The Review of DIABETIC STUDIES

REVIEW

Genes Mediating Environment Interactions in Type 1 Diabetes

Erik Biros, Margaret A. Jordan and Alan G. Baxter

Comparative Genomics Centre, Molecular Sciences Building 21, James Cook University, Townsville QLD 4811, Australia.
Address correspondence to: Alan G. Baxter, e-mail: alan.baxter@jcu.edu.au.

■ Abstract

The relative risk of type 1 (autoimmune) diabetes mellitus for a sibling of an affected patient is fifteen times that of the general population, indicating a strong genetic contribution to the disease. Yet, the incidence of diabetes in most Western communities has doubled every fifteen years since the Second World War - a rate of increase that can only possibly be explained by a major etiological effect of environment. Here, the authors provide a selective review of risk factors identified to date. Recent reports of linkage of type 1 diabetes to genes encoding pathogen pattern recognition molecules, such as toll-like receptors, are discussed, providing a testable hypothesis regarding a mechanism by which genetic and environmental influences on disease progress are integrated.

Keywords: type 1 diabetes · genes · environment

Introduction

Type 1 (autoimmune) diabetes (T1D) is an endocrine disease in which the body's immune system gradually destroys the insulin-producing β-cells in the pancreas, leading to a loss of insulin secretion and hyperglycemia. Progression from the pre-clinical stage of β-cell autoimmunity, known as insulitis, to established diabetes can take up to a decade [1,2]. Globally, T1D affects between 10-20 million people. Forty percent of cases develop the onset of the disease before the age of 20, making it one of the most common severe chronic childhood illnesses [3-5]. T1D is the leading cause of end-stage renal disease, blindness, and amputation in many communities, and is a major cause of cardiovascular disease and premature death in the general population [6].

It is not clear what initiates the autoimmune cascade leading to T1D, but it is certain that both genetic and environmental factors contribute to the risk of disease. Progress in the understanding of the pathogenesis of T1D has been complicated by the large number (>20) of genes involved. It appears that most linkage peaks located to date are the product of multiple linked loci, each of which confers relatively little risk. The limited effect of each locus has required very large sample sets to generate sufficient analytic power. Furthermore, many linkage peaks have not been replicated in subsequent studies, raising the possibility that they are dependent on local environmental conditions, or are affected by parental imprinting.

Animal models of human T1D, such as the NOD mouse [reviewed in 7] and BB rat [reviewed in 8], were originally applied to help develop paradigms and methodological tools for the dissection of this complex genetic trait. In several cases, correlation of syntenic linkage peaks has suggested, and in some cases molecular analysis has supported, the possibility that in

 DOI 10.1900/RDS.2005.2.192

both the animal model and the human disease, similar polymorphisms in the same genes affect disease risk. The animal models have three other advantages in this context. Firstly, as they are inbred, large numbers of segregating progeny can be produced, greatly increasing the statistical power of genetic analyses. Secondly, mouse strains, and to a lesser extent rat strains, are highly amenable to recombinant genetic approaches, permitting allele exchange experiments to formally test hypotheses relating to causality. Finally, as the housing conditions of laboratory animals can be exactly controlled, these models provide a platform to test hypotheses relating to gene/environment interactions in determining the risk of disease. This review provides a selective summary of proposed genetic and environmental risk factors for type 1 diabetes.

Monogenic autoimmunity

Valuable progress in the understanding of the pathogenesis of autoimmune disease (AID) has come with the recognition of several genes in humans that underlie Mendelian forms of the disease. In particular, two very rare syndromes are now genetically characterized: autoimmune polyendocrine syndrome type 1 (APS-1; also termed autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, APECED) and immune deregulation, polyendocrinopathy, enteropathy, X-linked (IPEX). Both syndromes are associated with the development of immune mediated diabetes - neonatal diabetes for the IPEX syndrome, and about 20% of patients with APS-I develop diabetes as children or young adults [9, 10].

APS-1

The APS-1 syndrome is inherited as an autosomal recessive disorder [11] and results from mutations of the *AIRE* (autoimmune regulator) gene [12] that is located on chromosome 21q22 [13, 14]. Through studies of targeted mutant *Aire*-deficient (knockout) mice, we know that *Aire*'s protein product acts as a thymic transcription factor for numerous genes [15], including some related to T1D, such as hormones (e.g. insulin) and growth factors (e.g. insulin-like growth factor 2). *Aire* knockout mice on a 129/C57BL/6 background develop high titres of autoantibodies reactive with multiple organs as well as T cell infiltrates of multiple organs, but do not develop diabetes. When backcrossed to the B10.Br background for 2 generations, *Aire* deficiency was associated with an increased incidence of diabetes in a model involving the transgenic expression of hen egg lysosyme (HEL) in the pancreatic islets and a HEL peptide-specific T cell receptor on T cells [16]. Thus, an attractive hypothesis is that mutations of the *AIRE* gene (e.g. APS-1 syndrome) in humans may cause reduced "peripheral antigen" expression in the thymus thereby decreasing the induction of thymic central tolerance to these antigens [17, 18].

IPEX

IPEX syndrome results from mutations of the *FOXP3* gene [19-21], which is located on chromosome Xp11.23. All disease-associated mutations appear to disrupt gene function causing a phenotype in hemizygous males but not in heterozygous females. *FOXP3* encodes the protein scurfin, which belongs to the *Forkhead* family of winged-helix transcription factors [22, 23]. Its expression is largely restricted to T cells with a regulatory function (Treg cells), suggesting a role in the development of these cells [24-26]. Human Hassall's corpuscles express thymic stromal lymphopoietin (TSLP), which activates thymic CD11c-positive dendritic cells to express high levels of CD80 and CD86 molecules. These TSLP-conditioned dendritic cells are then able to induce the proliferation and differentiation of $CD4^+CD8^-CD25^-$ thymic T cells into $CD4^+CD25^+Foxp3^+$ Tregs, which suppress autoimmune T cells and help to maintain tolerance to self [27].

At present, there is little or no indication that polymorphisms at either *AIRE* or *FOXP3* contribute to the common forms of T1D. While one small case-control study has reported an association of the *FOXP3* gene with T1D in Japanese patients [28], another study in Sardinian families found no evidence when com-pared with a case-control cohort [29].

Polygenic type 1 diabetes

To date, there is at least suggestive evidence that more than thirty genes contribute to the susceptibility of T1D development. Loci for which reasonable confirmation has been obtained, or show significance in one of the two major publications reporting multiple genome-wide linkage scans ([30] and [31]; reporting data from 767 and 1,435 multiplex families respectively), include 6p21 (*IDDM1*), 11p15 (*IDDM2*), 2q31-q33 (*IDDM12*), 10p11-q11 (*IDDM10*), 6q25 (*IDDM5*), 1p13 and 16q22-24. The three chromosomal regions with consistently significant evidence of association with T1D are the *HLA* region at 6p21.3 (*IDDM1*) [32, 33], the *INS* region at 11p15 (*IDDM2*) [34, 35] and the *CTLA4/CD28* region at 2q31-q33 [36-39].

The major histocompatibility complex (MHC)

The MHC region on human chromosome 6p21 has been identified as a critical susceptibility locus for many human AIDs, including T1D [40]. Through studies done on animals, it has been hypothesized that certain MHC alleles are less efficient at presenting self-peptides to the developing T cells in the thymus, so that negative selection fails [41-44]. Human genetic predisposition to T1D is strongly associated with *HLA-DQβ*, which is closely linked to *DR3* and *DR4* alleles [40]. T1D has a positive association with both *DR3* and *DR4,* where relative risk (RR) is 3 and 6, respectively; risk is the highest (RR=30) in individuals of the heterozygous *HLA-DR3/DR4* genotype. Susceptible *HLA-DQβ* alleles comprise non-charged amino acids (valine, serine, or alanine) at position 57 (*HLA-DQ8β*) instead of the common aspartic acid residue at this site (*HLA-DQ7β*). The autoimmune feature of this single amino acid change may be explained by the loss of a salt bridge between α and β chains making the MHC class II molecule less stable and affecting its peptide-binding affinity [45, 46]. On the other hand, resistance to disease is associated with *HLA DR2* and *DQB1*0602*; people with these alleles rarely develop diabetes even if they express *DR3* and/or *DR4* [47].

INS-associated variable number of tandem repeats (VNTR)

As HLA-mediated susceptibility does not explain all genetic susceptibility to T1D, it is evident that non-HLA loci must also be involved. There is a consistent association with T1D of the region near the insulin gene (*INS*) on chromosome 11p15, termed *IDDM2* [34, 48]. A major contributor to risk in this region seems to be a variable number of tandem repeats (VNTR) that is located within the regulatory sequences of the *INS* gene [49]. This polymorphism consists of a 14-15bp G-rich consensus sequence (ACAGGGGTC TGGGG) that clusters to 30-60 and 120-170 repeats in the class I and class III alleles, respectively. Intermediary class II alleles are uncommon in Caucasian populations [50]. A homozygous combination of class I alleles is found in about 85% of T1D patients in comparison to about 60% in the general population, suggesting it is an allele conferring susceptibility to T1D. Conversely, homozygosity for class III alleles is rarely found among diabetics [51, 50]. It is believed that T1D susceptibility and/or resistance associated with *IDDM2* may derive from the impact of VNTR alleles on insulin transcription in the thymus. An association between class III alleles and a higher steady-state level of (pro)insulin mRNA expression in the thymus has been identified and correlated with increased preproinsulin expression, which could mediate improved induction of central tolerance [52-54].

IGF2

Other loci in the 11p15 region may also contribute to *IDDM2*-encoded T1D susceptibility. Situated downstream from the *INS* VNTR, within the *IDDM2* region, is the *IGF2* (insulin-like growth factor 2) gene, which inhibits apoptosis and stimulates pancreatic β-cell proliferation [55]. IGF2 may also act as a positive selecting peptide for insulin reactive T cells because of its sequence homology to preproinsulin [56, 57]. Paquette *et al.* [58] found an association between class I alleles of the *INS* promoter VNTR and increased expression of *IGF2* in placenta, and it has been suggested that IGF2 expression may have some effect on intrauterine growth and birth size, both of which are known T1D risk factors [59]. However, this finding has not been confirmed in other studies [60, 61].

CTLA4

The *CTLA4/CD28* region at 2q31-q33 contains genes encoding crucial T cell regulatory molecules, including *CTLA4* (encoding the cytotoxic T lymphocyte associated protein 4, CD152) and *CD28*. These proteins have the opposite roles of regulator, and enhancer, of T cell effector function, respectively. The human *CTLA4* gene may contain several polymorphisms, making it a candidate susceptibility gene for AIDs. At present, the most informative polymorphism is a single peptide dimorphism at position 49 (A49G) in exon 1 [62]. This substitution leads to the exchange of a threonine to alanine residue [63]. Most case-control studies confirm the link between the G allele of this SNP and several AIDs, including T1D in various populations [36-39, 64]. Interestingly, increased proliferation of T cells was found in G/G homozygotes [65, 66] but further functional studies are needed.

IDDM10

A region on human chromosome 10 (10p11-q11; *IDDM10*) has been linked to T1D by the Type 1 Diabetes Genetics Consortium [31], supporting the prior association reported by Reed *et al.* [67] in an independent UK case control study. No candidate genes have been proposed.

SUMO4

A non-synonymous coding SNP in the *SUMO4* gene within *IDDM5* on chromosome 6q25 encodes a

methionine to valine substitution at codon 55, which is also associated with T1D [68-70]. The intronless gene *SUMO4* belongs to the family of small ubiquitin-like modifiers (*SUMO* family) encoding post-translational modifying proteins [71]. SUMO4 is believed to be involved in immune responses by modulating nuclear translation of nuclear factor-κB (NF-κB), affecting the transcription of genes encoding pro-inflammatory cytokines [68, 71].

LYP/PTPN22

Recently it was discovered that after *IDDM1* and *IDDM2*, the next gene which contributes the strongest effect of T1D risk is *LYP/PTPN22* mapping to chromosome 1p13.2 [72-74]. *PTPN22* codes for a key enzyme, protein tyrosine phosphatase N22, which associates with the molecular adaptor protein CBL and may be involved in regulating function in the T cell receptor (TCR) signaling pathway [75]. A functional single nucleotide polymorphism (SNP; C1858T) within *PTPN22* results in the exchange of arginine for a tryptophan residue at position 620 (R620W) in the proximal proline-rich SH3 domain of PTPN22. Bottini *et al.* [76] and Begovich *et al.* [77] showed that this substitution disrupts the interaction between PTPN22 and an intracellular C-terminal Src protein tyrosine kinase (CSK), a molecule that down-modulates TCR signaling [78]. The T allele (encoding a tryptophan residue) has positive association with T1D at a relative risk of approximately 3 [79, 80].

Other loci

The most recent data published by the Type 1 Diabetes Genetics Consortium did not find evidence for many of the previously published linkage regions (e.g. *IDDM3-9*, *IDDM11*, *IDDM13-18*, *PTPN22*) but found evidence suggestive of linkage at several other loci [31]. Given that some of the loci not identified have received significant prior support (such as *IDDM5* and *PTPN22*), local variations in allele frequencies or environmental conditions may play a significant role.

Parent-of-origin effects

In addition to the sheer number of *IDDM* loci identified, the genetics of T1D is further complicated by the action of parental imprinting on their expression. Parent-of-origin effects have been most intensively studied for the HLA (*IDDM1*) and the *INS* VNTR (*IDDM2*), both with conflicting results. Excessive transmission of paternal HLA alleles compared with maternal alleles have been observed by some groups [81-83], while others have either noted excessive transmission of the maternal alleles or identified no difference with respect to the parental origin of the alleles [84-86]. Similarly, paternal imprinting of *INS* VNTR-*IGF2* within *IDDM2* has been observed [87, 88], but this has also been disputed [89]. More recently, Bennett *et al.* proposed that the paternal effect was indeed present, but only when the father's untransmitted allele was of class III [90].

Other *IDDM* loci have been considered for effects of imprinting, including *IDDM5*, *IDDM8*, *IDDM10* and *IDDM15*. However, while Delepine and coworkers found no significant differences in the parental origin of alleles at *IDDM5*, *IDDM8* or *IDDM15* [91], Paterson *et al.* showed the paternal origin of *IDDM*8 to be significant for disease incidence, while there was a maternal effect at *IDDM10* [92].

Environment and T1D

The incidence of diabetes differs between different ethnic groups. Although this can partly be explained by differences in genetic predisposition between various populations, changes in diabetes incidence of migrating populations, as well as the rapidity of the increase in incidence of diabetes world wide [93] indicate that environmental factors play an important role.

Ante- and perinatal environment

Advanced maternal age (> 35 years), excessive weight gain in pregnancy and amniocentesis have all been reported as risk factors in the pathogenesis of diabetes [94]. The possibility of the intrauterine environment having an effect on subsequent T1D development requires further investigation.

An association between increased weight (BMI) gain in the first 12 months after birth and T1D was first noticed by Baum *et al.* [95] and has been confirmed by others [96, 97]. This may be caused by excess fat cell accumulation affecting insulin resistance. An increased growth in length in the following two years was also observed, possibly leading to increased demands for insulin secretion. This pattern of increased growth was associated with the presence of autoantibodies to tyrosine phosphatase-like protein (IA-2), at clinical diagnosis many years later [97], suggesting that the increased growth in infancy could result in faster subsequent pancreatic islet destruction once IA-2 autoantibodies are present.

Kagohashi *et al.* transplanted preimplantation-stage mouse embryos from the diabetes-prone NOD strain

into the uteruses of diabetes-resistant ICR and DBA/2J mice, to assess the role of maternal factors in the development of insulitis and overt diabetes [98]. The NOD→ICR and NOD→DBA offspring were observed to develop insulitis considerably earlier than NOD→NOD offspring but overt diabetes was significantly suppressed in these offspring in comparison to NOD→NOD offspring. Insulin autoantibodies (IAAs) were undetectable in ICR and DBA/2J surrogate mothers and in NOD→ICR and NOD→DBA offspring at the onset of insulitis, suggesting that maternal factors other than transmitted IAAs induced the earlier onset. The authors concluded that this was an indication that altered maternal factors other than transmitted IAAs, such as hormone levels or viral infections and/or other infectious agents, could be transmitted vertically from the mother to the offspring during the perinatal period, modifying the immune response to islets, which in turn might affect the pathogenic course from insulitis to overt diabetes.

Dietary factors

Gluten

The association between T1D and coeliac disease (CD), a condition affecting approximately 1 in 200 people [99] characterized by immune mediated damage to the jejunal mucosa, was first observed nearly 40 years ago [100, 101]. CD is an inflammatory disease triggered by gluten, a protein complex in wheat, rye and barley, and results in villous atrophy and crypt hyperplasia of the small bowel. As the symptoms of CD are often mild, atypical or absent, it often remains undiagnosed. Up until recently it was believed that the prevalence of CD in diabetics was similar to that of the general population [102], but with the advent of more sensitive screening methods, such as quantitation of anti-tissue transglutaminase (TTG) autoantibodies [103], it has become clear that the prevalence of CD in patients with T1D can be as high as 10% [104]. Furthermore, Bao *et al.* found that 33% of T1D patients homozygous for *DR3-DQ2* produce TTG autoantibodies [105]. Unstable diabetes and growth failure in children may be an indication of CD [106, reviewed in 107]. As complications of CD include development of lymphoma [108, 109] and osteoporosis [110], and the risk of these diseases can be reversed by eliminating gluten from the diet, screening and diagnosis of children with a high risk to diabetes is important.

Animal studies in BB rats in which gluten or the wheat protein gliadin were added to the diet, have shown an increased risk of diabetes [111, 112]. Most studies in NOD mice support a causative role for gluten, with increased incidence of disease associated with increased consumption, and protection associated with exclusion of gluten from the diet [113-116]. Hummel *et al.* fed a gluten exclusion diet to patients who were positive for at least two T1D-associated autoantibodies, but did not find any consistent alteration in the subsequent titers of these antibodies over a 12 month period [117]. They therefore concluded that gluten does not directly drive islet autoantibody production in T1D as it does for TTG autoantibodies in CD. Pastore *et al.* found that although autoantibody titers were not influenced by a gluten free diet, insulin secretion in subjects at high risk for T1D was improved [118], thus indicating that eliminating gluten from the diet may nevertheless have a beneficial effect on the preservation of β-cell mass or function.

Cow's milk (CM) proteins

An inverse correlation between breast-feeding frequency and T1D was observed in the early 1980s. Borch-Johnsen *et al.* reported that diabetic children were often either breast-fed for shorter periods of time, or not at all, in comparison to their non-diabetic siblings or the general population [119]. Several groups, working in both human and animal models, set about trying to confirm this finding with inconsistent results. Fort *et al.* could find no relationship between breast-feeding history and T1D [120] while others found that children with diabetes had been breast-fed over less time, on average, than non-diabetic children [121-123]. Similarly, a two-fold increase in diabetes incidence has been observed when the duration of breast-feeding was less than 3-4 months [reviewed in 124, 125, 107], or when the infant was exposed to CM formula before 2-3 months of age [126, 127]. More recently, the DIPP study (a Finnish birth-cohort study) observed an association between seropositivity for anti-IA-2 autoantibodies, or for all four diabetes-associated autoantibodies (anti-islet, anti-GAD65, anti-IA-2 and anti-insulin), with a breast-feeding time of less than 2 months [128], and an increased risk to T1D was also found to be associated with CM intake in children past the infancy stage [129, 130].

Patients with newly diagnosed T1D have been documented to have enhanced humoral and cellular immune responses to several CM proteins, including whey proteins, bovine serum albumin (BSA) [131, 132] and β-lactoglobulin (BLG) [133, 134]. Autoimmunity to ICA69 also occurs in some patients with other auto-

immune diseases such as rheumatoid arthritis, which shares some genetic background with T1D (i.e. *HLA DR0401* allele) [135]. While levels of antibodies to BSA and BLG decline with age in control subjects, patients with T1D showed no such reduction, indicating a failure of oral tolerance. Cytokines secreted by lymphocytes stimulated by BLG did not differ between T1D-patients and controls [136].

The immunogenic effect of dietary proteins such as CM has also been clearly demonstrated in studies in NOD mice [137] and BB rats [111, reviewed in 138]. Beppu *et al.* in 1987 reported elevated levels of antibodies to BSA (bovine serum albumin) in NOD mice compared to non-diabetic controls, indicating an exaggerated immune response to BSA [139]. It is possible that BSA mediates its effect on T1D through its structural similarity to an islet cell antigen termed ICA69 or p69 [132].

Vitamin D

The risk of onset of T1D correlates to seasonal variation, with the largest proportion of cases being diagnosed during winter and autumn and the smallest in summer, especially in countries with extreme seasonal variations [140]. The levels of serum 25-hydroxyvitamin D_3 ($25OHD_3$) shows reciprocal seasonal and geographic variations [141-145]. Vitamin D is not only essential for bone and mineral metabolism but also affects glucose metabolism and immune function. β-cell insulin synthesis and secretion are impaired in vitamin D-deficient animals [146] and glucose tolerance is restored when vitamin D levels are returned to normal. Human peripheral blood monocytes and activated T cells have high affinity receptors for vitamin D [147, 148] and NOD mice supplemented with the active form of vitamin D or its analogues do not develop insulitis [146] or diabetes [149]. Human studies have shown that the incidence of T1D is reduced significantly with vitamin D supplementation of either infants at risk [150], or their mothers during pregnancy [151].

Toxins

The β-cells of pancreatic islets are highly sensitive to metabolic stress, free radicals and cytokine exposure [152]. This issue was brought to the fore when a series of cases of attempted suicide with the rodenticide Vacor (containing N-3-pyridylmethyl-N'-p-nitrophenyl urea; RH-787) resulted in direct β-cell toxicity and onset of insulin-dependent diabetes [153]. Streptozotocin, derived from *Steptomyces spp,* also induces T1D through a direct toxic action on β-cells in certain inbred mouse strains [154]. A modification of the administration protocol was developed as a model of autoimmune diabetes, in which repeated, small doses of streptozotocin were administered, with a view to priming immune responses against chemically modified β-cell constituents released following tissue damage [155]. This model fell out of favor when Leiter *et al.* found that the resulting disease did not appear to be T cell-dependent, and it faced significant competition from the spontaneous autoimmune mouse model, the NOD mouse [156]. Ironically, results of recent experiments using anti-CD3 monoclonal antibodies in low-dose streptozotocin-treated mice suggest that islet destruction in this model is, at least in part, autoimmune [157].

Myers *et al.* found that nanogram quantities of the macrolide antibiotic bafilomycin A1 caused glucose intolerance and pancreatic islet disruption in mice [158]. This macrolide and others structurally similar are produced by the *Streptomyces* species ubiquitous in soil, which can infest tuberous vegetables, particularly potatoes and beet [124]. Myers *et al.* have proposed that dietary exposure to bacterial macrolides could therefore damage pancreatic islets, resulting in antigen shedding, priming autoimmune responses in susceptible individuals [158]. Consistent with this possibility is the high T1D incidence in Western countries where consumption of tuberous vegetables and sugar refined from beets is common.

Microorganisms and T1D

In both humans and animal models, strong associations have been identified between infectious agents and T1D [159-160]. Viruses are regarded as a prime initiating factor because they may show tissue tropism for islets, can cause tissue damage and antigen shedding, and can prime strong immune responses leading to local inflammation.

Congenital rubella infection

Prenatal rubella infection in the first trimester is the best-established example of viral initiation of T1D [161]. Twenty percent of patients with congenital rubella develop the disease [161, 162] and animal studies in rabbits [161] and hamsters [163] confirm the association. Although the onset of infection clearly precedes the development of T cells in humans, it remains possible that the role of the virus is one of immunomodification, rather than initiation, as the virus maintains a productive infection of the pancreas throughout life [164]. Despite the major impact on the prevalence

of congenital rubella by vaccination programs in Western countries, T1D incidence continues to increase [165], suggesting that this is not a common cause of T1D in these communities.

Enteroviruses

Enteroviruses are small (27 nm in diameter) viruses that are made of ribonucleic acid (RNA) and protein and include the polioviruses, coxsackieviruses, and echoviruses. There are 3 different polioviruses and more than 60 non-polio enteroviruses that can cause disease in humans, including 23 Coxsackie A viruses, 6 Coxsackie B viruses, 28 echoviruses, and 4 other enteroviruses. An association between enteroviruses and T1D was observed in both human [166, 167] and animal studies [168]. Prospective studies of enteroviruses in Finland, where diabetes incidence is high but enterovirus infections are uncommon, revealed that the viruses were more often detected in those children who later developed diabetes than in their siblings who did not [169-173] and Luppi *et al.* reported that host susceptibility to enteroviral infection may be influenced by *HLA* background [173] - potentially compounding any attempt at association studies. Despite this, a causal association is supported by the seroconversion to anti-islet antibodies after enterovirus infection found in children with the high risk *HLA-DQB1* genotype [170].

Coxsackie virus B4

Gamble and colleagues [166, 167] described a seasonal variation in T1D incidence following enterovirus infection, as well as increased antibodies against Coxsackie virus B4 serotypes, in newly diagnosed patients when compared to control subjects. These findings were confirmed by subsequent studies [174-178]. Sequence similarity between the enterovirus 2C protein of Coxsackie virus B4 and the 65 kDa form of glutamate decarboxylase, (GAD65), a GABA-producing neuroendocrine enzyme present in β-cells [179], raises the possibility that an anti-viral T cell response may cross react with the native protein, inducing an autoimmune response [180, 181]. To date, attempts to demonstrate direct cross-reactivity at the T cell clonal level have failed despite the relevant peptides being highly immunogenic [182, 183].

Although great interest was generated by the report of Yoon *et al.* that Coxsackie virus B4, isolated from the pancreas of a child who died from diabetic ketoacidosis, transferred diabetes to diabetes-resistant SJL mice, this feat has never been replicated [168]. Horwitz *et al.* revisited the issue by infecting recombinant mouse strains with Coxsackie virus B4 virus [184]. Although mice bearing the diabetes-associated MHC haplotype of NOD mice on a C57 strain background did not develop diabetes, and infected wild type NOD mice did not show evidence of exacerbated disease, infected NOD mice bearing a transgenic T cell receptor (BDC2.5), which was specific for an islet antigen, did. Serreze *et al.* subsequently found that infection with Coxsackie virus B4 can accelerate diabetes in NOD mice providing a certain level of autoreactive T cells have accumulated prior to infection [185].

Other viruses

Infection with other viruses, such as mumps, human cytomegalovirus (CMV) and rotaviruses have also been suggested to be diabetogenic in susceptible individuals [169, 186, 171, 187-189]. Although the mumps epidemics have been associated with an increase in T1D incidence [190], the introduction of a vaccination for mumps has not proven an effective primary preventative. The association between CMV and T1D was implied by a case report of an infant with CMV who presented with T1D at 13 months of age [191]. Others tried to verify this association in T1D patients, but could not substantiate it [192].

Rotaviruses are double-stranded RNA viruses of the reovirus family and are a predominant cause of gastroenteritis in infants. They were suspected to have an association with T1D due to a strong sequence similarity between rotavirus VP7 and both GAD65 and IA-2 [193, 194]. In the longitudinal Australian BabyDiab study of at-risk children, Honeyman *et al.* revealed that islet antibodies appeared in 24 of 300 children within the same 6 month period in which they had significant rises in rotavirus-specific IgA and IgG [194]. This association of rotavirus infections with development of diabetes was not confirmed in a Finnish study [195].

Gene/environment interactions

To date, most postulated mechanisms for environmental effects on the risk of T1D involve the initiation of disease; either due to cross reactivity of immune responses to food or microorganisms to β-cell constituents, or through tissue tropism and cytotoxicity of infectious agents, resulting in antigen shedding and priming of an autoimmune response. The data from the spontaneous animal models of T1D are partially consistent with such a mechanism. If protein antigens are excluded and the diet supplemented by protein hydro-

lysate as a source of amino actids, diabetes is prevented [137, 196].

As a generalization, these mechanisms do not appear to play a major role in the modulation of disease risk by infectious agents in these models, as the greater the microorganism burden of the mice, the lower the incidence of diabetes [197]. The exact nature of such interactions may be difficult to dissect as they could be due to multifaceted interactions [198]; several infections might have to act in concert to precipitate clinical autoimmunity and in some infections viruses may play a role in prevention rather than precipitation of disease [199]. Progress is unlikely to be achieved until the interactions between environmental component and genome are understood at the molecular level. For example, we have identified a molecular constituent of *Mycobacterium bovis* cell wall (mycolylarabino-galactan-peptidoglycan; MAPG) capable of preventing diabetes in NOD mice, providing it is administered after the onset of insulitis, but before end-stage tissue destruction (PCT/AU97/00770). This appears to be the molecular basis of the diabetes protection attributed to complete Freund's adjuvant (CFA) [200] and whole *Mycobacterium bovis* [201] in this model. In contrast, exposure of intact skin to bacterial robosylating exotoxin can exacerbate disease in NOD mice [202]. Again, the effect appears to be a modulation of on-going inflammation, rather than due to conventional antigen priming.

Toll-like receptors

One potential mechanism of such interactions is via toll-like receptor (TLR) signaling [203, 198]. Toll-like receptors are a family of homologous proteins involved in the afferent limb of vertebrate innate immune responses to components of bacteria, viruses and parasites. Signaling via TLR has major effects on the activities of antigen presenting cells (APCs), including the production of inflammatory cytokines and the upregulation of MHC products and costimulator molecules. Although TLR4, the first identified member of the family, appears to act at the cell surface as the signal transduction component of a multimeric lipopolysaccharide (LPS) receptor [204], it is likely that some members operate within the cell. For example, TLR3 is necessary for responses to viral dsRNA; TLR9 is necessary for the immunopotentiating effects of unmethylated CpG DNA [205], which is presumably only exposed after bacterial lysis in phagolysosomes; and TLR2 is recruited to macrophage phagosomes following stimulation with a yeast cell wall preparation [206].

TLR2 is involved in responses to a broad range of constituents of pathogen cell walls, especially hydrophobic or lipid containing components. In many cases, it appears to act as a heterodimer with TLR1 or TLR6 or, based on strong sequence homologies, TLR-10. In contrast, TLR3, 7, 8 and 9 share relatively little sequence homology with the TLR2-associated members, respond to nucleic acids [207, 208] and all but TLR8 are known to mediate IFN-α and IFN-β production. TLR4 lies somewhere between these two groups: it has intermediate sequence homologies, responds to the LPS of gram negative bacteria, but mediates IFN-β secretion [209].

Like IL-1, ligated TLR interact with MyD88 through association of shared TIR domains. The death domain of MyD88 interacts with that of IRAK, a serine/threonine protein kinase, resulting in the activation and phosphorylation of IRAK, allowing it, in turn, to interact with TRAF6, an E3 ligase. TRAF6 then undergoes stimulus-dependent autoubiquitination, activating the kinase TAK1, which phosphorylates and activates the complex of IKK α and β kinases, leading to IκB degredation [reviewed in 210]. The IκB protein family holds NFκB/Rel transcription factor dimers latently in the cytoplasm and their degradation results in NFκB/Rel translocation to the nucleus [reviewed in 211], resulting in the secretion of IL-1β and upregulation of the expression of CD80 and CD86. Although the pathway described is common to all TLR, the target specific differences in outcome of stimulation indicates additional regulatory mechanisms and signaling pathways. For example, TLR4 signaling also involves a novel TIR domain containing adapter protein, called TIRAP [212] and Nod2 modulates TLR-dependent responses to LPS and muramyl dipeptide [213].

The direct evidence for a role of TLR in mediating environmental effects on progression to T1D is currently scant. In mice bearing a transgene that induced expression of the costimulator molecule B7.1 (CD80) on their pancreatic β-cells, multiple injections of the TLR3 ligand polyinosinic-polycytidylic (poly(I:C)) precipitated diabetes [214]. Similarly, in a rat model of diabetes, in which disease is induced in BB-diabetes resistant rats by infection with Kilham rat virus, prior treatment with poly(I:C) increased the incidence of diabetes from 23% to 100% [215]. A suggestion of an association between T1D and TLR3 polymorphisms in South African blacks has also been reported [216], but as the gene encoding TLR3 does not map to a major linkage region for T1D in other populations, confirmation is needed.

The protective effect of both CFA and *Mycobacterium bovis* in NOD mice is mediated by IFN-γ [217], which is likely to be a downstream consequence of IL-12 production by APC [218]. This, in turn, is known to be produced following TLR ligation by components of mycobacterial cell wall [219]. Consistent with this hypothesis is the claim that CFA mediated protection is dependent on an adjuvant action [200] and the dependence on MyD88 of the adjuvant action of CFA [220]. It is therefore interesting that the gene encoding TLR2, which plays a critical role in innate immune responses to mycobacterial wall (including to MAPG specifically) [221], maps to the same chromosome 3 region as the NOD mouse diabetes susceptibility gene *Idd17* [222]. Similarly, the gene encoding mouse TLR12, identified only through sequence homology at this stage, maps to the same genomic region of chromosome 4 as the NOD mouse diabetes susceptibility gene *Idd25* [223].

Of greater clinical significance, is the colocalization of human genes. The gene encoding TLR4 maps to chromosome 9q33 [224], the same region Concannon *et al.* [31] mapped an unnamed T1D locus of suggestive genome wide significance. There is no clear published evidence of altered TLR4 function in diabetic patients. The gene encoding TLR5 in humans is located on chromosome 1q42, a T1D linkage region identified by Cox *et al.* [30] and subsequently confirmed by others [225, 226]. The systemic autoimmune disease systemic lupus erythematosus also maps to this locus in both humans [227, 228] and mice [229-232], including lupus induced in NOD mice by mycobacteria [233]. In this case, a polymorphism introducing a premature stop codon into the sequence of TLR5 in man has been associated with disease [234]. Clearly, in the case of diabetes, there is work to be done!

■ References

1. **Johnston C, Millward BA, Hoskins P, Leslie RD, Bottazzo GF, Pyke DA.** Islet-cell antibodies as predictors of the later development of type 1 (insulin-dependent) diabetes. A study in identical twins. *Diabetologia* 1989. 32(6):382-386.
2. **Bonifacio E, Bingley PJ, Shattock M, Dean BM, Dunger D, Gale EA, Bottazzo GF.** Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet* 1990. 335(8682):147-149.
3. **Rewers M, LaPorte RE, King H, Tuomilehto J.** Trends in the prevalence and incidence of diabetes: insulin-dependent diabetes mellitus in childhood. *World Health Stat Q* 1988;41(3-4):179-189.
4. **Rewers M.** The changing face of the epidemiology of insulin-dependent diabetes mellitus (IDDM): research designs and models of disease causation. *Ann Med* 1991. 23(4):419-426.
5. **Libman I, Songer T, LaPorte R.** How many people in the U.S. have IDDM? *Diabetes Care* 1993. 16(5):841-842.
6. **Chase HP, MacKenzie TA, Burdick J, Fiallo-Scharer R, Walravens P, Klingensmith G, Rewers M.** Redefining the clinical remission period in children with type 1 diabetes. *Pediatr Diabetes* 2004. 5(1):16-19.
7. **Serreze DV, Chen YG.** Of mice and men: use of animal models to identify possible interventions for the prevention of autoimmune type 1 diabetes in humans. *Trends Immunol* 2005. 26(11):603-607.
8. **Mordes JP, Bortell R, Blankenhorn EP, Rossini AA, Greiner DL.** Rat models of type 1 diabetes: genetics, environment, and autoimmunity. *ILAR J* 2004. 45(3):278-291.
9. **Bakke AC, Purtzer MZ, Wildin RS.** Prospective immunological profiling in a case of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX). *Clin Exp Immunol* 2004. 137(2):373-378.
10. **Perheentupa J.** APS-I/APECED: the clinical disease and therapy. *Endocrinol Metab Clin North Am* 2002. 31(2):295-320.
11. **Myhre AG, Halonen M, Eskelin P, Ekwall O, Hedstrand H, Rorsman F, Kampe O, Husebye ES.** Autoimmune polyendocrine syndrome type 1 (APS I) in Norway. *Clin Endocrinol (Oxf)* 2001. 54(2):211-217.
12. **Kumar PG, Laloraya M, She JX.** Population genetics and functions of the autoimmune regulator (AIRE). Endocrinol Metab Clin North Am 2002. 31(2):321-338.
13. **Aaltonen J, Bjorses P, Sandkuijl L, Perheentupa J, Peltonen L.** An autosomal locus causing autoimmune disease: autoimmune polyglandular disease type I assigned to chromosome 21. *Nat Genet* 1994. 8(1):83-87.
14. **Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, Krohn KJ, Lalioti MD, Mullis PE, Antonarakis SE, et al.** Positional cloning of the APECED gene. *Nat Genet* 1997. 17(4):393-398.
15. **Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D.** Projection of an immunological self shadow within the thymus by the AIRE protein. *Science* 2002. 298(5597):1395-1401.
16. **Liston A, Gray DH, Lesage S, Fletcher AL, Wilson J, Webster KE, Scott HS, Boyd RL, Peltonen L, Goodnow CC.** Gene dosage--limiting role of AIRE in thymic expression, clonal deletion, and organ-specific autoimmunity. *J Exp Med* 2004. 200(8):1015-1026.
17. **Mathis D, Benoist C.** Back to central tolerance. *Immunity* 2004. 20(5):509-516.
18. **Sabater L, Ferrer-Francesch X, Sospedra M, Caro P, Juan M, Pujol-Borrell R.** Insulin alleles and autoimmune regulator (AIRE) gene expression both influence insulin expression in the thymus. *J Autoimmun* 2005. 25(4):312-318.
19. **Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD.** The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001. 27(1):20-21.
20. **Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, et al.** X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001. 27(1):18-20.
21. **Myers AK, Perroni L, Costigan C, Reardon W.** Clinical

and molecular findings in IPEX syndrome. *Arch Dis Child* 2006. 91(1):63-64.

22. **Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM.** JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J Clin Invest* 2000. 106(12):R75-R81.
23. **Roncador G, Brown PJ, Maestre L, Hue S, Martinez-Torrecuadrada JL, Ling KL, Pratap S, Toms C, Fox BC, Cerundolo V, Powrie F, Banham AH.** Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. *Eur J Immunol* 2005. 35(6):1681-1691.
24. **Fontenot JD, Gavin MA, Rudensky AY.** Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003. 4(4):330-336.
25. **Hori S, Nomura T, Sakaguchi S.** Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003. 299(5609):1057-1061.
26. **Khattri R, Cox T, Yasayko SA, Ramsdell F.** An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol* 2003. 4(4):337-342.
27. **Watanabe N, Wang YH, Lee HK, Ito T, Wang YH, Cao W, Liu YJ.** Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature* 2005. 436(7054):1181-1185.
28. **Bassuny WM, Ihara K, Sasaki Y, Kuromaru R, Kohno H, Matsuura N, Hara T.** A functional polymorphism in the promoter/enhancer region of the FOXP3/Scurfin gene associated with type 1 diabetes. Immunogenetics 2003. 55(3):149-156.
29. **Zavattari P, Deidda E, Pitzalis M, Zoa B, Moi L, Lampis R, Contu D, Motzo C, Frongia P, Angius E, Maioli M, Todd JA, Cucca F.** No association between variation of the FOXP3 gene and common type 1 diabetes in the Sardinian population. *Diabetes* 2004. 53(7):1911-1914.
30. **Cox NJ, Wapelhorst B, Morrison VA, Johnson L, Pinchuk L, Spielman RS, Todd JA, Concannon P.** Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am J Hum Genet* 2001. 69(4):820-830.
31. **Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Pociot F, Todd JA, Rich SS.** Type 1 Diabetes Genetics Consortium. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. *Diabetes* 2005. 54(10):2995-3001.
32. **Nerup J, Platz P, Andersen OO, Christy M, Lyngsoe J, Poulsen JE, Ryder LP, Nielsen LS, Thomsen M, Svejgaard A.** HL-A antigens and diabetes mellitus. *Lancet* 1974. 2(7885):864-866.
33. **Barbosa J, Chern MM, Noreen H, Anderson VE.** Analysis of linkage between the major histocompatibility system and juvenile, insulin-dependent diabetes in multiplex families. Reanalysis of data. *J Clin Invest* 1978. 62(2):492-495.
34. **Bell GI, Horita S, Karam JH.** A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes* 1984. 33(2):176-183.
35. **Hitman GA, Tarn AC, Winter RM, Drummond V, Williams LG, Jowett NI, Bottazzo GF, Galton DJ.** Type 1 (insulin-dependent) diabetes and a highly variable locus close to the insulin gene on chromosome 11. *Diabetologia* 1985. 28(4):218-222.
36. **Lee YJ, Huang FY, Lo FS, Wang WC, Hsu CH, Kao HA, Yang TY, Chang JG.** Association of CTLA4 gene A-G polymorphism with type 1 diabetes in Chinese children. *Clin Endocrinol (Oxf)* 2000. 52(2):153-157.
37. **Ongagna JC, Sapin R, Pinget M, Belcourt A.** Markers for risk of type 1 diabetes in relatives of Alsacian patients with type 1 diabetes. *Int J Exp Diabetes Res* 2002. 3(1):1-9.
38. **Zalloua PA, Abchee A, Shbaklo H, Zreik TG, Terwedow H, Halaby G, Azar ST.** Patients with early onset of type 1 diabetes have significantly higher GG genotype at position 49 of the CTLA4 gene. *Hum Immunol* 2004. 65(7):719-724.
39. **Golden B, Levin L, Ban Y, Concepcion E, Greenberg DA, Tomer Y.** Genetic analysis of families with autoimmune diabetes and thyroiditis: evidence for common and unique genes. *J Clin Endocrinol Metab* 2005. 90(8):4904-4911.
40. **Sheehy MJ, Scharf SJ, Rowe JR, Neme de Gimenez MH, Meske LM, Erlich HA, Nepom BS.** A diabetes-susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. *J Clin Invest* 1989. 83(3):830-835.
41. **Reich EP, Sherwin RS, Kanagawa O, Janeway CA Jr.** An explanation for the protective effect of the MHC class II I-E molecule in murine diabetes. *Nature* 1989. 341(6240):326-328.
42. **Uehira M, Uno M, Kurner T, Kikutani H, Mori K, Inomoto T, Uede T, Miyazaki J, Nishimoto H, Kishimoto T, Yamamura KI.** Development of autoimmune insulitis is prevented in E alpha d but not in A beta k NOD transgenic mice. *Int Immunol* 1989. 1(2):209-213.
43. **Lund T, O'Reilly L, Hutchings P, Kanagawa O, Simpson E, Gravely R, Chandler P, Dyson J, Picard JK, Edwards A, Kioussis D, Cooke A.** Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A beta-chain or normal I-E alpha-chain. *Nature* 1990. 345(6277):727-729.
44. **Slattery RM, Kjer-Nielsen L, Allison J, Charlton B, Mandel TE, Miller JF.** Prevention of diabetes in non-obese diabetic I-Ak transgenic mice. *Nature* 1990. 345(6277):724-726.
45. **Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, Wiley DC.** Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 1993. 364(6432):33-39.
46. **Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC.** Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* 1994. 368(6468):215-221.
47. **Redondo MJ, Kawasaki E, Mulgrew CL, Noble JA, Erlich HA, Freed BM, Lie BA, Thorsby E, Eisenbarth GS, Undlien DE, Ronningen KS.** DR- and DQ-associated protection from type 1A diabetes: comparison of DRB1*1401 and DQA1*0102-QB1*0602*. *J Clin Endocrinol Metab* 2000. 85(10):3793-3797.
48. **Owerbach D, Gabbay KH.** Localization of a type 1 diabetes susceptibility locus to the variable tandem repeat region flanking the insulin gene. *Diabetes* 1993. 42(12):1708-1714.
49. **Stead JD, Jeffreys AJ.** Allele diversity and germline mutation at the insulin minisatellite. *Hum Mol Genet* 2000. 9(5):713-723.
50. **Vafiadis P, Ounissi-Benkalha H, Palumbo M, Grabs R, Rousseau M, Goodyer CG, Polychronakos C.** Class III alleles of the variable number of tandem repeat insulin polymorphism associated with silencing of thymic insulin predispose to type 1 diabetes. *J Clin Endocrinol Metab* 2001. 86(8):3705-3710.

51. **Bennett ST, Wilson AJ, Cucca F, Nerup J, Pociot F, McKinney PA, Barnett AH, Bain SC, Todd JA.** IDDM2-VNTR-encoded susceptibility to type 1 diabetes: dominant protection and parental transmission of alleles of the insulin gene-linked minisatellite locus. *J Autoimmun* 1996. 9(3):415-421.
52. **Pugliese A, Zeller M, Fernandez A Jr, Zalcberg LJ, Bartlett RJ, Ricordi C, Pietropaolo M, Eisenbarth GS, Bennett ST, Patel DD.** The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat Genet* 1997. 15(3):293-297.
53. **Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, Wickramasinghe S, Colle E, Polychronakos C.** Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet* 1997. 15(3):289-292.
54. **Chentoufi AA, Polychronakos C.** Insulin expression levels in the thymus modulate insulin-specific autoreactive T-cell tolerance: the mechanism by which the IDDM2 locus may predispose to diabetes. *Diabetes* 2002. 51(5):1383-1390.
55. **Ilieva A, Yuan S, Wang RN, Agapitos D, Hill DJ, Rosenberg L.** Pancreatic islet cell survival following islet isolation: the role of cellular interactions in the pancreas. *J Endocrinol* 1999. 161:357-364.
56. **Geenen V, Achour I, Robert F, Vandersmissen E, Sodoyez JC, Defresne MP, Boniver J, Lefebvre PJ, Franchimont P.** Evidence that insulin-like growth factor 2 (IGF2) is the dominant thymic peptide of the insulin superfamily. *Thymus* 1993. 21(2):115-127.
57. **Martens H, Goxe B, Geenen V.** The thymic repertoire of neuroendocrine self-antigens: physiological implications in T-cell life and death. *Immunol Today* 1996. 17(7):312-317.
58. **Paquette J, Giannoukakis N, Polychronakos C, Vafiadis P, Deal C.** The INS 5' variable number of tandem repeats is associated with IGF2 expression in humans. *J Biol Chem* 1998. 273(23):14158-14164.
59. **Dahlquist G, Bennich SS, Kallen B.** Intrauterine growth pattern and risk of childhood onset insulin-dependent (type I) diabetes: population based case-control study. *BMJ* 1996. 313(7066):1174-1177.
60. **Vafiadis P, Bennett ST, Todd JA, Grabs R, Polychronakos C.** Divergence between genetic determinants of IGF2 transcription levels in leukocytes and of IDDM2-encoded susceptibility to type 1 diabetes. *J Clin Endocrinol Metab* 1998. 83(8):2933-2939.
61. **Vafiadis P, Grabs R, Goodyer CG, Colle E, Polychronakos C.** A functional analysis of the role of IGF2 in IDDM2-encoded susceptibility to type 1 diabetes. *Diabetes* 1998. 47(5):831-836.
62. **Agarwal K, Jones DE, Daly AK, James OF, Vaidya B, Pearce S, Bassendine MF.** CTLA-4 gene polymorphism confers susceptibility to primary biliary cirrhosis. *J Hepatol* 2000. 32(4):538-541.
63. **Harper K, Balzano C, Rouvier E, Mattei MG, Luciani MF, Golstein P.** CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. *J Immunol* 1991. 147(3):1037-1044.
64. **Zhernakova A, Eerligh P, Barrera P, Weseloy JZ, Huizinga TW, Roep BO, Wijmenga C, Koeleman BP.** CTLA4 is differentially associated with autoimmune diseases in the Dutch population. *Hum Genet* 2005. 118(1):58-66.
65. **Kouki T, Sawai Y, Gardine CA, Fisfalen ME, Alegre ML, DeGroot LJ.** CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J Immunol* 2000. 165(11):6606-6611.
66. **Maurer M, Loserth S, Kolb-Maurer A, Ponath A, Wiese S, Kruse N, Rieckmann P.** A polymorphism in the human cytotoxic T-lymphocyte antigen 4 (CTLA4) gene (exon 1 +49) alters T-cell activation. *Immunogenetics* 2002. 54(1):1-8.
67. **Reed P, Cucca F, Jenkins S, Merriman M, Wilson A, McKinney P, Bosi E, Joner G, Ronningen K, Thorsby E, Undlien D, Merriman T, Barnett A, Bain S, Todd J.** Evidence for a type 1 diabetes susceptibility locus (IDDM10) on human chromosome 10p11-q11. *Hum Mol Genet* 1997. 6(7):1011-1016.
68. **Guo D, Li M, Zhang Y, Yang P, Eckenrode S, Hopkins D, Zheng W, Purohit S, Podolsky RH, Muir A, et al.** A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nat Genet* 2004. 36(8):837-841.
69. **Park Y, Park S, Kang J, Yang S, Kim D.** Assessing the validity of the association between the SUMO4 M55V variant and risk of type 1 diabetes. *Nat Genet* 2005. 37(2):112.
70. **Qu H, Bharaj B, Liu XQ, Curtis JA, Newhook LA, Paterson AD, Hudson TJ, Polychronakos C.** Assessing the validity of the association between the SUMO4 M55V variant and risk of type 1 diabetes. *Nat Genet* 2005. 37(2):111-112.
71. **Noso S, Ikegami H, Fujisawa T, Kawabata Y, Asano K, Hiromine Y, Tsurumaru M, Sugihara S, Lee I, Kawasaki E, Awata T, Ogihara T.** Genetic heterogeneity in association of the SUMO4 M55V variant with susceptibility to type 1 diabetes. *Diabetes* 2005. 54(12):3582-3586.
72. **Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM, Vella A, Nutland S, Rance HE, Maier L, et al.** Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 2004. 53(11):3020-3023.
73. **Onengut-Gumuscu S, Ewens KG, Spielman RS, Concannon P.** A functional polymorphism (1858C/T) in the PTPN22 gene is linked and associated with type 1 diabetes in multiplex families. *Genes Immun* 2004. 5(8):678-680.
74. **Qu H, Tessier MC, Hudson TJ, Polychronakos C.** Confirmation of the association of the R620W polymorphism in the protein tyrosine phosphatase PTPN22 with type 1 diabetes in a family based study. *J Med Genet* 2005. 42(3):266-270.
75. **Hasegawa K, Martin F, Huang G, Tumas D, Diehl L, Chan AC.** PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 2004. 303(5658):685-689.
76. **Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T.** A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat Genet* 2004. 36(4):337-338.
77. **Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, et al.** A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phos-

phatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004. 75(2):330-337.
78. **Cloutier JF, Veillette A.** Association of inhibitory tyrosine protein kinase p50csk with protein tyrosine phosphatase PEP in T cells and other hemopoietic cells. *EMBO J* 1996. 15(18):4909-4918.
79. **Gomez LM, Anaya JM, Gonzalez CI, Pineda-Tamayo R, Otero W, Arango A, Martin J.** PTPN22 C1858T polymorphism in Colombian patients with autoimmune diseases. *Genes Immun* 2005. 6(7):628-631.
80. **Zheng W, She JX.** Genetic association between a lymphoid tyrosine phosphatase (PTPN22) and type 1 diabetes. *Diabetes* 2005. 54(3):906-908.
81. **Jos J, Farkas D, de Tand MF, Cartron J, Cohen-Hagenhauer O, Tozzo E, Deschamps I.** DNA polymorphism analysis of HLA class II genes in unrelated children and in first-degree relatives with type 1 diabetes. *Diabetes Res* 1991. 18(2):53-59.
82. **Margaritte-Jeannin P, Clerget-Darpoux F, Hors J, Deschamps I.** Testing parental imprinting in insulin-dependent diabetes mellitus by the marker-association-segregation-chi 2 method. *Am J Hum Genet* 1995. 56(5):1080-1087.
83. **Feugeas JP, Tortosa P, Dulay S, Augustin-Pascalis I, Charron D, Krishnamoorthy R, Caillens H, Montchamp-Moreau C.** Analysis of HLA haplotypes in families with type 1 diabetes mellitus on La Reunion island. *Eur J Immunogenet* 1996. 23(6):459-470.
84. **Deschamps I, Hors J, Clerget-Darpoux F, Gardais E, Robert JJ, Marcelli-Berge A, Lestradet H, Dausset J.** Excess of maternal HLA-DR3 antigens in HLA DR3,4 positive type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1990. 33(7):425-430.
85. **Bain SC, Rowe BR, Barnett AH, Todd JA.** Parental origin of diabetes-associated HLA types in sibling pairs with type 1 diabetes. *Diabetes* 1994. 43(12):1462-1468.
86. **Undlien DE, Akselsen HE, Joner G, Dahl-Jorgensen K, Aagenaes O, Sovik O, Thorsby E, Ronningen KS.** No difference in the parental origin of susceptibility HLA class II haplotypes among Norwegian patients with insulin-dependent diabetes mellitus. *Am J Hum Genet* 1995. 57(6):1511-1514.
87. **Bennett ST, Lucassen AM, Gough SC, Powell EE, Undlien DE, Pritchard LE, Merriman ME, Kawaguchi Y, Dronsfield MJ, Pociot F, et al.** Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat Genet* 1995. 9(3):284-292.
88. **Kim HS, Lee DW, Lee SJ, Choi BH, Chang SI, Yoon HD, Lee IK.** The effect of parental imprinting on the INS-IGF2 locus of Korean type 1 diabetic patients. *Korean J Intern Med* 2001. 16(4):223-229.
89. **Polychronakos C, Giannoukakis N, Deal CL.** Imprinting of IGF2, insulin-dependent diabetes, immune function, and apoptosis: a hypothesis. *Dev Genet* 1995. 17(3):253-262.
90. **Bennett ST, Wilson AJ, Esposito L, Bouzekri N, Undlien DE, Cucca F, Nistico L, Buzzetti R, Bosi E, Pociot F, et al.** Insulin VNTR allele-specific effect in type 1 diabetes depends on identity of untransmitted paternal allele. The IMDIAB Group. *Nat Genet* 1997. 17(3):350-352.
91. **Delepine M, Pociot F, Habita C, Hashimoto L, Froguel P, Rotter J, Cambon-Thomsen A, Deschamps I, Djoulah S, Weissenbach J, Nerup J, Lathrop M, Julier C.** Evidence of a non-MHC susceptibility locus in type 1 diabetes linked to HLA on chromosome 6. *Am J Hum Genet* 1997. 60(1):174-187.
92. **Paterson AD, Naimark DM, Petronis A.** The analysis of parental origin of alleles may detect susceptibility loci for complex disorders. *Hum Hered* 1999. 49(4):197-204.
93. **Onkamo P, Vaananen S, Karvonen M, Tuomilehto J.** Worldwide increase in incidence of type 1 diabetes--the analysis of the data on published incidence trends. *Diabetologia* 1999. 42(12):1395-1403.
94. **McKinney PA, Parslow R, Gurney K, Law G, Bodansky HJ, Williams DR.** Antenatal risk factors for childhood diabetes mellitus; a case-control study of medical record data in Yorkshire, UK. *Diabetologia* 1997. 40(8):933-939.
95. **Baum JD, Ounsted M, Smith MA.** Weight gain in infancy and subsequent development of diabetes mellitus in childhood. *Lancet* 1975. 2(7940):866.
96. **Johansson C, Samuelsson U, Ludvigsson J.** A high weight gain early in life is associated with an increased risk of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1994. 37(1):91-94.
97. **Bruining GJ.** Association between infant growth before onset of juvenile type 1 diabetes and autoantibodies to IA-2. Netherlands Kolibrie study group of childhood diabetes. *Lancet* 2000. 356(9230):655-656.
98. **Kagohashi Y, Udagawa J, Abiru N, Kobayashi M, Moriyama K, Otani H.** Maternal factors in a model of type 1 diabetes differentially affect the development of insulitis and overt diabetes in offspring. *Diabetes* 2005. 54(7):2026-2031.
99. **Ivarsson A, Persson LA, Juto P, Peltonen M, Suhr O, Hernell O.** High prevalence of undiagnosed celiac disease in adults: a Swedish population-based study. *J intern Med* 1999. 245:63-68.
100. **Walker-Smith JA, Grigor W.** Coeliac disease in a diabetic child. *Lancet* 1969. 1(7603):1021.
101. **Visakorpi JK.** Intestinal disaccharide decomposition and its cogenital disturbances. Monatsschr Kinderheilkd 1969. 117(1):25-27.
102. **Walsh CH, Cooper BT, Wright AD, Malins JM, Cooke WT.** Diabetes mellitus and coeliac disease: a clinical study. *Q J Med* 1978. 47(185):89-100.
103. **Anderson RP, Degano P, Godkin AJ, Jewell DP, Hill AV.** In vivo antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. *Nat Med* 2000. 6(3):337-342.
104. **De Vitis I, D'Addesa S, D'Agostino G, Cotroneo P, Ghirlanda G.** Celiac sprue and insulin-dependent diabetes mellitus. *J Clin Gastroenterol* 1993. 17(4):354-355.
105. **Bao F, Yu L, Babu S, Wang T, Hoffenberg EJ, Rewers M, Eisenbarth GS.** One third of HLA DQ2 homozygous patients with type 1 diabetes express celiac disease-associated transglutaminase autoantibodies. *J Autoimmun* 1999. 13(1):143-148.
106. **Sires JM.** Diabetes mellitus in childhood and adolescence. Clinical types. *Bol Med Hosp Infant Mex* 1979. 36(6):1153-1162.
107. **Akerblom HK, Vaarala O, Hyoty H, Ilonen J, Knip M.** Environmental factors in the etiology of type 1 diabetes. *Am J Med Genet* 2002. 115(1):18-29.
108. **Holmes GK, Prior P, Lane MR, Pope D, Allan RN.** Malignancy in coeliac disease - effect of a gluten free diet. *Gut* 1989. 30(3):333-338.
109. **O'Connor TM, Cronin CC, Loane JF, O'Meara NM, Firth RG, Shanahan F, O'Halloran DJ.** Type 1 diabetes

mellitus, coeliac disease, and lymphoma: a report of four cases. *Diabet Med* 1999. 16(7):614-617.
110. **Holmes GK.** Screening for coeliac disease in type 1 diabetes. *Arch Dis Child* 2002. 87(6):495-498.
111. **Elliott RB, Martin JM.** Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? *Diabetologia* 1984. 26(4):297-299.
112. **Scott FW, Sarwar G, Cloutier HE.** Diabetogenicity of various protein sources in the diet of the diabetes-prone BB rat. *Adv Exp Med Biol* 1988. 246:277-285.
113. **Schmid S, Koczwara K, Schwinghammer S, Lampasona V, Ziegler AG, Bonifacio E.** Delayed exposure to wheat and barley proteins reduces diabetes incidence in non-obese diabetic mice. *Clin Immunol* 2004. 111(1):108-118.
114. **Hoorfar J, Buschard K, Dagnaes-Hansen F.** Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice. *Br J Nutr* 1993. 69(2):597-607.
115. **Funda DP, Kaas A, Bock T, Tlaskalova-Hogenova H, Buschard K.** Gluten-free diet prevents diabetes in NOD mice. *Diabetes Metab Res Rev* 1999. 15(5):323-327.
116. **Maurano F, Mazzarella G, Luongo D, Stefanile R, D'Arienzo R, Rossi M, Auricchio S, Troncone R.** Small intestinal enteropathy in non-obese diabetic mice fed a diet containing wheat. *Diabetologia* 2005. 48(5):931-937.
117. **Hummel M, Bonifacio E, Naserke HE, Ziegler AG.** Elimination of dietary gluten does not reduce titers of type 1 diabetes-associated autoantibodies in high-risk subjects. *Diabetes Care* 2002. 25(7):1111-1116.
118. **Pastore MR, Bazzigaluppi E, Belloni C, Arcovio C, Bonifacio E, Bosi E.** Six months of gluten-free diet do not influence autoantibody titers, but improve insulin secretion in subjects at high risk for type 1 diabetes. *J Clin Endocrin Metabol* 2006. 88(1):162-165.
119. **Borch-Johnsen K, Joner G, Mandrup-Poulsen T, Christy M, Zachau-Christiansen B, Kastrup K, Nerup J.** Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. A hypothesis. *Lancet* 1984. 2(8411):1083-1086.
120. **Fort P, Lanes R, Dahlem S, Recker B, Weyman-Daum M, Pugliese M, Lifshitz F.** Breast feeding and insulin-dependent diabetes mellitus in children. *J Am Coll Nutr* 1986. 5(5):439-441.
121. **Glatthaar C, Whittall DE, Welborn TA, Gibson MJ, Brooks BH, Ryan MM, Byrne GC.** Diabetes in Western Australian children: descriptive epidemiology. *Med J Aust* 1988. 148(3):117-123.
122. **Mayer EJ, Hamman RF, Gay EC, Lezotte DC, Savitz DA, Klingensmith GJ.** Reduced risk of IDDM among breast-fed children. The Colorado IDDM Registry. *Diabetes* 1988. 37(12):1625-1632.
123. **Blom L, Dahlquist G, Nystrom L, Sandstrom A, Wall S.** The Swedish childhood diabetes study--social and perinatal determinants for diabetes in childhood. *Diabetologia* 1989. 32(1):7-13.
124. **Akerblom HK, Knip M.** Putative environmental factors in Type 1 diabetes. *Diabetes Metab Rev* 1998. 14(1):31-67.
125. **Harrison LC, Honeyman MC.** Cow's milk and type 1 diabetes: the real debate is about mucosal immune function. *Diabetes* 1999. 48(8):1501-1507.
126. **Virtanen SM, Saukkonen T, Savilahti E, Ylonen K, Rasanen L, Aro A, Knip M, Tuomilehto J, Akerblom HK.** Diet, cow's milk protein antibodies and the risk of IDDM in Finnish children. Childhood Diabetes in Finland Study Group. *Diabetologia* 1994. 37(4):381-387.
127. **Gerstein HC.** Cow's milk exposure and type 1 diabetes mellitus. A critical overview of the clinical literature. *Diabetes Care* 1994. 17(1):13-19.
128. **Kimpimaki T, Kupila A, Hamalainen AM, Kukko M, Kulmala P, Savola K, Simell T, Keskinen P, Ilonen J, Simell O, Knip M.** The first signs of beta-cell autoimmunity appear in infancy in genetically susceptible children from the general population: the Finnish Type 1 Diabetes Prediction and Prevention Study. *J Clin Endocrinol Metab* 2001. 86(10):4782-4788.
129. **Verge CF, Howard NJ, Irwig L, Simpson JM, Mackerras D, Silink M.** Environmental factors in childhood IDDM. A population-based, case-control study. *Diabetes Care* 1994. 17(12):1381-1389.
130. **Virtanen SM, Laara E, Hypponen E, Reijonen H, Rasanen L, Aro A, Knip M, Ilonen J, Akerblom HK.** Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: a nested case-control study of siblings of children with diabetes. Childhood Diabetes in Finland Study Group. *Diabetes* 2000. 49(6):912-917.
131. **Karjalainen J, Saukkonen T, Savilahti E, Dosch HM.** Disease-associated anti-bovine serum albumin antibodies in type 1 (insulin-dependent) diabetes mellitus are detected by particle concentration fluoroimmunoassay, and not by enzyme linked immunoassay. *Diabetologia* 1992. 35(10):985-990.
132. **Miyazaki I, Cheung RK, Gaedigk R, Hui MF, Van der Meulen J, Rajotte RV, Dosch HM.** T cell activation and anergy to islet cell antigen in type 1 diabetes. *J Immunol* 1995. 154(3):1461-1469.
133. **Savilahti E, Akerblom HK, Tainio VM, Koskimies S.** Children with newly diagnosed insulin-dependent diabetes mellitus have increased levels of cow's milk antibodies. *Diabetes Res* 1988. 7(3):137-140.
134. **Vaarala O, Klemetti P, Savilahti E, Reijonen H, Ilonen J, Akerblom HK.** Cellular immune response to cow's milk beta-lactoglobulin in patients with newly diagnosed IDDM. *Diabetes* 1996. 45(2):178-182.
135. **Roep BO.** T-cell responses to autoantigens in IDDM. The search for the Holy Grail. Diabetes 1996. 45(9):1147-1156.
136. **Karlsson CA, Wahlgren MC, Tragardh AC.** Non-invasive monitoring of protein adsorption and removal in a turbulent flow cell. *Colloids Surf B Biointerfaces* 2001. 20(1):9-25.
137. **Elliott RB, Reddy SN, Bibby NJ, Kida K.** Dietary prevention of diabetes in the nonobese diabetic mouse. *Diabetologia* 1988. 31(1):62-64.
138. **Martin JM, Trink B, Daneman D, Dosch HM, Robinson B.** Milk proteins in the etiology of insulin-dependent diabetes mellitus (IDDM). *Ann Med* 1991. 23(4):447-452.
139. **Beppu H, Winter WE, Atkinson MA, Maclaren NK, Fujita K, Takahashi H.** Bovine albumin antibodies in NOD mice. *Diabetes Res* 1987. 6(2):67-69.
140. **Dahlquist G, Mustonen L.** Childhood onset diabetes--time trends and climatological factors. *Int J Epidemiol* 1994. 23(6):1234-1241.
141. **Fairney A, Fry J, Lipscomb A.** The effect of darkness on vitamin D in adults. *Postgrad Med J* 1979. 55(642):248-250.
142. **Stryd RP, Gilbertson TJ, Brunden MN.** A seasonal variation study of 25-hydroxyvitamin D3 serum levels in normal humans. *J Clin Endocrinol Metab* 1979. 48(5):771-775.

143. **Webb AR, Kline L, Holick MF.** Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* 1988. 67(2):373-378.
144. **McKenna MJ.** Differences in vitamin D status between countries in young adults and the elderly. *Am J Med* 1992. 93(1):69-77.
145. **Barger-Lux MJ, Heaney RP.** Effects of above average summer sun exposure on serum 25-hydroxyvitamin D and calcium absorption. *J Clin Endocrinol Metab* 2002. 87(11):4952-4956.
146. **Mathieu C, Laureys J, Sobis H, Vandeputte M, Waer M, Bouillon R.** 1,25-Dihydroxyvitamin D3 prevents insulitis in NOD mice. *Diabetes* 1992. 41(11):1491-1495.
147. **Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM.** Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab* 1983. 57(6):1308-1310.
148. **Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC.** 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science* 1983. 221(4616):1181-1183.
149. **Mathieu C, Laureys J, Waer M, Bouillon R.** Prevention of autoimmune destruction of transplanted islets in spontaneously diabetic NOD mice by KH1060, a 20-epi analog of vitamin D: synergy with cyclosporine. *Transplant Proc* 1994. 26(6):3128-3129.
150. **EURODIAB.** Vitamin D supplement in early childhood and risk for type 1 (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. *Diabetologia* 1999. 42(1):51-54.
151. **Stene LC, Ulriksen J, Magnus P, Joner G.** Use of cod liver oil during pregnancy associated with lower risk of type 1 diabetes in the offspring. *Diabetologia* 2000. 43(9):1093-1098.
152. **Amrani A, Verdaguer J, Thiessen S, Bou S, Santamaria P.** IL-1alpha, IL-1beta, and IFN-gamma mark beta cells for Fas-dependent destruction by diabetogenic CD4(+) T lymphocytes. *J Clin Invest* 2000. 105(4):459-468.
153. **Karam JH, Lewitt PA, Young CW, Nowlain RE, Frankel BJ, Fujiya H, Freedman ZR, Grodsky GM.** Insulinopenic diabetes after rodenticide (Vacor) ingestion: a unique model of acquired diabetes in man. *Diabetes* 1980. 29(12):971-978.
154. **Ganda OP, Rossini AA, Like AA.** Studies on streptozotocin diabetes. *Diabetes* 1976. 25(7):595-603.
155. **Like AA, Rossini AA.** Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science* 1976. 193(4251):415-417.
156. **Leiter EH, Beamer WG, Shultz LD.** The effect of immunosuppression on streptozotocin-induced diabetes in C57BL/KsJ mice. *Diabetes* 1983. 32(2):148-155.
157. **Vallera DA, Carroll SF, Brief S, Blazar BR.** Anti-CD3 immunotoxin prevents low-dose STZ/interferon-induced autoimmune diabetes in mouse. *Diabetes* 1992. 41(4):457-464.
158. **Myers MA, Mackay IR, Rowley MJ, Zimmet PZ.** Dietary microbial toxins and type 1 diabetes--a new meaning for seed and soil. *Diabetologia* 2001. 44(9):1199-1200.
159. **Nakagawa K, Harrison LC.** The potential roles of endogenous retroviruses in autoimmunity. *Immunol Rev* 1996. 152:193-236.
160. **Tsumura H, Miyazawa M, Ogawa S, Wang JZ, Ito Y, Shimura K.** Detection of endogenous retrovirus antigens in NOD mouse pancreatic beta-cells. Lab Anim 1998. 32(1):86-94.
161. **Menser MA, Forrest JM, Bransby RD.** Rubella infection and diabetes mellitus. *Lancet* 1978. 1(8055):57-60.
162. **Shaver KA, Boughman JA, Nance WE.** Congenital rubella syndrome and diabetes: a review of epidemiologic, genetic, and immunologic factors. *Am Ann Deaf* 1985. 130(6):526-532.
163. **Rayfield EJ, Kelly KJ, Yoon JW.** Rubella virus-induced diabetes in the hamster. *Diabetes* 1986. 35(11):1278-1281.
164. **Phillips CA, Melnick JL, Yow MD, Bayatpour M, Burkhardt M.** Persistence of virus in infants with congenital rubella and in normal infants with a history of maternal rubella. *JAMA* 1965. 193:1027-1029.
165. **Hummel M, Fuchtenbusch M, Schenker M, Ziegler AG.** No major association of breast-feeding, vaccinations, and childhood viral diseases with early islet autoimmunity in the German BABYDIAB Study. *Diabetes Care* 2000. 23(7):969-974.
166. **Gamble DR, Taylor KW.** Seasonal incidence of diabetes mellitus. *Br Med J* 1969. 3(671):631-633.
167. **Gamble DR, Kinsley ML, FitzGerald MG, Bolton R, Taylor KW.** Viral antibodies in diabetes mellitus. *Br Med J* 1969. 3(671):627-630.
168. **Yoon JW, Austin M, Onodera T, Notkins AL.** Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. *N Engl J Med* 1979. 300(21):1173-1179.
169. **Hyoty H, Hiltunen M, Knip M, Laakkonen M, Vahasalo P, Karjalainen J, Koskela P, Roivainen M, Leinikki P, Hovi T, et al.** A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes* 1995. 44(6):652-657.
170. **Hiltunen M, Hyoty H, Knip M, Ilonen J, Reijonen H, Vahasalo P, Roivainen M, Lonnrot M, Leinikki P, Hovi T, Akerblom HK.** Islet cell antibody seroconversion in children is temporally associated with enterovirus infections. Childhood Diabetes in Finland (DiMe) Study Group. *J Infect Dis* 1997. 175(3):554-560.
171. **Lonnrot M, Salminen K, Knip M, Savola K, Kulmala P, Leinikki P, Hyypia T, Akerblom HK, Hyoty H.** Enterovirus RNA in serum is a risk factor for beta-cell autoimmunity and clinical type 1 diabetes: a prospective study. Childhood Diabetes in Finland (DiMe) Study Group. *J Med Virol* 2000. 61(2):214-220.
172. **Bruserud O, Jevell J, Thorsby E.** HLA-DR3 and -DR4 control T-lymphocyte responses to mumps and coxsackie B4 virus: studies on patients with type 1 (insulin-dependent) diabetes and healthy subjects. *Diabetologia* 1985. 28:420-426.
173. **Luppi P, Alexander A, Bertera S, Noonan K, Trucco M.** The same HLA-DQ alleles determine either susceptibility or resistance to different coxsackievirus-mediated autoimmune diseases. *J Biol Regul Homeost Agents* 1999. 13:14-26.
174. **Banatvala JE, Bryant J, Schernthaner G, Borkenstein M, Schober E, Brown D, De Silva LM, Menser MA, Silink M.** Coxsackie B, mumps, rubella, and cytomegalovirus specific IgM responses in patients with juvenile-onset insulin-dependent diabetes mellitus in Britain, Austria, and Australia. *Lancet* 1985. 1(8443):1409-1412.
175. **Frisk G, Friman G, Tuvemo T, Fohlman J, Diderholm H.** Coxsackie B virus IgM in children at onset of type 1 (insulin-dependent) diabetes mellitus: evidence for IgM induction by a recent or current infection. *Diabetologia* 1992. 35(3):249-253.
176. **Szopa TM, Titchener PA, Portwood ND, Taylor KW.**

Diabetes mellitus due to viruses-some recent developments. *Diabetologia* 1993. 36(8):687-695.
177. **Clements GB, Galbraith DN, Taylor KW.** Coxsackie B virus infection and onset of childhood diabetes. *Lancet* 1995. 346(8969):221-223.
178. **Helfand RF, Gary HE Jr, Freeman CY, Anderson LJ, Pallansch MA.** Serologic evidence of an association between enteroviruses and the onset of type 1 diabetes mellitus. Pittsburgh Diabetes Research Group. *J Infect Dis* 1995. 172(5):1206-1211.
179. **Okada Y, Taniguchi H, Schimada C.** High concentration of GABA and high glutamate decarboxylase activity in rat pancreatic islets and human insulinoma. *Science* 1976. 194(4265):620-622.
180. **Atkinson MA, Bowman MA, Campbell L, Darrow BL, Kaufman DL, Maclaren NK.** Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. *J Clin Invest* 1994. 94(5):2125-2129.
181. **Varela-Calvino R, Sgarbi G, Arif S, Peakman M.** T-Cell reactivity to the P2C nonstructural protein of a diabetogenic strain of coxsackievirus B4. *Virology* 2000. 274(1):56-64.
182. **Marttila J, Juhela S, Vaarala O, Hyoty H, Roivainen M, Hinkkanen A, Vilja P, Simell O, Ilonen J.** Responses of coxsackievirus B4-specific T-cell lines to 2C protein-characterization of epitopes with special reference to the GAD65 homology region. *Virology* 2001. 284(1):131-141.
183. **Schloot NC, Willemen SJ, Duinkerken G, Drijfhout JW, de Vries RR, Roep BO.** Molecular mimicry in type 1 diabetes mellitus revisited: T-cell clones to GAD65 peptides with sequence homology to Coxsackie or proinsulin peptides do not crossreact with homologous counterpart. *Hum Immunol* 2001. 62(4):299-309.
184. **Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvetnick N.** Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nat Med* 1998. 4(7):781-785.
185. **Serreze DV, Ottendorfer EW, Ellis TM, Gauntt CJ, Atkinson MA.** Acceleration of type 1 diabetes by a coxsackievirus infection requires a preexisting critical mass of autoreactive T-cells in pancreatic islets. *Diabetes* 2000. 49:708-711.
186. **Nairn C, Galbraith DN, Taylor KW, Clements GB.** Enterovirus variants in the serum of children at the onset of type 1 diabetes mellitus. *Diabet Med* 1999. 16(6):509-513.
187. **Hyoty H.** Enterovirus infections and type 1 diabetes. *Ann Med* 2002. 34(3):138-147.
188. **Lammi N, Karvonen M, Tuomilehto J.** Do microbes have a causal role in type 1 diabetes? *Med Sci Monit* 2005. 11(3):RA63-69.
189. **Honeyman M.** How robust is the evidence for viruses in the induction of type 1 diabetes? *Curr Opin Immunol* 2005. 17(6):616-623.
190. **Chalew SA, Rappazzo JA, McLaughlin JV, Maclaren N.** The increasing incidence of juvenile onset diabetes in the Baltimore area: lack of correlation with secular incidence of mumps. *Md State Med* J 1982. 31(4):67-70.
191. **Ward KP, Galloway WH, Auchterlonie IA.** Congenital cytomegalovirus infection and diabetes. *Lancet* 1979. 1(8114):497.
192. **Ivarsson SA, Lindberg B, Nilsson KO, Ahlfors K, Svanberg L.** The prevalence of type 1 diabetes mellitus in follow-up of Swedish infants congenitally infected with cytomegalovirus. *Diabet Med* 1993. 10(6):521-523.
193. **Honeyman MC, Stone NL, Harrison LC.** T-cell epitopes in type 1 diabetes autoantigen tyrosine phosphatase IA-2: potential for mimicry with rotavirus and other environmental agents. *Mol Med* 1998. 4(4):231-239.
194. **Honeyman MC, Coulson BS, Stone NL, Gellert SA, Goldwater PN, Steele CE, Couper JJ, Tait BD, Colman PG, Harrison LC.** Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes* 2000. 49(8):1319-1324.
195. **Blomqvist M, Juhela S, Erkkila S, Korhonen S, Simell T, Kupila A, Vaarala O, Simell O, Knip M, Ilonen J.** Rotavirus infections and development of diabetes-associated autoantibodies during the first 2 years of life. *Clin Exp Immunol* 2002. 128(3):511-515.
196. **Coleman DL, Kuzava JE, Leiter EH.** Effect of diet on incidence of diabetes in nonobese diabetic mice. *Diabetes* 1990. 39(4):432-436.
197. **Pozzilli P, Signore A, Williams AJ, Beales PE.** NOD mouse colonies around the world--recent facts and figures. *Immunol Today* 1993. 14(5):193-196.
198. **Filippi C, von Herrath M.** How viral infections affect the autoimmune process leading to type 1 diabetes. *Cell Immunol* 2005. 233(2):125-132.
199. **Bach JF.** Protective role of infections and vaccinations on autoimmune diseases. *J Autoimmun* 2001. 16(3):347-353.
200. **Sadelain MW, Qin HY, Lauzon J, Singh B.** Prevention of type 1 diabetes in NOD mice by adjuvant immunotherapy. *Diabetes* 1990. 39(5):583-589.
201. **Harada M, Kishimoto Y, Makino S.** Prevention of overt diabetes and insulitis in NOD mice by a single BCG vaccination. *Diabetes Res Clin Pract* 1990. 8(2):85-89.
202. **Riminton DS, Kandasamy R, Dravec D, Basten A, Baxter AG.** Dermal enhancement: bacterial products on intact skin induce and augment organ-specific autoimmune disease. *J Immunol* 2004. 172(1):302-309.
203. **Turley S, Poirot L, Hattori M, Benoist C, Mathis D.** Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. *J Exp Med* 2003. 198(10):1527-1537.
204. **Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B.** Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998. 282(5396):2085-2088.
205. **Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S.** A Toll-like receptor recognizes bacterial DNA. *Nature* 2000. 408(6813):740-745.
206. **Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, Aderem A.** The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* 1999. 401:811-815.
207. **Alexopoulou L, Holt AC, Medzhitov R, Flavell RA.** Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 2001. 413(6857):732-738.
208. **Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, Bauer S.** Species-specific recognition of single-stranded RNA via Toll-like receptor 7 and 8. *Science* 2004. 303(5663):1526-1529.
209. **Kirschning CJ, Schumann RR.** TLR2: cellular sensor for

microbial and endogenous molecular patterns. *Curr Top Microbiol Immunol* 2002. 270:121-144.
210. **Mushegian A and Medzhitov R.** Evolutionary perspective on innate immune recognition. *J Cell Biol* 2001. 155:705-710.
211. **Baeuerle PA, Baltimore D.** NFκB: ten years after. *Cell* 1996. 87:13-20.
212. **Horng T, Barton GM, Medzhitov R.** TIRAP: an adapter molecule in the Toll signaling pathway. *Nat Immunol* 2001. 2:835-841.
213. **Pauleau AL, Murray PJ.** Role of nod2 in the response of macrophages to Toll-like receptor agonists. *Mol Cell Biol* 2003. 23:7531-7539.
214. **Wen L, Peng J, Li Z, Wong FS.** The effect of innate immunity on autoimmune diabetes and the expression of Toll-like receptors on pancreatic islets. *J Immunol* 2004. 172(5):3173-3180.
215. **Zipris D, Lien E, Xie JX, Greiner DL, Mordes JP, Rossini AA.** TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats. *J Immunol* 2005. 174(1):131-142.
216. **Pirie FJ, Pegoraro R, Motala AA, Rauff S, Rom L, Govender T, Esterhuizen TM.** Toll-like receptor 3 gene polymorphisms in South African Blacks with type 1 diabetes. *Tissue Antigens* 2005. 66(2):125-130.
217. **Serreze DV, Chapman HD, Post CM, Johnson EA, Suarez-Pinzon WL, Rabinovitch A.** Th1 to Th2 cytokine shifts in nonobese diabetic mice: sometimes an outcome, rather than the cause, of diabetes resistance elicited by immunostimulation. *J Immunol* 2001. 166(2):1352-1359.
218. **Chan SH, Perussia B, Gupta JW, Kobayashi M, Pospisil M, Young HA, Wolf SF, Young D, Clark SC, Trinchieri G.** Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J Exp Med* 1991. 173(4):869-879.
219. **Dao DN, Kremer L, Guerardel Y, Molano A, Jacobs WR Jr, Porcelli SA, Briken V.** Mycobacterium tuberculosis lipomannan induces apoptosis and interleukin-12 production in macrophages. *Infect Immun* 2004. 72(4):2067-2074.
220. **Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R.** Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2001. 2:947-950.
221. **Underhill DM, Ozinsky A, Smith KD, Aderem A.** Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc Natl Acad Sci U S A* 1999. 96(25):14459-14463.
222. **Podolin PL, Denny P, Lord CJ, Hill NJ, Todd JA, Peterson LB, Wicker LS, Lyons PA.** Congenic mapping of the insulin-dependent diabetes (Idd) gene, Idd10, localizes two genes mediating the Idd10 effect and eliminates the candidate Fcgr1. *J Immunol* 1997. 159(4):1835-1843.
223. **Reifsnyder PC, Li R, Silveira PA, Churchill G, Serreze DV, Leiter EH.** Conditioning the genome identifies additional diabetes resistant loci in Type 1 diabetes resistant NOR/Lt mice. *Genes Immun* 2005. 6(6):528-538.
224. **Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF.** A family of human receptors structurally related to Drosophila Toll. *Proc Nat Acad Sci U S A* 1998. 95:588-593.
225. **Ewens KG, Johnson LN, Wapelhorst B, O'Brien K, Gutin S, Morrison VA, Street C, Gregory SG, Spielman RS, Concannon P.** Linkage and association with Type 1 diabetes on chromosome 1q42. *Diabetes* 2002. 51(11):3318-3325.
226. **Chistiakov DA, Chernisheva A, Savost'anov KV, Turakulov RI, Kuraeva TL, Dedov II, Nosikov VV.** The TAF5L gene on chromosome 1q42 is associated with type 1 diabetes in Russian affected patients. *Autoimmunity* 2005. 38(4):283-293.
227. **Moser KL, Neas BR, Salmon JE, Yu H, Gray-McGuire C, Asundi N, Bruner GR, Fox J, Kelly J, Henshall S, et al.** Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc Natl Acad Sci U S A* 1998. 95:14869-14874.
228. **Gaffney PM, Kearns GM, Shark KB, Ortmann WA, Selby SA, Malmgren ML, Rohlf KE, Ockenden TC, Messner RP, King RA, Rich SS, Behrens TW.** A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc Natl Acad Sci U S A* 1998. 95:14875-14879.
229. **Kono DH, Burlingame RW, Owens DG, Kuramochi A, Balderas RS, Balomenos D, Theofilopoulos AN.** Lupus susceptibility loci in New Zealand mice. *Proc Natl Acad Sci U S A* 1994. 91:10168-10172.
230. **Drake CG, Babcock SK, Palmer E, Kotzin BL.** Genetic analysis of the NZB contribution to lupus-like autoimmune disease in (NZB x NZW)F1 mice. *Proc Natl Acad Sci U S A* 1994. 91:4062-4066.
231. **Rozzo SJ, Vyse TJ, Drake CG, Kotzin BL.** Effect of genetic background on the contribution of New Zealand Black loci to autoimmune lupus nephritis. *Proc Natl Acad Sci U S A* 1996. 93:15164-15168.
232. **Morel L, Mohan C, Yu Y, Croker BP, Tian N, Deng A, Wakeland EK.** Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J Immunol* 1997. 158:6019-6028.
233. **Jordan MA, Silveira PA, Shepherd DP, Chu C, Kinder SJ, Chen J, Palmisano LJ, Poulton LD, Baxter AG.** Linkage analysis of systemic lupus erythematosus induced in diabetes-prone nonobese diabetic mice by Mycobacterium bovis. *J Immunol* 2000. 165(3):1673-1684.
234. **Hawn TR, Wu H, Grossman JM, Hahn BH, Tsao BP, Aderem A.** A stop codon polymorphism of Toll-like receptor 5 is associated with resistance to systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 2005. 102(30):10593-10597.