnet (1-800-HALT-DM1).

Conclusion

There has been great progress in understanding type 1 diabetes in the last two decades. We can predict disease through genetic testing of alleles of genes in the MHC region combined with analysis of islet autoantibodies and metabolic function. The realization that type 1 diabetes is an autoimmune disorder associated with a series of additional autoimmune diseases, many with shared genetic loci (e.g. celiac disease, Addison's disease, thyroid autoimmunity), has led many centers to screen for these associated disorders. Current knowledge has also led to progress in trials of preventive therapies through manipulation of the immune response. It is hoped that in the years to come diabetes will be a preventable disease and the results of current phase III clinical trials will hopefully inform clinical care. ■

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