Cell. Mol. Life Sci. 64 (2007) 865 – 872
1420-682X/07/070865-8
DOI 10.1007/s00018-007-6469-4

Cellular and Molecular Life Sciences

Review

Cellular and molecular pathogenesis of type 1A diabetes

M.M. Jahromi[a, b] and G.S. Eisenbarth[a, *]

[a] Barbara Davis Center for Childhood Diabetes, University of Colorado, Health sciences Center, Aurora, Colorado 80010 (USA), e-mail: George.Eisenbarth@UCHSC.edu
[b] Pathology Department, Salmaniya Medical Complex, Ministry of Health (Kingdom of Bahrain)

Received 1 November 2006; received after revision 27 November 2006; accepted 8 January 2007
Online First 13 February 2007

Abstract. Type 1A diabetes is an organ-specific autoimmune disease resulting from destruction of insulin-producing pancreatic β-cells. The main susceptibility genes code for polymorphic HLA molecules and in particular alleles of class II MHC genes (DR, DQ and DP). Polymorphisms of individual genes outside the MHC also contribute to diabetes risk but recent evidence suggests that there are additional non-HLA genes determining susceptibility linked to the MHC. It is now possible using genetic and autoantibody assays to predict the development of type 1A diabetes in the majority of individuals, and trials of diabetes prevention are underway.

Keywords. T1AD, MHC, pathogenesis, autoantibody, β-cell, polyendocrinopathy, polygenic, oligogenic.

Introduction

The current American Diabetes Association classification system defines four major forms of diabetes mellitus. The four major subgroups are: type 1 and type 2, diabetes due to other known causes and gestational diabetes (Table 1). Type 1 diabetes is defined in etiologic terms, i.e. as diabetes caused by the destruction of insulin-producing β-cells, the consequence of which is an absolute dependence on insulin for survival. Type 1 diabetes is further subdivided according to whether β-cell destruction is caused by an immune (type 1A) or other (type 1B) processes [1].

Type 1 diabetes, formerly known as either juvenile-onset diabetes (because of the early age of onset) or insulin-dependent diabetes mellitus (because of the clinical need for insulin), is now widely thought to be an organ-specific autoimmune disease. Here we wish to summarize some generally accepted observations about this disease and then give our perspectives on unresolved issues surrounding the prediction, pathogenesis and treatment. In particular, we will discuss markers of the disease, such as autoantibodies against pancreatic islet cell antigens.

Prevalence and etiology of type 1A diabetes

Type 1A diabetes accounts for approximately 90% of childhood-onset diabetes and 5–10% of adult onset diabetes [2]. About 40% of persons with type 1A diabetes develop the disease before 20 years of age, thus making it one of the most common severe chronic diseases of childhood. In the USA, where 30,000 new cases occur each year [3], type 1A diabetes affects 1 in 300 children and as many as 1 in 100 adults. Diabetes (of all causes) is a leading cause of end-stage renal disease, blindness and amputation, and a major cause of cardiovascular disease and premature death in the general US population [3]. Approximately one-half of

* Corresponding author.

Table 1. Classification of diabetes mellitus

Types of Diabetes	Old nomenclature	Etiology
Type 1 Type 1A Type 1B	Juvenile-onset insulin-dependent diabetes mellitus	β-cell destruction. autoimmune unknown
Type 2	Non-insulin-dependent diabetes mellitus	insulin resistance/β-cell loss
Gestational		onset during pregnancy
Other types	secondary diabetes	specific genetic defects pancreas disease Endocrinopathies, etc.

Table 2. Defined Genes Associated with Genetic Risk

	Chromosome	λS	O.R.	LOD	p Genome-wide(GM)
IDDM1	6p21	3.35	"App 30"	116.3	1×10^{-4}
IDDM2	11p15	1.16		1.87	0.37
PTP22	1p13	1.05			ns
IDDM12	2q31-33	1.19 (CTLA1.01)	3 (CTLA1.1)	3.34	0.016
IL2RA/CD25	10p15.1		<0.13		1×10^{-10}(not GM)
IFIH1	2q24.3		0.82		1×10^{-10}(not GM)
ITPR3	6q21		2.9		1.3×10^{-6}(not GM)

value not GM = not corrected for genome wide analysis.

patients develop type 1 diabetes within the first two decades of life, but an increasing number of cases are being recognized in older individuals. The geographic incidence varies widely from less than 0.1/100,000 per year in China to more than 37/100,000 in Finland [4]. Of note, the incidence of type 1A diabetes is increasing in many Western countries, doubling approximately every 20–30 years [5].

Molecular genetics of Type 1A diabetes

The largest contribution to genetic susceptibility (Table 2) from a single locus comes from several genes located in the major histocompatibility complex (MHC) on chromosome 6p21.3 [6], accounting for at least 40% of the familial aggregation of this disease [7]. Depending on age of onset 30% to almost 50% of type 1 diabetes patients are heterozygous for HLA-DQA1*0501DQB1*0201 (termed DQ2)/ DQA1*0301DQB1*0302 (termed DQ8) with associated DR3 and DR4 alleles The DR2-associated molecule [HLA-DQA1*0102DQB1*0602 (DQ6)] is associated with dominant protection from the disease (DQ6 is carried by approximately 20% of the US population but less than 1% of children with type 1A diabetes). Residues important for the structure of the peptide binding groove of both HLA-DQ and DR molecules are associated with disease susceptibility and resistance.

Polymorphisms of a variable nucleotide tandem repeat (VNTR) 5 of the proinsulin gene are associated with risk (termed IDDM2) [8], and the long-form of the VNTR is associated both with protection from diabetes and increasesed insulin messenger RNA expression within the thymus [9]. Of note, whereas humans have a single insulin gene, mice have two insulin genes, termed insulin 1 and insulin 2 [10]. Both murine insulin genes are expressed in islets, while only insulin 2 is expressed in the thymus, and knocking out the insulin 2 gene (not the insulin 1 gene) greatly accelerates the development of diabetes [11, 12]. Thus for both human and mouse diabetes, risk relates to lower insulin expression within the thymus and is potentially related to the development of central T cell tolerance to insulin.

After the MHC and insulin gene, a single amino acid change of a gene termed PTPN22, a tyrosine phosphatase that influences T cell receptor signaling, influences diabetes risk [12]. The same polymorphism increases the risk of a series of autoimmune disorders including rheumatoid arthritis and lupus erythematosus. Polymorphisms of another gene associated with T cell regulation, the CTLA-4 gene, have a minor influence on genetic risk [13, 14]. A recent genome SNP screen confirmed the PTPN22 association with type 1 diabetes ($p<10^{-17}$) and implicated several other loci, including a locus with several potential associated genes, including an interferon-induced helicase gene (IFIH1) [15] in several populations, and independent replication is awaited. Similarly, a large study has

provided evidence for association with interleukin-2 receptor polymorphisms [16].

Over the past decade, there has been a major effort to define non-MHC-linked loci that together have a major influence on the development of type 1A diabetes. Recently, studies have provided evidence that additional loci, linked to the MHC but not classical HLA genes, may have a major influence on susceptibility [14, 17–20]. In particular, the DAISY study that has HLA typed from cord blood more than 30,000 newborns has found a risk of activating anti-islet autoimmunity approaching 80% (Fig. 1) for siblings of patients with the highest risk DR-DQ genotype (DR3/4-DQ2/8) who have also inherited both HLA haplotypes identical by descent with their diabetic sibling proband [17].

These extremely high risk children rapidly progress to diabetes following the appearance of anti-islet autoantibodies. Such a high risk, only fixing the MHC region, suggests that for this HLA genotype, genetic risk is almost totally determined by genes within or linked to the MHC region on chromosome 6, and with the current ability to readily analyze thousands of SNPs across the MHC, an intense effort is underway to define 'diabetogenic' genes in this region. A recent report indicates that a polymorphism of the ITPR3 (inositol tripohosphate receptor 3) gene [19], centromeric of the MHC, may be one such gene, and we and others have evidence that loci telomeric of the MHC may also contribute to risk [19, 21–23]. Of note, the MHC has a number of remarkable conserved and relatively common haplotypes, where for as much as 7 million base pairs unrelated haplotypes may be identical for more than 99% of their single nucleotide polymorphisms [17, 24, 25]. Such extended haplotypes were described more than two decades ago, with the most common being the haplotype with HLA DR3, A1 and B8 alleles, termed haplotype 8.1 [24]. Another DR3 haplotype that provides greater diabetes risk is the DR3, A30, B18 'Basque' haplotype [26]. The existence of such haplotypes with such remarkable conservation over such a long genomic distance needs to be factored in to the search for polymorphisms within the MHC associated with type 1A diabetes. For example, a polymorphism millions of base pairs from the DR gene can be in complete linkage disequilibrium with high-risk DR3-DQ2 alleles, and before an independent influence of that polymorphism can be accepted, analysis correcting for these extended haplotypes is essential.

In addition to the common form of type 1A diabetes, autoimmune diabetes also develops associated with other autoimmune disorders in three major syndromes [autoimmune polyendocrine syndrome (APS)-1, APS-2, and IPEX] [27, 28]. Type 1A diabetes

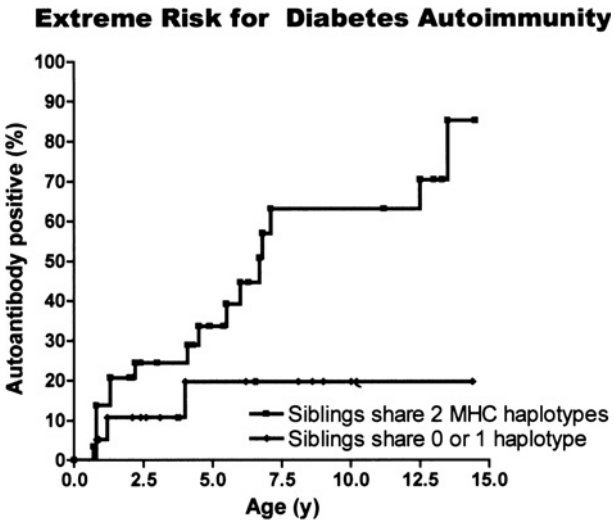

Figure 1. Life table analysis of DR3/4-DQ2/8 siblings of patients with type 1 diabetes in the DAISY study followed from birth for the development of anti-islet autoantibodies. These relatives with the highest-risk DR3/4-DQ2/8 HLA genotype were subdivided by the number of HLA haplotypes inherited identical by descent to their proband diabetic sibling [21].

is associated with immunoendocrinopathies, including primary adrenal insufficiency (Addison's disease), Graves' disease, autoimmune thyroiditis, myasthenia gravis, and celiac disease [29–32]. The presence in the same individual of two or more of the above disorders (as well as additional autoimmune diseases) usually defines APS-2. The genetic determinants of the component conditions and the APS-2 syndrome are not completely defined. Most of the component immunoendocrinopathies are associated with an [33, 34] increased frequency of DR3 in patients with or without type 1A diabetes. There is a preponderance of females among diabetes patients with APS-2, similar to what is observed in patients with thyroid and gastric autoimmunity in general, but unlike what is known for patients with isolated type 1A diabetes [35].

APS-1 is a rare autosomal recessive disorder with a characteristic triad of diseases of mucocutaneous candidiasis, hypoparathyroidism and Addison's disease. These patients also develop hepatitis and multiple additional autoimmune disorders (type 1A diabetes in approximately 15%). APS-1 results from mutations of the AIRE gene (autoimmune regulator). Though the disorder is almost always inherited in an autosomal recessive manner, at least one family with dominant inheritance has been described. The AIRE gene has a major influence on thymic deletion of T lymphocytes, at least in part by controlling expression of 'peripheral antigens' such as insulin in the thymus. A recent report documents the remarkable occurrence of anti-interferon-α autoantibodies in 100% of patients with the syndrome (first autoantibody of

APS-1 patients to appear and the anti-interferon autoantibodies persist) [36].

Another rare syndrome associated with autoimmune diabetes is the IPEX syndrome (immune dysfunction, polyendocrinopathy, exteropathy, X-linked). The IPEX syndrome results from a mutation of the FoxP3 gene. FoxP3 is an essential transcription factor for CD4+CD25+ regulatory T cells [37, 38]. That these children can develop type 1 diabetes in the first days of life illustrates the importance of regulatory T cells.

Heterogeneity of autoimmune diabetes is also apparent in three spontaneous animal models of type 1A diabetes, the BB (Biobreeding) [39], the NOD (non-obese diabetic) [40] and the Tokushima [41] rat. For all three strains, polymorphisms of genes of the MHC (the homologues of DR and DQ) are essential for disease [39, 41, 42].

For the BB and Tokushima rat, there are major loci outside the MHC that contribute to disease (oligogenic inheritance) and in particular for the BB rat, a recessive T cell lymphopenia gene [39] is necessary for disease in the absence of specific environmental factors that activate the innate immune system. The NOD mouse develops diabetes with more than 20 non-MHC loci contributing to susceptibility (polygenic inheritance) [42].

Environmental factors and type 1A diabetes

Despite decades of study, environmental factors triggering the development of anti-islet autoimmunity and type 1 diabetes have been difficult to delineate. Multiple environmental candidates have been proposed, in particular foods such as bovine milk and cereals, and viral infections. Congenital rubella infection clearly increases the development of type 1 diabetes, but only the rare congenital infection. Studies from Finland did not confirm in US and German studies, implicate enteroviral infections [43–45]. A large study (TRIGR) is evaluating the elimination of bovine milk in the first months of life [46]. It is clear that environmental changes can influence the development of type 1 diabetes, with the incidence in developed countries showing a significant increase (doubling approximately every 20–30 years) [47]. One hypothesis to account for the increase is termed the hygiene hypothesis, namely as infectious diseases decrease in populations, immune-mediated disorders such as asthma and diabetes increase [48].

Predicting type 1A diabetes

The first autoantibodies utilized for prediction of type 1A diabetes [49–52] were cytoplasmic islet cell autoantibodies (ICAs) and insulin antibodies. Currently, multiple sequenced autoantigens have been discovered and autoantibodies are measured with recombinant autoantibody assays [1, 2, 53]. The standard assays measure autoantibodies reacting with insulin [54–60], glutamic acid decarboxylase (GAD) [61], ICA512/IA-2 [62, 63], and I-A2 beta (phogrin) [64, 65]. Despite the importance of autoantibodies for disease prediction, anti-islet autoantibodies do not by themselves cause β-cell destruction. Infants of diabetic mothers, with transplacental passage of anti-islet autoantibodies, do not develop diabetes. A report indicates that anti-islet autoantibodies at birth (GAD65 and IA-2 autoantibodies, but not insulin autoantibodies) in offspring of mothers with type 1A diabetes are associated with a decrease in the development of anti-islet autoantibodies for children followed in the BabyDiab study [66, 67]. In addition, a child with genetic B cell deficiency and no antibodies progressed to type 1 diabetes [68]. On the other hand, studies in the NOD mouse indicate that both B lymphocytes and transplacental autoantibodies (insulin autoantibodies detected) are necessary for development of diabetes in this animal model [69, 70].

The simplest rule for the prediction of type 1A diabetes is that the presence of multiple anti-islet autoantibodies confers high risk. For relatives of patients with type 1A diabetes expressing a single anti-islet autoantibody, the risk of progression is approximately 20% with a decade of follow up. In contrast almost all relatives expressing at least two of the autoantibodies (of GAD65, IA-2 and insulin) eventually progress to overt diabetes (Fig. 2) [71].

At the onset of type 1A diabetes, invasion of pancreatic islets by T lymphocytes is often found and animal experiments prove that T lymphocytes can transfer disease. As expected for an immune-mediated disorder, immunosuppressive regimens such as antibodies to CD3 and cyclosporine A [72] delay the loss of insulin secretion. It has proven difficult to detect autoreactive T cells in blood of patients in comparison to normal controls. Improved tracking reagents such as tetramers and ELISPOT assays might help in quantitation as they do in the NOD mouse model for specific autoantigenic peptides [73]. The numbers of autoreactive lymphocytes in type 1A diabetes are low, and it is likely that they are mostly localized in the pancreas and draining lymph node [53]. A recent study by Kent and coworkers [74] found marked oligoclonal expansions of CD4 T lymphocytes in pancreatic lymph nodes of

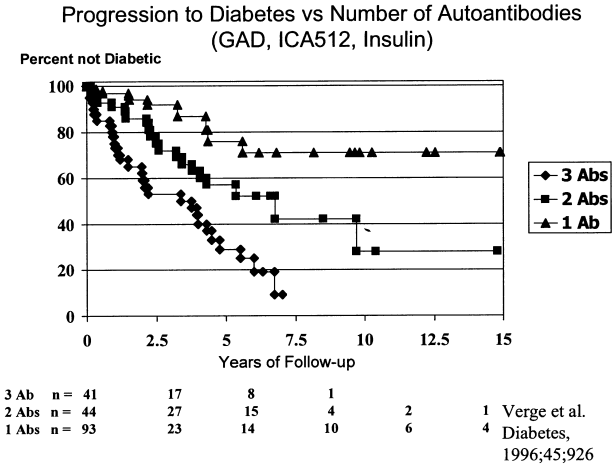

Figure 2. Progression to type 1 diabetes of relatives of patients subdivided by the number of anti-islet autoantibodies expressed (of GAD65, IA-2 and insulin) [71].

two patients. Current evidence from animal models indicates that autoreactive lymphocytes can have either destructive or regulatory effector functions [75, 76] and generation of regulatory T lymphocytes is an important avenue being pursued for the prevention of type 1 diabetes.

The β-cell target

In the NOD mouse model, target autoantigens of autoreactive T cells are being defined [77–79]. There are multiple different molecules targeted, including insulin and IGRP, that are β-cell specific, while studies also implicate non-islet-specific molecules [80, 81]. The molecule GAD65 has been administered to NOD mice with protection from progression to diabetes, but a number of non-specific immunizations also prevent diabetes, and blocking immune responses to GAD does not alter progression to diabetes [82]. Similarly knocking out genes for IA-2 and IA-2 beta does not inhibit progression to diabetes [83]. In contrast, a series of studies indicate that blocking responses to insulin prevent diabetes, and eliminating the B:9-23 peptide [knockouts of both insulin genes, with mutation of proinsulin gene (alanine for tyrosine at position 16 of the B chain)] prevents diabetes [10, 84]. We favor the hypothesis that there is a primary autoantigen for type 1 diabetes of the NOD mouse, and the insulin B:9-23 determinant is dominant. The evidence suggesting a similar dominant peptide for humans comes from the marked HLA specificity across populations in terms of high-risk alleles and gentoypes (e.g. DR3/4-DQ2/8) and the initial studies indicating that oligoclonal expansions of T cells reacting with insulin A1-15 may predominante in pancreatic lymph nodes [74]. In the NOD mouse model, the CD4 T lymphocytes that recognize insulin peptide B:9-23 utilize a restricted alpha chain T cell receptor variable and J region sequences [85], and we hypothesize that this specific T cell receptor sequence plus the insulin B:9-23 peptide creates an underlying susceptibility to type 1 diabetes in mice.

Prevention of type 1A diabetes

It is now possible to design trials for the prevention of type 1 diabetes, targeting multiple stages of the disease. The major therapies being studied involve immunosuppression, immunomodulation, antigen-specific therapies, or a combination of these approaches. Multiple protocols can prevent diabetes in animal models, in particular therapies that expand regulatory T cells to specific islet antigens. Translating these findings to humans has proven difficult but there is preliminary evidence that at-risk relatives with high levels of insulin autoantibodies may have a delay of progression to diabetes when treated with oral insulin (to induce 'mucosal tolerance') [86], and recently the company Diamyd has presented results of a phase II trial where simple injections of GAD65 in alum in patients with new-onset diabetes slowed progressive loss of C-peptide secretion. Multiple studies have utilized immunosuppressive medications/immunomodulating therapies such as antibodies to the T cell surface molecule CD3. Two phase II studies demonstrated delay of loss of C-peptide secretion [87, 88] and larger studies are planned.

Conclusion

T 1A diabetes is almost certainly a 'complex' genetically determined autoimmune disease with T lymphocyte destruction of pancreatic β-cells. Studies in animal models if relevant to humans have defined many of the pathways important for β-cell destruction. At present, we can predict type 1A diabetes, prevent the disorder in animal models, but safe prevention in humans remains to be achieved.

Acknowledgements. This work was supported by the National Institutes of Health (DK32083, DK32493, DK057538), Autoimmunity Prevention Center (AI50964), Diabetes Endocrine Research Center (P30 DK57516), Clinical Research Centers (MO1 RR00069, MO1 RR00051), the Immune Tolerance Network (AI15416), the American Diabetes Association, the Juvenile Diabetes Research Foundation and the Children's Diabetes Foundation. G. S. Eisenbarth is a consultant for Bayhill Therapeutics and Macrogenics for the development of therapies to prevent beta-cell destruction.

1 American Diabetes Association (2006) Diagnosis and classification of diabetes mellitus. Diabetes Care 29 (Suppl 1), S43–S48.
2 LaPorte, R. E., Matsushima, M. and Chang, Y-F. (1995) Prevalence and incidence of Insulin-Dependent Diabetes. National Institutes of Health; Bethesda.
3 LaPorte, R. E., Matsushima, M. and Chang, Y. F. (1995) Prevalence and incidence of insulin-dependent diabetes, 2 ed. Bethesda, National Institutes of Health, NIH publ. No. 95-1468.
4 Karvonen, M., Viik-Kajander, M., Moltchanova, E., Libman, I., LaPorte, R. and Tuomilehto, J. (2000) Incidence of childhood type 1 diabetes. Diabetes Care 23, 1516–1526.
5 Green, A. and Patterson, C. C. (2001) Trends in the incidence of childhood-onset diabetes in Europe. Diabetologia 44, B3–B8.
6 Sheehy, M. J., Scharf, S. J., Rowe, J. R., Neme de Gimenez, M. H., Meske, L. M., Erlich, H. A. and Nepom, B. S. (1989) A diabetes-susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. J. Clin. Invest. 83, 830–835.
7 Morel, P. A., Dorman, J. S., Todd, J. A., McDevitt, H. O. and Trucco, M. (1988) Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study. Proc. Natl. Acad. Sci. USA 85, 8111–8115.
8 Barratt, B. J., Payne, F., Lowe, C. E., Hermann, R., Healy, B. C., Harold, D., Concannon, P., Gharani, N., McCarthy, M. I., Olavesen, M. G., McCormack, R., Guja, C., Ionescu-Tirgoviste, C., Undlien, D. E., Ronningen, K. S., Gillespie, K. M., Tuomilehto-Wolf, E., Tuomilehto, J., Bennett, S. T., Clayton, D. G., Cordell, H. J. and Todd, J. A. (2004) Remapping the insulin gene/IDDM2 locus in type 1 diabetes. Diabetes 53, 1884–1889.
9 Kralovicova, J., Gaunt, T. R., Rodriguez, S., Wood, P. J., Day, I. N. and Vorechovsky, I. (2006) Variants in the human insulin gene that affect pre-mRNA splicing: is –23HphI a functional single nucleotide polymorphism at IDDM2? Diabetes 55, 260–2644.
10 Nakayama, M., Abiru, N., Moriyama, H., Babaya, N., Liu, E., Miao, D., Yu, L., Wegmann, D. R., Hutton, J. C., Elliott, J. F. and Eisenbarth, G. S. (2005) Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. Nature 435, 220–223.
11 Babaya, N., Nakayama, M., Moriyama, H., Gianani, R., Still, T., Miao, D., Yu, L., Hutton, J. C. and Eisenbarth, G. S. (2006) A new model of insulin-deficient diabetes: male NOD mice with a single copy of Ins1 and no Ins2. Diabetologia 49, 1222–1228.
12 Bottini, N., Muscumeci L, Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M., MacMurray, J., Meloni, G., Lucarelli, P., Pellechia, M., Eisenbarth, G., Comings, D. and Mustelin, T. (2004) A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat. Genet. 36, 337–338.
13 Fife, B. T., Griffin, M. D., Abbas, A. K., Locksley, R. M. and Bluestone, J. A. (2006) Inhibition of T cell activation and autoimmune diabetes using a B cell surface-linked CTLA-4 agonist. J Clin. Invest. 116, 2252–2261.
14 Van Belzen, M. J., Mulder, C. J., Zhernakova, A., Pearson, P. L., Houwen, R. H. and Wijmenga, C. (2004) CTLA4 +49 A/G and CT60 polymorphisms in Dutch coeliac disease patients. Eur. J. Hum. Genet. 12, 782–785.
15 Smyth, D. J., Cooper, J. D., Bailey, R., Field, S., Burren, O., Smink, L. J., Guja, C., Ionescu-Tirgoviste, C., Widmer, B., Dunger, D. B., Savage, D. A., Walker, N. M., Clayton, D. G. and Todd, J. A. (2006) A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nat. Genet. 38, 617–619.
16 Vella, A., Cooper, J. D., Lowe, C. E., Walker, N., Nutland, S., Widmer, B., Jones, R., Ring, S. M., McArdle, W., Pembrey, M. E., Strachan, D. P., Dunger, D. B., Twells, R. C., Clayton, D. G. and Todd, J. A. (2005) Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. Am. J. Hum. Genet. 76, 773–779.
17 Aly, T. A., Eller, E., Ide, A., Gowan, K., Babu, S. R., Erlich, H. A., Rewers, M. J., Eisenbarth, G. S. and Fain, P. R. (2006) Multi-SNP analysis of MHC region: remarkable conservation of HLA-A1-B8-DR3 haplotype. Diabetes 55, 1265–1269.
18 Lee, C., Maull, E., Chapman, N., Tracy, S. and Gauntt, C. (1997) Genomic regions of coxsackievirus B3 associated with cardiovirulence. J. Med. Virol. 52, 341–347.
19 Roach, J. C., Deutsch, K., Li, S., Siegel, A. F., Bekris, L. M., Einhaus, D. C., Sheridan, C. M., Glusman, G., Hood, L., Lernmark, A. and Janer, M. (2006) Genetic mapping at 3-kilobase resolution reveals inositol 1,4,5-triphosphate receptor 3 as a risk factor for type 1 diabetes in Sweden. Am. J. Hum. Genet. 79, 614–627.
20 Velaga, M. R., Wilson, V., Jennings, C. E., Owen, C. J., Herington, S., Donaldson, P. T., Ball, S. G., James, R. A., Quinton, R., Perros, P. and Pearce, S. H. (2004) The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. J Clin. Endocrinol. Metab 89, 5862–5865.
21 Aly, T. A., Ide, A., Jahromi, M. M., Barker, J. M., Fernando, M. S., Babu, S. R., Yu, L., Miao, D., Erlich, H. A., Fain, P. R., Barriga, K. J., Norris, J. M., Rewers, M. J. and Eisenbarth, G. S. (2006) Extreme genetic risk for type 1A diabetes. Proc. Natl. Acad. Sci. USA 103, 14074–14079.
22 Lie, B. A., Sollid, L. M., Ascher, H., Ek, J., Akselsen, H. E., Ronningen, K. S., Thorsby, E. and Undlien, D. E. (1999) A gene telomeric of the HLA class I region is involved in predisposition to both type 1 diabetes and coeliac disease. Tissue Antigens 54, 162–168.
23 Johansson, S., Lie, B. A., Todd, J. A., Pociot, F., Nerup, J., Cambon-Thomsen, A., Kockum, I., Akselsen, H. E., Thorsby, E. and Undlien, D. E. (2003) Evidence of at least two type 1 diabetes susceptibility genes in the HLA complex distinct from HLA-DQB1, -DQA1 and -DRB1. Genes Immun. 4, 46–53.
24 Alper, C. A., Awdeh, Z. and Yunis, E. J. (1992) Conserved, extended MHC haplotypes. Exp. Clin. Immunogenet. 9, 58–71.
25 Yunis, E. J., Larsen, C. E., Fernandez-Vina, M., Awdeh, Z. L., Romero, T., Hansen, J. A. and Alper, C. A. (2003) Inheritable variable sizes of DNA stretches in the human MHC: conserved extended haplotypes and their fragments or blocks. Tissue Antigens 62, 1–20.
26 Bilbao, J. R., Calvo, B., Aransay, A. M., Martin-Pagola, A., Perez, d. N., Aly, T. A., Rica, I., Vitoria, J. C., Gaztambide, S., Noble, J., Fain, P. R., Awdeh, Z. L., Alper, C. A. and Castano, L. (2006) Conserved extended haplotypes discriminate HLA-DR3-homozygous basque patients with type 1 diabetes mellitus and celiac disease. Genes Immun. 7, 550–554.
27 Ahonen, P., Myllarniemi, S., Sipila, I. and Perheentupa, J. (1990) Clinical variation of autoimmune polyendocrinopathy – candidiasis – ectodermal dystrophy (APECED) in a series of 68 patients. N. Engl. J. Med. 322, 1829–1836.
28 Patel, D. D. (2001) Escape from tolerance in the human X-linked autoimmunity-allergic disregulation syndrome and the scurfy mouse. J. Clin. Invest. 107, 155–157.
29 Nishimaki, K., Kawamura, T., Inada, H., Yagawa, K., Nose, Y., Nabeya, N., Isshiki, G., Tatsumi, N. and Niihira, S. (2000) HLA DPB1*0201 Gene confers disease susceptibility in Japanese with childhood onset type I diabetes, independent of HLA-DR and DQ genotypes. Diabetes Res. Clin. Pract. 47, 49–55.
30 Blomhoff, A., Lie, B. A., Myhre, A. G., Kemp, E. H., Weetman, A. P., Akselsen, H. E., Huseby, E. S. and Undlien, D. E. (2004) Polymorphisms in the cytotoxic T lymphocyte antigen-4 gene region confer susceptibility to Addison's disease. J Clin. Endocrinol. Metab 89, 3474–3476.
31 Gambelunghe, G., Falorni, A., Ghaderi M, Laureti S, Tortoioli, C., Santeusanio, F., Brunetti, P. and Sanjeevi, C. B. (1999) Microsatellite polymorphism of the MHC class I chain-related (MIC-A and MIC-B) genes marks the risk for autoimmune Addison's disease. J. Clin. Endocrinol. Metab. 84, 3701–3707.
32 Yu, L., Brewer, K. W., Gates, S., Wu, A., Wang, T., Babu, S.,

Gottlieb, P., Freed, B. M., Noble, J., Erlich, H., Rewers, M. and Eisenbarth, G. (1999) DRB1*04 and DQ alleles: expression of 21-hydroxylase autoantibodies and risk of progression to Addison's disease. J. Clin. Endocrinol. Metab. 84, 328–335.
33 Wallaschofski, H., Meyer, A., Tuschy, U. and Lohmann, T. (2003) HLA-DQA1*0301-associated susceptibility for autoimmune polyglandular syndrome type II and III. Horm. Metab. Res. 35, 120–124.
34 Robles, D. T., Fain, P. R., Gottlieb, P. A. and Eisenbarth, G. S. (2002) The genetics of autoimmune polyendocrine syndrome type II. In Autoimmune Polyendocrine Syndromes, 31, Eisenbarth, G. S. (Ed.), pp. 353–368, Saunders, Philadelphia.
35 Bosi, E., Becker, F., Bonifacio, E., Wagner, R., Collins, P., Gale, E. A. and Bottazzo, G. F. (1991) Progression to type I diabetes in autoimmune endocrine patients with islet cell antibodies. Diabetes 40, 977–984.
36 Meager, A., Visvalingam, K., Peterson, P., Moll, K., Murumagi, A., Krohn, K., Eskelin, P., Perheentupa, J., Husebye, E., Kadota, Y. and Willcox, N. (2006) Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med. 3, e289.
37 Wildin, R. S. and Freitas, A. (2005) IPEX and FOXP3: Clinical and research perspectives. J. Autoimmun. 25 (Suppl), 56–62.
38 Bindl, L., Torgerson, T., Perroni, L., Youssef, N., Ochs, H. D., Goulet, O. and Ruemmele, F. M. (2005) Successful use of the new immune-suppressor sirolimus in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). J. Pediatr. 147, 256–259.
39 Hornum, L., Romer, J. and Markholst, H. (2002) The diabetes-prone BB rat carries a frameshift mutation in Ian4, a positional candidate of Iddm1. Diabetes 51, 1972–1979.
40 Makino, S., Kunimoto, K., Muraoka, Y., Mizushima, Y., Katagiri, K. and Tochino, Y. (1980) Breeding of a non-obese, diabetic strain of mice. Exp. Anim. 29, 1–13.
41 Lenzen, S., Tiedge, M., Elsner, M., Lortz, S., Weiss, H., Jorns, A., Kloppel, G., Wedekind, D., Prokop, C. M. and Hedrich, H. J. (2001) The LEW.1AR1/Ztm-Iddm Rat: a new model of spontaneous insulin-dependent diabetes mellitus. Diabetologia 44, 1189–1196.
42 Ikegami, H., Makino, S. (1993) Genetic susceptibility to insulin-dependent diabetes mellitus: from NOD mice to humans. In: Lessons from animal diabetes, Shafrir, E. (Ed.), pp. 39–50, Smith-Gordon, London.
43 Lonnrot, M., Salminen, K., Knip, M., Savola, K., Kulmala, P., Leinikki, P., Hyypia, T., Akerblom, H. K. and Hyoty, H. (2000) Enterovirus RNA in serum is a risk factor for beta-cell autoimmunity and clinical type 1 diabetes: a prospective study. J. Med. Virol. 61, 214–220.
44 Graves, P. M., Rotbart, H. A., Nix, W. A., Pallansch, M. A., Erlich, H. A., Norris, J. M., Hoffman, M., Eisenbarth, G. S. and Rewers, M. (2003) Prospective study of enteroviral infections and development of beta-cell autoimmunity. Diabetes Autoimmunity Study in the Young (DAISY). Diabetes Res. Clin. Pract. 59, 51–61.
45 Viskari, H., Ludvigsson, J., Uibo, R., Salur, L., Marciulionyte, D., Hermann, R., Soltesz, G., Fuchtenbusch, M., Ziegler, A. G., Kondrashova, A., Romanov, A., Knip, M. and Hyoty, H. (2004) Relationship between the incidence of type 1 diabetes and enterovirus infections in different European populations: Results from the EPIVIR project. J. Med. Virol. 72, 610–617.
46 Akerblom, H. K., Virtanen, S. M., Ilonen, J., Savilahti, E., Vaarala, O., Reunanen, A., Teramo, K., Hamalainen, A. M., Paronen, J., Riikjarv, M. A., Ormisson, A., Ludvigsson, J., Dosch, H. M., Hakulinen, T. and Knip, M. (2005) Dietary manipulation of beta cell autoimmunity in infants at increased risk of type 1 diabetes: a pilot study. Diabetologia 48, 829–837.
47 Hermann, R., Knip, M., Veijola, R., Simell, O., Laine, A. P., Akerblom, H. K., Groop, P. H., Forsblom, C., Pettersson-Fernholm, K. and Ilonen, J. (2003) Temporal changes in the frequencies of HLA genotypes in patients with type 1 diabetes-indication of an increased environmental pressure? Diabetologia 46, 420–425.
48 Bach, J. F. (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. N. Engl. J. Med. 347, 911–920.
49 Devendra, D., Liu, E. and Eisenbarth, G. S. (2004) Type 1 diabetes: recent developments. BMJ 328, 750–754.
50 Eisenbarth, G. S. (2002) Autoimmune polyendocrine syndromes. In: Type 1 Diabetes: Molecular, Cellular and Clinical Immunology. Plenum, New York.
51 Daaboul, J. and Schatz, D. (2003) Overview of prevention and intervention trials for type 1 diabetes. Rev. Endocr. Metab. Disord. 4, 317–323.
52 Abiru, N., Kawasaki, E. and Eguch, K. (2002) Current knowledge of Japanese type 1 diabetic syndrome. Diabetes Metab. Res. Rev. 18, 357–366.
53 Rabin, D. U., Pleasic, S. M., Shapiro, J. A., Yoo-Warren, H., Oles, J., Hicks, J. M., Goldstein, D. E. and Rae, P. M. M. (1994) Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. J. Immunol. 152, 3183–3188.
54 Leslie, R. D., Atkinson, M. A. and Notkins, A. L. (1999) Autoantigens IA-2 and GAD in type I (insulin-dependent) diabetes. Diabetologia 42, 3–14.
55 Bach, J. F. (1999) Etiology and pathogenesis of human insulin-dependent diabetes mellitus. In: Contemporary Endocrinology: Autoimmune Endocrinopathies, , Volpe, R. (Ed.), pp. 293–307. Humana, Totowa.
56 Abiru, N. and Eisenbarth, G. S. (1999) Autoantibodies and autoantigens in type 1 diabetes: role in pathogenesis, prediction and prevention. Can. J. Diabetes Care 23, 59–65.
57 Bonifacio, E., Scirpoli, M., Kredel, K., Fuchtenbusch, M. and Ziegler, A. G. (1999) Early autoantibody responses in prediabetes are IgG1 dominated and suggest antigen-specific regulation. J. Immunol. 163, 525–532.
58 Colman, P. G., McNair, P., Margetts, H., Schmidli, R. S., Werther, G. A., Alford, F. P., Ward, G. M., Tait, B. D., Honeyman, M. C. and Harrison, L. C. (1998) The Melbourne pre-diabetes study: prediction of type 1 diabetes mellitus using antibody and metabolic testing. Med. J. Aust. 169, 81–84.
59 Bingley, P. J., Bonifacio, E., Williams, A. J. K., Genovese, S., Bottazzo, G. F. and Gale, E. A. M. (1997) Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. Diabetes 46, 1701–1710.
60 Greenbaum, C. J., Palmer, J. P., Kuglin, B. and Kolb, H. (1992) Insulin autoantibodies measured by radioimmunoassay methodology are more related to insulin-dependent diabetes mellitus than those measured by enzyme-linked immunosorbent assay: results of the Fourth International Workshop on the Standardization of Insulin Autoantibody Measurement. J. Clin. Endocrinol. Metab. 74, 1040–1044.
61 Baekkeskov, S., Aanstoot, H.-J., Christgau, S., Reetz, A., Solimena, M., Cascalho, M., Folli, F., Richter-Olesen, H. and DeCamilli, P. (1990) Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347, 151–156.
62 Rabin, D. U., Pleasic, S. M., Palmer-Crocker, R. and Shapiro, J. A. (1992) Cloning and expression of IDDM-specific human autoantigens. Diabetes 41, 183–186.
63 Lan, M. S., LU, J., Goto, Y. and Notkins, A. L. (1994) Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. DNA Cell Biol. 13, 505–514.
64 Wasmeier, C. and Hutton, J. C. (1996) Molecular cloning of phogrin, a protein-tyrosine phosphatase homologue localized to insulin secretory granule membranes. J. Biol. Chem. 271, 18161–18170.
65 Lu, J., Li, Q., Xie, H., Chen, Z.-J., Borovitskaya, A. E., Maclaren, N. K., Notkins, A. L. and Lan, M. S. (1996) Identification of a second transmembrane protein tyrosine phosphatase, IA-2β, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37-KDa tryptic fragment. Proc. Natl. Acad. Sci. USA 93, 2307–2311.
66 Koczwara, K., Bonifacio, E. and Ziegler, A. G. (2004) Trans-

mission of maternal islet antibodies and risk of autoimmune diabetes in offspring of mothers with type 1 diabetes. Diabetes 53, 1–4.
67 Roll, U., Christie, M. R., Füchtenbusch, M., Payton, M. A., Hawkes, C. J. and Ziegler, A. G. (1996) Perinatal autoimmunity in offspring of diabetic parents. The German Multi-Center BABY-DIAB Study: detection of humoral immune responses to islet antigens in early childhood. Diabetes 45, 967–973.
68 Martin, S., Wolf-Eichbaum, D., Duinkerken, G., Scherbaum, W. A., Kolb, H., Noordzij, J. G. and Roep, B. O. (2001) Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. N. Engl. J. Med. 345, 1036–1040.
69 Serreze, D. V., Fleming, S. A., Chapman, H. D., Richard, S. D., Leiter, E. H. and Tisch, R. M. (1998) B Lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. J. Immunol. 161, 3912–3918.
70 Greeley, S. A., Katsumata, M., Yu, L., Eisenbarth, G. S., Moore, D. J., Goodarzi, H., Barker, C. F., Naji, A. and Noorchashm, H. (2002) Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. Nat. Med. 8, 399–402.
71 Verge, C. F., Gianani, R., Kawasaki, E., Yu, L., Pietropaolo, M., Jackson, R. A., Chase, H. P. and Eisenbarth, G. S. (1996) Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes 45, 926–933.
72 Bach, J. F. (1994) Insulin-dependent diabetes mellitus as an autoimmune disease. [Review]. Endocr. Rev. 15, 516–542.
73 Reijonen, H., Mallone, R., Heninger, A. K., Laughlin, E. M., Kochik, S. A., Falk, B., Kwok, W. W., Greenbaum, C. and Nepom, G. T. (2004) GAD65-specific CD4+ T-cells with high antigen avidity are prevalent in peripheral blood of patients with type 1 diabetes. Diabetes 53, 1987–1994.
74 Kent, S. C., Chen, Y., Bregoli, L., Clemmings, S. M., Kenyon, N. S., Ricordi, C., Hering, B. J. and Hafler, D. A. (2005) Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. Nature 435, 224–228.
75 Du, W., Wong, F. S., Li, M. O., Peng, J., Qi, H., Flavell, R. A., Sherwin, R. and Wen, L. (2006) TGF-beta signaling is required for the function of insulin-reactive T regulatory cells. J. Clin. Invest. 116, 1360–1370.
76 Jasinski, J. M., Yu, L., Nakayama, M., Li, M. M., Lipes, M. A., Eisenbarth, G. S. and Liu, E. (2006) Transgenic insulin (B:9–23) T-cell receptor mice develop autoimmune diabetes dependent upon RAG genotype, H-2g7 homozygosity, and insulin 2 gene knockout. Diabetes 55, 1978–1984.
77 Bresson, D., Togher, L., Rodrigo, E., Chen, Y. L., Bluestone, J. A., Herold, K. C. and von Herrath, M. (2006) Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing tregs. J. Clin. Invest. 116, 1371–1381.
78 Dai, Y. D., Jensen, K. P., Lehuen, A., Masteller, E. L., Bluestone, J. A., Wilson, D. B. and Sercarz, E. E. (2005) A peptide of glutamic acid decarboxylase 65 can recruit and expand a diabetogenic T cell clone, BDC2.5, in the pancreas. J. Immunol. 175, 3621–3627.
79 Andre, I., Gonzalez, A., Wang, B., Katz, J., Benoist, C. and Mathis, D. (1996) Checkpoints in the progression of autoimmune disease: lessons from diabetes models. Proc. Natl. Acad. Sci. USA 93, 2260–2263.
80 Frigeri, C., Martin, C. C., Svitek, C. A., Oeser, J. K., Hutton, J. C., Gannon, M. and O'Brien, R. M. (2004) The proximal islet-specific glucose-6-phosphatase catalytic subunit-related protein autoantigen promoter is sufficient to initiate but not maintain transgene expression in mouse islets in vivo. Diabetes 53, 1754–1764.
81 DiLorenzo, T. P. and Serreze, D. V. (2005) The Good Turned Ugly: Immunopathogenic basis for diabetogenic CD8+ T cells in NOD mice. Immunol. Rev. 204, 250–263.
82 Jaeckel, E., Klein, L., Martin-Orozco, N. and von Boehmer, H. (2003) Normal Incidence of Diabetes in NOD Mice Tolerant to Glutamic Acid Decarboxylase. J Exp. Med 197, 1635–44.
83 Kubosaki, A., Nakamura, S. and Notkins, A. L. (2005) Dense core vesicle proteins IA-2 and IA-2{beta}: metabolic alterations in double knockout mice. Diabetes 54 (Suppl 2), S46–S51.
84 Moriyama, H., Abiru, N., Paronen, J., Sikora, K., Liu, E., Miao, D., Devendra, D., Beilke, J., Gianani, R., Gill, R. G. and Eisenbarth, G. S. (2003) Evidence for a primary islet autoantigen (preproinsulin 1) for insulitis and diabetes in the nonobese diabetic mouse. Proc. Natl. Acad. Sci. USA 100, 10376–10381.
85 Homann, D. and Eisenbarth, G. S. (2006) An immunologic homunculus for type 1 diabetes. J. Clin. Invest. 116, 1212–1215.
86 Skyler, J. S., Krischer, J. P., Wolfsdorf, J., Cowie, C., Palmer, J. P., Greenbaum, C., Cuthberturn, D., Rafkin-(2005) Effects of oral insulin in relatives of patients with type 1 diabetes: the diabetes prevention trial-type 1. Diabetes Care 28, 1068–1076.
87 Keymeulen, B., Vandemeulebroucke, E., Ziegler, A. G., Mathieu, C., Kaufman, L., Hale, G., Gorus, F., Goldman, M., Walter, M., Candon, S., Schandene, L., Crenier, L., De Block, C., Seigneurin, J. M., De Pauw, P., Pierard, D., Weets, I., Rebello, P., Bird, P., Berrie, E., Frewin, M., Waldmann, H., Bach, J. F., Pipeleers, D. and Chatenoud, L. (2005) Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N. Engl. J. Med. 352, 2598–2608.
88 Herold, K. C., Hagopian, W., Auger, J. A., Poumian-Ruiz, E., Taylor, L., Donaldson, D., Gitelman, S. E., Harlan, D. M., Xu, D., Zivin, R. A. and Bluestone, J. A. (2002) Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N. Engl. J. Med. 346, 1692–1698.