Reviews in Endocrine & Metabolic Disorders 2003;4:213–223

Environmental Triggers and Determinants of Beta-Cell Autoimmunity and Type 1 Diabetes

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Key Words. **dietary factors, growth, viruses, toxins, standard of hygiene**

Introduction

Type 1 diabetes mellitus is perceived as a chronic immune-mediated disease with a subclinical prodromal period characterized by selective loss of insulin-producing beta cells in the pancreatic islets in genetically susceptible subjects. The most important genes contributing to disease susceptibility are located in the HLA-DQ locus on the short arm of chromosome 6 [1]. Nevertheless only a relatively small proportion, i.e. less than 10%, of genetically susceptible individuals progress to clinical disease. This implies that additional factors are needed to trigger and drive beta-cell destruction in genetically predisposed subjects. Clinical Type 1 diabetes represents end-stage insulitis, and it has been estimated that at the time of diagnosis only 10–20% of the insulin-producing beta cells are still functioning. Environmental factors have been implicated in the pathogenesis of Type 1 diabetes both as triggers and potentiators of beta-cell destruction [2–4].

Several lines of evidence support a critical role of environmental factors in the pathogenesis of type 1 diabetes. Studies in monozygotic twins indicate that only 13–33% are pair-wise concordant for Type 1 diabetes [5,6], suggesting that there is either acquired post-conceptional genetic discordance, or differential exposure to the putative environmental factor(s). The geographic variation in the incidence of Type 1 diabetes in children is conspicuous even among Caucasians, with one of the lowest annual rates reported from northern Greece amounting to less than 5/100,000 children under the age of 15 years [7] and the highest rate observed in Finland reaching 50 in 1998 [Reunanen, personal communication]. This tenfold difference in incidence can hardly be explained by genetic factors. A substantial increase in the incidence of Type 1 diabetes among children has been documented over the last decades particularly in Europe, and e.g. in Finland the incidence has increased more than four times from the early 1950's [8]. Such an increase cannot be the consequence only of enhanced genetic disease susceptibility in the population but must mostly be due to changes in life style and environment. Migrant studies have been little utilized in epidemiological surveys of Type 1 diabetes. Data available indicate, however, that the incidence of Type 1 diabetes has increased in population groups who have moved from a low incidence region to a high incidence area emphasizing the influence of environmental conditions [see 3]. This review aims at providing a brief account of the environmental factors that have been implicated as triggers and risk factors in human Type 1 diabetes (Table 1) with an emphasis on the most recent candidates.

Viral Infections

Viral infections have been implicated in the etiology of Type 1 diabetes for more than 100 years. More recently, several studies have been published showing that certain viruses, such as enteroviruses are capable of inducing diabetes in experimental animals, and seroepidemiological studies have suggested their role in human Type 1 diabetes as well [see 3]. Viruses may act by at least two possible mechanisms, either via a direct cytolytic effect, or by triggering an autoimmune process leading gradually to beta-cell destruction [9]. The role of molecular mimicry in diabetes-associated autoimmune responses has been indicated by the observations of structural and functional homology between viral structures and beta-cell antigens. Persistent or slow virus infections, like in the congenital rubella syndrome and cytomegalovirus infections may also be important in the induction of the autoimmune response. The role of viral infections in the etiopathogenesis of human Type 1 diabetes has been elucidated by serological and epidemiological studies, and case histories [10]. A meritorious review on the possible role of viruses in

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Table 1. *Environmental factors implicated in the pathogenesis of Type 1 diabetes*

Viral infections
Mumps
Rubella
Cytomegalovirus
Epstein-Barr virus
Enteroviruses
Retroviruses
Rotavirus
Dietary factors
Cow's milk proteins
– Casein
– Bovine serum albumin
– Beta-lactoglobulin
– Bovine insulin
Gluten and other plant proteins
Fats
Nitrate and nitrite
Coffee, tea
Deficiency of zinc
Vitamin D deficiency
Frequent intake of solid foods rich in carbohydrate and protein
Standard of hygiene and vaccinations
Toxins
Alloxan
Streptozotocin
N-nitroso compounds
Bafilomycin A1
Growth
Infant growth
Childhood growth
Psycosocial factors
Latitude and temperature
Antenatal and perinatal risk factors

the pathogenesie of Type 1 diabetes has been published recently [11].

Enteroviruses

Enteroviruses (EV) belong to the picornavirus family comprising small, naked icosahedral RNA viruses. The EV subfamily consists of four subgroups: polioviruses, coxsackie B viruses (CBV), coxsackie A viruses (CAV) and echoviruses, and includes more than 60 distinct serotypes. There are both epidemiological, serological and biological indications suggesting that EV may be involved in the pathogenesis of Type 1 diabetes. Gamble and Taylor [12] reported in 1969 parallel changes in the seasonal variation in the incidence of Type 1 diabetes and in the frequency of CBV infections. A series of serological case-control studies have shown an increased prevalence of elevated levels of CBV antibodies in patients with newly diagnosed Type 1 diabetes [13,14]. There are, however, also contradictory results, since some other reports have been unable to find any difference between patients with diabetes and controls [15,16] or even demonstrated decreased levels of CBV antibodies in patients [17].

The first serological studies measured neutralizing EV antibodies that are good markers of infection immunity but poor indicators of a recent infection, if the analyses do not include IgM antibodies. More recent studies have assessed the occurrence of recent or current EV infections by quantifying IgM antibodies with μ-antibody capture methods based on enzyme or radioimmunoassays. With such a methodology patients with newly diagnosed Type 1 diabetes were found to have increased IgM class antibodies against EV suggesting an excess of recent infections [see 18]. A Swedish group detected IgM class antibodies to CBV in 40% of children with newly diagnosed Type 1 diabetes and in none of the controls [19]. The majority of those, who had IgM class antibodies at the diagnosis of diabetes, had experienced previously an enterovirus infection caused by a different serotype, indicated by IgG class antibodies. As increased IgM titers reflect an ongoing or recent infection, Fohlman and Friman [20] concluded that these observations suggest that successive infections by different CBV and other EV increase the risk of manifestation of overt diabetes in genetically susceptible individuals. Such a process fits well into the "Copenhagen model" for the pathogenesis of Type 1 diabetes, i.e. the multiple hit hypothesis [21].

Two studies from Northern Europe have indicated that maternal enteroviral infections during pregnancy may be associated with later development of Type 1 diabetes in the offspring. Dahlquist and her coworkers [22] analyzed maternal sera taken at delivery and observed the closest relation between IgM to CBV3 and Type 1 diabetes in the children. A Finnish survey tested sera obtained at the end of the first trimester and showed the strongest association between CBV5 and Type 1 diabetes in offspring under the age of 3 years at diagnosis but not in those older than 3 [23]. In addition unaffected siblings of children with diabetes were observed, and those who progressed to clinical diabetes experienced on an average one serologically verified enterovirus infection per follow-up year, while the corresponding frequency was 0.6 among siblings who remained unaffected. Another report showed a temporal relationship between serological evidence of an EV infection and seroconversion to ICA positivity or significant increases in existing ICA titers in non-diabetic siblings [24]. In more recent studies a reverse transcriptase polymerase chain reaction (RT-PCR) method has been applied to detect EV specific RNA in the sera or circulating mononuclear cells of patients with newly diagnosed Type 1 diabetes. Diabetic children have been observed to test positive for viral RNA more frequently than control subjects [25–27]. Taken together, most cross-sectional studies in patients with newly diagnosed Type 1 diabetes

support the hypothesis that EV can precipitate clinical disease in subjects with signs of beta-cell autoimmunity. The prospective studies performed to date suggest that EV may trigger beta-cell autoimmunity and potentiate existing beta-cell autoimmunity.

The tropism phenomenon (the characteristics of a virus to infect a particular tissue or cell type), in which the attachment of virus to the viral receptors on cell membranes is a central feature, is thought to explain why some variants of EV are diabetogenic and some are not [20]. It has been proposed that pancreatic beta-cell tropic variants of CBV are present in the general population and that they are able to induce beta-cell damage in susceptible individuals [28].

Other viruses

Gundersen [29] reported in his classical study from 1927 an increase in the number of cases with Type 1 diabetes 2–4 years after a mumps epidemic. Subsequently there have been numerous case reports describing a temporal relationship between mumps and clinical onset of diabetes [see 3]. In epidemiological studies peaks in the incidence of childhood Type 1 diabetes have been observed 2–4 years after mumps epidemics. Serological evidence of an association between mumps infection and Type 1 diabetes has been difficult to obtain due to the long interval between the infection and the clinical manifestation of Type 1 diabetes. A Finnish study reported decreased IgG class mumps antibody titers in children with newly diagnosed Type 1 diabetes compared with those in controls, the finding being interpreted as indicative of an abnormal immunological response to mumps infection [30]. Interestingly, in patient series collected earlier, when natural mumps was still common in Finland, IgG class mumps virus antibodies were not decreased, and IgA antibodies were elevated in diabetic children. Accordingly the decline in mumps antibody levels may reflect the elimination of cases with mumps induced Type 1 diabetes by the MMR vaccine.

Diabetes has been observed in 10–20% of patients with the congenital rubella syndrome (CRS) with a latent period of 5–25 years [see 3]. A persistent rubella infection has been detected in the pancreases of some but not all subjects with congenital rubella. The rubella virus may modify membrane proteins of the infected beta cell leading to an immune attack against beta cells, since such modified proteins can be recognized as foreign antigens. As an alternative pathway cytotoxic T cells specific for a viral antigen may recognize a beta-cell specific antigen by molecular mimicry. This latter option is supported by the observation of Karounas et al. [31] that one out of several monoclonal antibodies raised against rubella virus capsid and envelope glycoproteins recognizes a 52 kD protein in islet cell extracts. To date this protein has not been biochemically characterized. The CRS-induced diabetes is a model for how other maternal infections transmitted to the fetus during pregnancy may be involved in the pathogenesis of Type 1 diabetes in the offspring.

The human cytomegalovirus (CMV) can be transmitted before birth, like the rubella virus, either transplacentally or at conception from an infected parent carrying the CMV genome in his or her genomic DNA. CMV infections may also be transmitted prenatally or postnatally through close contact or breast milk. CMV has been implicated in the development of Type 1 diabetes by a case report of an infant with congenital CMV infection who presented with diabetes at the age of 13 months [32]. In a Swedish prospective study 16,474 newborn infants were screened for congenital CMV infections by virus isolation from the urine, and 76 infants were found to be infected. Only one out of 73 infected individuals (1.4%) manifested Type 1 diabetes, when observed up to the age of 7 years or more, whereas 38 of 19,483 controls (0.2%) became affected by diabetes [33]. This observation suggests that congenital CMV infection is not a major trigger of Type 1 diabetes.

Hiltunen et al. [34] found comparable levels of CMV IgG and IgM antibodies in children with newly diagnosed Type 1 diabetes and in control children, while the patients had higher IgA antibodies than the controls. The latter observation may reflect reactivated or persistent CMV infections in children with recent-onset diabetes. No association was observed between ICA and CMV antibodies. Neither could any differences be seen in the CMV antibodies in early pregnancy between mothers whose offspring later presented with clinical Type 1 diabetes and control mothers. During prospective follow-up of unaffected siblings of children with diabetes no seroconversions could be detected in CMV antibodies, and no changes could be seen in CMV antibodies in relation to seroconversion to positivity for ICA or progression to clinical diabetes in the siblings. Accordingly no evidence was found in favor of the hypothesis that primary CMV infections *in utero* or in childhood could promote or precipitate Type 1 diabetes. If CMV infections play a role in the pathogenesis of this disease, it must be limited to a very small proportion of cases.

The human genome contains numerous retroviral sequences, a majority of which are non-infectious. Endogenous retroviruses exist as viral DNA integrated into the genome of every cell in the host, and they are transmitted vertically to the next generation via germ-line DNA. Retroviruses have been associated with autoimmune diabetes in animal models such as the NOD mouse [35]. Retroviruses have not been consistently shown to be involved in the development of human Type 1 diabetes, although insulin autantibodies (IAA) from patients with

Type 1 diabetes and unaffected first-degree relatives have been observed to cross-react with the retroviral antigen p73 [36], indicating that IAA-positive sera contain antibodies that recognize both insulin and p73.

Honeyman et al. [37] reported some time ago molecular homology between the VP7 protein of rotavirus and T-cell epitopes in the protein tyrosine phosphatase related IA-2 molecule and in the 65 kD isoform of glutamic acid decarboxylase. In a prospective study of infants genetically predisposed to Type 1 diabetes they observed that the appearance of diabetes-associated autoantibodies was associated with significant rises in rotavirus antibodiesy titers, indicating that rotavirus infections may induce beta-cell autoimmunity in genetically susceptible infants [38]. A prospective Finnish birth cohort study was, however, unable to confirm any association between rotavirus infections and the initiation of beta-cell autoimmunity in young children with increased genetic susceptibility to Type 1 diabetes [39].

Dietary Factors

The first reports on the effect of a dietary compound possibly affecting the incidence of Type 1 diabetes were published in the early 1980's. Helgason and Jonasson [40] made an interesting observation of a conspicuously high incidence of Type 1 diabetes in Icelandic boys born in October, and they proposed N-nitroso compounds being the etiological factor, mediated via parental germ cells. The finding was confirmed later in animal experiments [41]. Scott and Trick published the first study suggesting that dietary constituents may markedly affect the expression of diabetes in BB rats in 1983 [42]. More recently data have accumulated suggesting that cow's milk (CM) and its protein components may be involved in the pathogenesis of Type 1 diabetes [see 3].

CM proteins

Experiments in BB rats and NOD mice have clearly demonstrated that the exposure to CM proteins increases the incidence of diabetes. Prompted by anecdotal reports suggesting a low incidence of Type 1 diabetes in people from countries with a low protein intake, Elliott and Martin [43] were the first to report that manipulation of the protein component in the diet of BB rats affects the natural history of autoimmune diabetes: feeding rats a semi-synthetic amino acid diet from the onset of weaning led to a considerable reduction in the incidence of diabetes from 52% on milk protein supplementation to 15%. Subsequent studies by the Toronto group confirmed that the effect of CM proteins is established during a relatively narrow, early phase in the postnatal (weaning) period [44]. The prevention of diabetes by a synthetic diet in which CM proteins were replaced by a purified casein hydrolysate before weaning has subsequently been confirmed in the NOD mouse [45].

The main differences in protein composition between cow's and human milk are that (a) the protein concentration is higher in CM, principally due to the larger casein content; (b) the main whey protein component in CM is beta-lactoglobulin (BLG), which is not an endogenous component in human milk; and (c) the primary serum albumin amino acid sequence differs from that of human albumin and rodents in a small, circumscribed area [46]. An additional intriguing fact is that there is a three amino acid difference between bovine insulin present in CM and human insulin.

The association between CM consumption and the incidence of Type 1 diabetes in children has been dealt with in two ecological studies. Scott [47] reported in 1990 a close correlation ($r = 0.86$) between the per capita consumption of unfermented milk proteins in the whole population and the incidence of diabetes, and Dahl-Jørgensen et al. [48] confirmed one year later the same trend using incidence data from children aged 0–14 years and validated registries from the Diabetes Epidemiology Research International Study Group (1978–85), the correlation coefficient being 0.96. Studies like these are prone to various types of biases but can serve as background information to hypotheses linking Type 1 diabetes to exposure to CM. Data from three population-based case-control studies on CM intake prior to diagnosis of Type 1 diabetes are conflicting: Dahlquist et al. [49] found in a Swedish series a lower frequency of milk intake among diabetic children, whereas in New South Wales, Australia the CM intake had been higher in prediabetic children than in the controls [50]. In our Finnish nationwide "Childhood Diabetes in Finland" (DiMe) study, we observed that a high consumption of CM in childhood was associated with a more frequent appearance of diabetes-associated autoantibodies in initially unaffected siblings of children with Type 1 diabetes [51]. There was also an almost significant association between high CM consumption and progression to clinical Type 1 diabetes.

An inverse correlation between the duration of breastfeeding and Type 1 diabetes in childhood was first observed in a Scandinavian study almost 20 years ago [52]. This association has been confirmed in several but not all studies from various countries [see 3]. In a Finnish population-based study, the duration of exclusive breastfeeding and age at start of supplementary feeding with regular CM-based formulas were both related to an increased risk for Type 1 diabetes [53]. In comparison with controls matched for sex and age, young diabetic children had been more often predominantly breast-fed for less than 6 months and exclusively breast-fed for less than 3 months. In addition, a greater proportion of the affected children

had received supplementary CM-based formula over the first 3 months. These findings have been confirmed subsequently in a larger series of diabetic children [54]. A multivariate analysis of the total series of Finnish children with Type 1 diabetes indicated that early CM exposure was a more important risk factor than short breastfeeding [55].

Two meta-analyses have been performed with the aim to critically review and summarize the clinical evidence for the possible role of a short duration of breastfeeding or early CM exposure in the pathogenesis of Type 1 diabetes. Gerstein [56] analyzed in 1994 13 case-control studies and found that the risk of diabetes was 1.4 times higher in children who were breast-fed for less than 3 months and 1.6 times higher in those exposed to CM before the age of 3 months. The author concluded that early CM exposure might be an important determinant of subsequent Type 1 diabetes. Norris and Scott [57] reported quite similar risk ratios, 1.2 and 1.6, respectively, but emphasized that the increased risk of Type 1 diabetes associated with any of the infant diet exposures is low. They pointed out that retrospectively collected infant diet data might have their limitations due to possible recall bias and different response rates for cases and controls. The above mentioned risk ratios most likely underestimate the association between Type 1 diabetes and early CM exposure, however. Firstly, the breastfeeding data did not reflect exclusive breastfeeding in most studies. Secondly, controls for these studies were drawn from the general population, and such controls will include a majority of individuals not genetically susceptible to Type 1 diabetes.

Four birth cohort studies have assessed the relationship between infant feeding patterns and the appearance of diabetes-associated autoantibodies [58–61]. The statistical power of these studies is so far low, since the number of seroconverters is limited in all of them. None of the studies reported any association between breastfeeding or age at exposure to CM proteins and the emergence of maximally three diabetes-associated autoantibodies. In the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Study short exclusive breastfeeding and early introduction of CM-based formula were, however, related to an increased risk of developing progressive beta-cell autoimmunity reflected by positivity for IA-2 autoantibodies and all four autoantibody specificities analyzed. These observations suggest that early exposure to CM proteins is rather a promoter of beta-cell autoimmunity than a trigger of the process.

Immune responses to CM proteins in patients with newly diagnosed Type 1 diabetes

Savilahti et al. [62] reported in 1988 that children with newly diagnosed Type 1 diabetes had significantly higher levels of serum IgA antibodies to CM and BLG, and IgG antibodies to BLG than age-matched controls. The authors inferred alternatively that the pattern of CM consumption is altered in children who will develop Type 1 diabetes, the immunological reactivity to CM proteins is enhanced, or the permeability of their intestines to CM proteins is higher than normal. This finding has been confirmed in the nationwide "Childhood Diabetes in Finland" study, comprising 706 children with newly diagnosed Type 1 diabetes, 456 non-diabetic siblings and 105 unrelated age-matched controls below 7 years of age [63]. A Swedish nationwide case-control study showed that most CM antibody levels tended to be increased in diabetic children when compared with controls, the difference being significant for IgA antibodies to CM, bovíne serum albumin (BSA) and BLG [64,65]. The differences in these antibodies were more pronounced among young children. In a multiple logistic regression analysis, the authors observed that IgA antibodies to BLG were significantly associated with an increased risk of diabetes at young age independent of ICA status and of early weaning to CM-based formula. The authors concluded that in genetically susceptible children early exposure to BLG might be one of the triggers of the autoimmune process leading to the development of Type 1 diabetes.

The humoral immunity to various CM proteins has subsequently been studied in children and adults with Type 1 diabetes by many groups as reviewed by Åkerblom and Knip [3]. A majority of these studies have reported increased antibody levels to one or more protein components in CM. An enhanced humoral immune response to CM proteins seem to be specific for Type 1 diabetes, since elevated levels have not been generally observed in other autoimmune diseases. The active immune response has been reported to be restricted to CM proteins, since patients with newly diagnosed Type 1 diabetes have not been shown to have increased antibody levels to other dietary antigens, such as ovalbumin or gliadin [62,65].

Observations on the cellular immunity to CM proteins are of potential interest, as all pathogenetic models of Type 1 diabetes ascribe a crucial role to T cells as actively involved in beta-cell destruction. The published data on T-cell responses to CM proteins are controversial. Enhanced T-cell responses have been reported to BSA, the ABBOS fragment of BSA (amino acids 152–168), BLG and beta-casein [see 3]. These observations have not been consistently confirmed in other studies, however.

Possible mechanisms involved in the beta-cell lesions related to CM proteins

Several mechanisms have been proposed to explain how CM proteins may be related to beta-cell lesions. The BSA hypothesis, according to which structural homology

between BSA and an islet protein p69 leads to a misdirected immune response against p69, was introduced in 1992 by Karjalainen et al. [66]. Another hypothesis is based on the observation that digestion of bovine beta-casein results in a bioactive peptide, beta-casomorphin-7, with immunosuppresive activity [67]. A third alternative is that subjects who develop Type 1 diabetes have a dysregulated mucosal immune response predisposing to autoimmune diabetes [68,69].

Recently Vaarala et al. [70,71] suggested that early feeding with CM-based formulas results in immunization to bovine insulin that differs structurally from human insulin in three amino acid positions (amino acids 8 and 10 in the A-chain and amino acid 30 in the B-chain). Infants fed with CM-based formulas had significantly higher IgG antibodies to bovine insulin than breast-fed infants at the age of 3 months. No such difference was seen any more between these two groups at the age of 12 and 18 months, but as a matter of fact the antibody levels decreased in both groups reflecting the induction of oral tolerance to bovine insulin. There were, however, 11 deviant infants who developed signs of beta-cell autoimmunity over their first 2 years of life and whose IgG class antibodies to bovine insulin increased during longitudinal follow-up. Infants fed a CM-based formula have also been shown to have a higher T-cell response to bovine insulin at the age of 3 months than exclusively breast-fed infants [72]. These observations suggest that the immune response initially induced by bovine insulin may later be diverted into autoaggressive immunity against the beta cells in a few unfortunate individuals. This hypothesis goes along with other observations suggesting that immunization to insulin plays a key role in the autoimmune process leading to the loss of pancreatic beta cells and the development of Type 1 diabetes. Insulin is the only known beta-cell specific autoantigen in Type 1 diabetes and insulin autoantibodies are frequently detected in young children with newly diagnosed disease [73,74]. In prospective birth-cohort studies insulin autoantibodies appear most frequently as the first sign of beta-cell autoimmunity [75,76], indicating that insulin may be the primary or one of the primary autoantigens in human Type 1 diabetes.

Regardless of the mechanism the only strategy to definitely assess the prevailing controversy whether early exposure to CM proteins is a risk factor for Type 1 diabetes in man is to perform a dietary intervention trial [77]. Therefore such a trial (Trial to Reduce IDDM in Genetically at Risk, TRIGR) has been initiated in May 2003 as an international multicenter study after the study design had been tested in two pilot studies. The objective of the second pilot study was to explore whether elimination of CM proteins over the first 6–8 months of life would decrease the cumulative incidence of diabetes-associated autoantibodies by the age of 2 years. The intervention resulted in an almost significant reduction in the range of 40–60% in the cumulative incidence of the various diabetes-associated autoantibodies except for antibodies to glutamic acid decarboxylase [78]. This provides the first hint that it may be possible to manipulate spontaneous beta-cell autoimmunity in young children with nutritional intervention in infancy.

Other dietary factors

Some experimental studies indicate that gluten may be diabetogenic [45,79], but there is limited human data favoring this idea. Studies in BB rats have suggested a diabetogenic effect of soy protein [80,81], and Fort et al. [82] reported that children progressing to Type 1 diabetes had been given soy-based formulas in infancy more often than the controls. Recently a protein with a high amino acid sequence homology with a wheat storage globulin was identified, and antibodies to this molecule were detected both in diabetic BB rats and a few patients with newly diagnosed Type 1 diabetes [83]. The significance of this molecule in the development of human Type 1 diabetes remains to be defined. No consistent data are available on the possible role of dietary fats in the development of autoimmune diabetes [see 3].

A significant correlation between the incidence of Type 1 diabetes and the national coffee consumption per person has been reported in an ecological study [84]. Later studies have indicated that the maternal coffee consumption during pregnancy does not affect the risk of diabetes in the offspring [85,86]. A Swedish study found that a high groundwater zinc concentration was associated with a significantly reduced risk for Type 1 diabetes, and the authors concluded that zinc deficiency may lead to Type 1 diabetes [87].

A European multicenter study observed that vitamin D supplementation in early childhood was associated with a decreased risk of Type 1 diabetes [88]. Recently a Finnish birth cohort study reported that regular or irregular vitamin D supplementation in infancy is associated with a reduced risk of Type 1 diabetes later in childhood, while a suspicion of rickets was linked with an increased disease risk [89]. These observations are theoretically interesting from that point of view that vitamin D has been shown to prevent experimental thyroiditis [90] and autoimmune diabetes in the NOD mouse [91].

Other Environmental Factors

As listed in Table 1 there are a series of other environmental factors that have been proposed to be involved in the pathogenesis of Type 1 diabetes. Some new developments

in this area deserve to be mentioned. An Australian study reported recently that bafilomycin A1, a macrolide antibiotic produced by *Streptomyces* species ubiquitous in soil, may induce glucose intolerance and pancreatic islet disruption in mice [92]. Tuberous vegetables, potatoes and beets in particular, may be infested by such *Strepomyces* species and thereby humans could be exposed to high concentrations of bafilomycin A1. The potential diabetogenicity of this compound is open in man, however.

Increased weight gain in infancy has repeatedly been reported to be a risk factor for Type 1 diabetes later in childhood [see 4]. A Finnish study showed that those children who presented with Type 1 diabetes had been not only heavier but also taller in infancy [93]. Increased height and weight later in childhood turned as well out to be definite risk factors for Type 1 diabetes [94]. Accelerated linear growth and weigh gain results in an enhanced beta-cell load and increasing insulin resistance. It has been shown experimentally that active beta cells are more prone to cytokine-induced damage than resting cells. This suggest that rapid growth induces beta-cell stress. According to the accelerator hypothesis recently presented by Wilkin [95] insulin resistance is an important factor affecting the rising incidence of both Type 1 and Type 2 diabetes, the only differences between these two forms of diabetes being the pace of progression to overt disease and the fact that those who present with Type 1 diabetes carry genetic predisposition to autoimmunity.

A Pathogenetic Model of Type 1 Diabetes

A series of observations suggest that beta-cell autoimmunity may be triggered by an environmental culprit at any age, although a majority of the processes appear to start early in childhood [96]. Figure 1 presents a pathogenetic

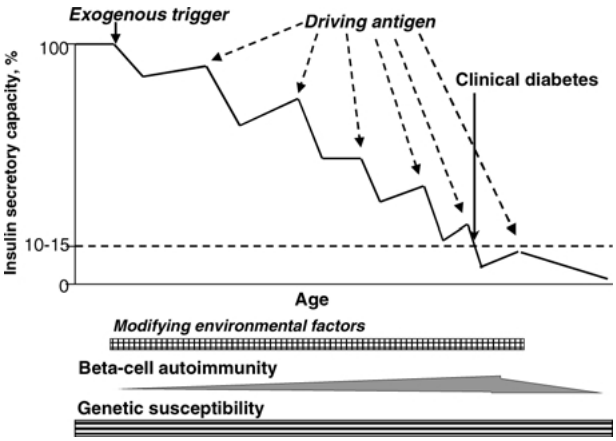

Fig. 1. *Progression from genetic susceptibility to clinical Type 1 diabetes initiated by an exogenous trigger, driven by an exogenous antigen and modified by a series of environmental factors.*

Table 2. *Essential elements in the development of Type 1 diabetes*

Genetic diabetes susceptibility	Exogenous trigger (Critically timed diabetogenic enterovirus)	Driving antigen (High exposure to bovine insulin)	Type 1 diabetes as outcome
−	−	−	No
+	−	−	No
−	+	−	No
+	+	−	No
−	−	+	No
+	−	+	No
−	+	+	No
+	+	+	Yes

model of Type 1 diabetes according to which the genetic disease susceptibility allows the initiation of a beta-cell destructive process resulting in the presentation of clinical Type 1 diabetes in some unfortunate individuals. What might be the most likely environmental trigger of beta-cell autoimmunity? Based on present knowledge a critically timed diabetogenic enterovirus infection is the most likely candidate. Initiation of the process does not necessarily lead to progression to clinical disease, however. According to the hypothesis favored by this author there is a need for a driving exogenous antigen playing the same role as gluten in celiac disease. Bovine insulin present in most CM-based products could be such a driving antigen, high exposure to the antigen resulting in a progressive destructive process. A Finnish study have shown that a high CM consumption (more than two glasses of milk/day) is associated with an increased risk of seroconversion to autoantibody positivity and progression to clinical Type 1 diabetes in initially non-diabetic siblings of affected children [54] supporting the idea that a CM component could be the driving dietary antigen in Type 1 diabetes. In addition there are most likely a series of environmental factors modifying the fate and pace of the beta-cell destructive process. Such factors may include e.g. non-specific infections, weight gain, accelerated linear growth, and vitamin D supplementation. This hypothesis holds that progression to clinical diabetes requires the combination of genetic disease susceptibility, a critically timed diabetogenic enterovirus infection and high exposure to dietary bovine insulin (Table 2). If any of these determinants is missing or any of the exogenous factors inappropriately timed the risk of Type 1 diabetes is minimal even in the presence of the other predisposing elements. Such a model can also explain why only about 10% of those with HLA-conferred genetic susceptibility to Type 1 diabetes do progress to overt disease.

Conclusions and Future Directions

Why is it important to pursue studies on the role of environmental factors in the pathogenesis of Type 1 diabetes?

The identification of exogenous factors triggering and potentiating beta-cell destruction offers potentially means for intervention aimed at the prevention of Type 1 diabetes. Environmental modification offers likely the most powerful strategy for effective prevention of this disease, since such an approach can target the whole population or at least that proportion of the population carrying increased genetic disease susceptibility and would therefore prevent both sporadic and familial Type 1 diabetes, if successful. This consideration is crucial, since the sporadic cases comprise 83–98% of all children with newly diagnosed diabetes according to a recent European survey [9]. The preliminary results of the second pilot study of the TRIGR project, suggesting that it is possible to manipulate the spontaneous appearance of beta-cell autoimmunity by dietary modification early in life in high risk individuals, represent the first indication that environmental modification may affect the natural history of preclinical Type 1 diabetes.

The scientific challenges in the near future are to define the most likely environmental culprits and potentiators of beta-cell autoimmunity and to delineate how exogenous factors affect the natural history of Type 1 diabetes in the preclinical phase. A new consortium comprising six prospective birth cohort studies, the German BABYDIAB Study, the American Diabetes Autoimmunity Study in the Young (DAISY) and the Finnish DIPP Study among others, and observing risk individuals from birth through signs of beta-cell autoimmunity to clinical disease provides an optimal setting for successful detective work. This TEDDY (The Environmental Determinants in Diabetes of the Young) consortium has been funded by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) for a 5-year period (2003–2007). We have also to keep our eyes and minds open for potential protective environmental factors, since family studies have shown that all high risk individuals do not progress to clinical diabetes within a foreseeable period of time [98,99].

References

1. Ilonen J, Sjöroos M, Knip M, Veijola R, Simell O, Åkerblom HK, Paschou P, Bozas E, Havarini B, Malamitsi-Puchner A, Thymelli J, Vazeou A, Bartsocas CS. Estimation of genetic risk for type 1 diabetes. *Am J Med Genet* 2002;115:30–36.
2. Dahlquist G. Environmental risk factors in human type 1 diabetes: An epidemiological perspective. *Diabetes Metab Rev* 1995;11:37–46.
3. Åkerblom HK, Knip M. Putative environmental factors in type 1 diabetes. *Diabetes Metab Rev* 1998;14:31–67.
4. Åkerblom HK, Vaarala O, Hyöty H, Ilonen J, Knip M. Environmental factors in the etiology of type 1 diabetes. *Am J Med Genet* 2002;115:18–29.
5. Barnett AH, Eff C, Leslie RDG, Pyke DA. Diabetes in identical twins. A study of 200 pairs. *Diabetologia* 1981;20:87–93.
6. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengård J, Kesäniemi YA. Concordance for Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia* 1992;35:1060–1067.
7. The EURODIAB ACE Study Group. Variation and trends in incidence of childhood diabetes in Europe. *Lancet* 2000;355:873–876.
8. Gale EA. The rise of childhood type 1 diabetes in the 20th century. *Diabetes* 2002;51:3353–3361.
9. Yoon JW. Role of viruses in the pathogenesis of IDDM. *Ann Med* 1991;23:437–445.
10. Szopa TM, Titchener PA, Portwood ND, Taylor KW. Diabetes mellitus due to viruses—some recent developments. *Diabetologia* 1993;36:687–695.
11. Hyöty H, Taylor KW. The role of viruses in human diabetes. *Diabetologia* 2002;45:1353–1361.
12. Gamble DR, Taylor KW. Seasonal incidence of diabetes mellitus. *Br Med J* 1969;3:631–633.
13. Barrett-Connor E. Is insulin-dependent diabetes mellitus caused by Coxsackie B infection? A review of the epidemiologic evidence. *Rev Inf Dis* 1985;7:207–215.
14. Banatvala JE. Insulin-dependent (juvenile-onset, type 1) diabetes mellitus. Coxsackie B viruses revisited. *Prog Med Virol* 1987;34:33–54.
15. Orchard TJ, Atchison RW, Becker D, Rabin B, Eberhardt M, Kuller LH, LaPorte RE, Cavender D. Coxsackie infection and diabetes. *Lancet* 1983;2:631.
16. Mertens T, Grüneklee D, Eggers HJ. Neutralizing antibodies against Coxsackie B viruses in patients with recent onset of type 1 diabetes. *Eur J Pediatr* 1983;140:293–294.
17. Palmer JP, Cooney MK, Ward RH, Hansen JA, Brodsky JB, Ray CG, Crossley JR, Asplin CM, Williams RH. Reduced Coxsackie antibody titres in Type 1 (insulin-dependent) diabetic patients presenting during an outbreak of Coxsackie B3 and B4 infection. *Diabetologia* 1982;22:426–429.
18. Graves PM, Norris JM, Pallansch MA, Gerling IC, Rewers M. The role of enteroviral infections in the development of IDDM: Limitations of current approaches. *Diabetes* 1997;46:161–168.
19. Frisk G, Fohlman J, Kobbah M, Ewald U, Tuvemo T, Diderholm H, Friman G. High frequency of Coxsackie-B-virus-specific IgM in children developing type 1 diabetes during a period of high diabetes morbidity. *J Med Virol* 1985;17:219–227.
20. Fohlman J, Friman G. Is juvenile diabetes a viral disease? *Ann Med* 1993;25:569–574.
21. Nerup J, Mandrup-Poulsen T, Mølvig J, Helqvist S, Wogensen L, Egeberg J. Mechanisms of pancreatic beta-cell destruction in Type 1 diabetes. *Diabetes Care* 1988;11(Suppl 1):16–23.
22. Dahlquist CG, Ivarsson S, Lindberg B, Forsgren M. Maternal enteroviral infection during pregnancy. *Diabetes* 1995;44:408–413.
23. Hyöty H, Hiltunen M, Knip M, Laakkonen M, Vähäsalo P, Karjalainen J, Koskela P, Roivainen M, Leinikki P, Hovi T, Åkerblom HK, The Childhood Diabetes in Finland Study Group. A prospective study of the role of Coxsackie B and other enterovirus infections in the pathogenesis of IDDM. *Diabetes* 1995;44:652–657.
24. Hiltunen M, Hyöty H, Knip M, Ilonen J, Reijonen H, Vähäsalo P, Roivainen M, Leinikki P, Hovi T, Åkerblom HK, The Childhood Diabetes in Finland Study Group. ICA seroconversion in children is temporally associated with enterovirus infections. *J Infect Dis* 1997;175:554–560.
25. Clements CB, Galbraith D, Taylor KW. Coxsackie B virus infection and onset of childhood diabetes. *Lancet* 1995;346:221–223.

26. Andrealotti L, Hober D, Hober-Vandenberghe C, Belaich S, Vantyghem MC, Lefebvre J, Wattre P. Detection of Coxsackie B virus RNA sequences in whole blood samples from adult patients at the onset of type 1 diabetes mellitus. *J Med Virol* 1997;52:121–127.
27. Yin H, Berg AK, Tuvemo T, Frisk G. Enterovirus RNA is found in peripheral blood mononuclear cells in a majority of type 1 diabetic children at onset. *Diabetes* 2002;51:1964–1971.
28. Szopa TM, Ward T, Dronfield DM, Portwood ND, Taylor KW. Coxsackie B4 viruses with the potential to damage beta cells of the islets are present in clinical isolates. *Diabetologia* 1990;33:325–328.
29. Gundersen E. Is diabetes of infectious origin? *J Inf Dis* 1927;41:197–202.
30. Hyöty H, Hiltunen M, Reunanen A, Leinikki P, Vesikari T, Lounamaa R, Tuomilehto J, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Decline of mumps antibodies in Type 1 (insulin-dependent) diabetic children and a plateau in the rising incidence of Type 1 diabetes after introduction of the mumps-measles-rubella vaccine in Finland. *Diabetologia* 1993;36:1303–1308.
31. Karounas DG, Wolinsky JS, Thomas JW. Monoclonal antibody to rubella virus capsid recognizes a beta-cell antigen. *J Immunol* 1993;150:3080–3085.
32. Ward KP, Galloway WH, Auchterlonie IA. Congenital cytomegalovirus infection and diabetes. *Lancet* 1979;1:497.
33. Ivarsson SA, Lindberg B, Nilsson KO, Ahlfors K, Svanberg L. The prevalence of type 1 diabetes mellitus at follow-up of Swedish infants congenitally infected with cytomegalovirus. *Diabetic Med* 1993;10:521–523.
34. Hiltunen M, Hyöty H, Karjalainen J, Leinikki P, Knip M, Lounamaa R, Åkerblom HK. The Childhood Diabetes in Finland Study Group. Serological evaluation of the role of cytomegalovirus in the pathogenesis of IDDM: A prospective study. *Diabetologia* 1995;38:705–710.
35. Suenaga K, Yoon JW. Association of beta-cell specific expression of endogenous retrovirus with the development of insulitis and diabetes in NOD mice. *Diabetes* 1988;37:1722–1726.
36. Hao W, Serreze DV, McCulloch DK, Neifing JL, Palmer JP. Insulin (auto) antibodies from human IDDM cross-react with retroviral antigen p73. *J Autoimmun* 1993;6:787–798.
37. 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;4:231–239.
38. 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;49:1319–1324.
39. Blomquist M, Juhela S, Erkkilä 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;128:511–515.
40. Helgason T, Jonasson MR. Evidence for a food additive as a cause of ketosis-prone diabetes. *Lancet* 1981;2:716–720.
41. Helgason T, Ewen SWB, Ross IS, Stowers JM. Diabetes produced in mice by smoked/cured mutton. *Lancet* 1982;2:1017–1022.
42. Scott FW, Trick KD. Dietary modification of spontaneous diabetes in the BB Wistar rat. *Proc Canad Fed Biol Soc* 1983;26:222.
43. Elliott RB, Martin JM. Dietary protein: A trigger of insulin-dependent diabetes in the BB rat? *Diabetologia* 1984;26:297–299.
44. Daneman D, Fishman L, Clarson C, Martin JM. Dietary triggers of insulin-dependent diabetes in the BB rat. *Diabetes Res* 1987;5:93–97.
45. Coleman DL, Kuzava JE, Leiter EH. Effect of diet on incidence of diabetes in non-obese diabetic mice. *Diabetes* 19990;39:432–436.
46. Martin JM, Trink B, Daneman D, Dosch H-M, Robinson B. Milk proteins in the etiology of insulin-dependent diabetes mellitus. *Ann Med* 1991;23:447–452.
47. Scott FW. Cow milk and insulin-dependent diabetes mellitus: Is there a relationship? *Am J Clin Nutr* 1990;51:489–491.
48. Dahl-Jorgensen K, Joner G, Hanssen KF. Relationship between cow's milk consumption and incidence of IDDM in childhood. *Diabetes Care* 1991;14:1081–1083.
49. Dahlquist GG, Blom L, Persson L-Å, Sandström AIM, Wall SG. Dietary factors and the risk of developing insulin dependent diabetes in childhood. *Br Med J* 1990;300:1302–1306.
50. 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;17:1381–1389.
51. Virtanen SM, Hyppönen E, Läärä E, Vähäsalo P, Kulmala P, Savola K. Räsänen L, Knip M, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Cow's milk consumption, disease associated autoantibodies and Type 1 diabetes mellitus: A follow-up study in siblings of diabetic children. *Diabetic Med* 1998;15:730–738.
52. 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;II:1083–1086.
53. Virtanen SM, Räsänen L, Aro A, Lindström J, Sippola H, Lounamaa R, Toivanen L, Tuomilehto J, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Infant feeding in Finnish children <7 yr of age with newly diagnosed IDDM. *Diabetes Care* 1991;14:415–417.
54. Virtanen SM, Räsänen L, Aro A, Ylönen K, Lounamaa R, Tuomilehto J, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Feeding in infancy and the risk of Type 1 diabetes mellitus in Finnish children. *Diabetic Med* 1992;9:815–819.
55. Virtanen SM, Räsänen L, Ylönen K, Aro A, Clayton D, Langholz B, Pitkäniemi J, Savilahti E, Lounamaa R, Tuomilehto J, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Early introduction of dairy products associated with increased risk for insulin-dependent diabetes mellitus in Finnish children. *Diabetes* 1993;42:1786–1790.
56. Gerstein HM. Cow's milk exposure and Type 1 diabetes mellitus: A critical overview of the clinical literature. *Diabetes Care* 1994;17:13–19.
57. Norris JM, Scott FW. A meta-analysis of infant diet and insulin-dependent diabetes mellitus: Do biases play a role. *Epidemiology* 1996;7:87–92.
58. Norris JM, Beaty B, Klingensmith G, Yu L, Hoffman M, Chase HP, Erlich HA, Hamman RF, Eisenbarth GS, Rewers M. Lack of association between early exposure to cow's milk protein and β-cell autoimmunity. *JAMA* 1996;276:609–614.
59. Couper JJ, Steele C, Beresford S, Powell T, McCaul K, Pollard A, Gellert S, Tait B, Harrison LC, Colman PG. Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. *Diabetes* 1999;48:2145–2149.
60. Hummel M, Fuchtenbush M, Schenker M, Ziegler A-G. No major association of breast-feeding, vaccinations, and childhood viral diseases with early islet autoimmunity in the German BABYDIAB Study. *Diabetes Care* 2000;23:969–974.
61. Kimpimäki T, Erkkola M, Korhonen S, Kupila A, Virtanen SM, Ilonen J, Simell O, Knip M. Short exclusive breast-feeding predisposes young children with increased genetic risk of Type 1 diabetes to progressive beta-cell autoimmunity. *Diabetologia* 2001;44:63–69.
62. Savilahti E, Åkerblom HK, Tainio V-M, Koskimies S. Children with newly diagnosed insulin-dependent diabetes mellitus have increased levels of cow's milk antibodies. *Diabetes Res* 1988;7:137–140.

63. Savilahti E, Saukkonen TT, Virtala ET, Tuomilehto J, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Increased levels of cow's milk and ß-lactoglobulin antibodies in young children with newly diagnosed IDDM. *Diabetes Care* 1993;16:984–989.
64. Dahlquist G, Savilahti E, Landin-Olsson M. An increased level of antibodies to beta-lactoglobulin is a risk determinant for early-onset Type 1 (insulin-dependent) diabetes mellitus independent of islet cell antibodies and early introduction of cow's milk. *Diabetologia* 1992;35:980–984.
65. Saukkonen T, Savilahti E, Vaarala O, Virtala ET, Tuomilehto J, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Children with newly diagnosed IDDM have increased levels of antibodies to bovine serum albumin but not to ovalbumin. *Diabetes Care* 1994;17:970–976.
66. Karjalainen J, Martin JM, Knip M, Ilonen J, Robinson BH, Savilahti E, Åkerblom HK, Dosch H-M. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med* 1992;327:302–307.
67. Elliott RB, Harris DP, Hill JP, Bibby NJ, Wasmuth HE. Type I (insulin-dependent) diabetes mellitus and cow milk: Casein variant consumption. *Diabetologia* 1999;42:292–296.
68. Vaarala O, Saukkonen T, Savilahti E, Klemola T, Åkerblom HK. Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula. *J Allergy Clin Immunol* 1995;96:917–923.
69. Paronen J, Klemetti P, Kantele JM, Savilahti E, Perheentupa J, Åkerblom HK, Vaarala O. Glutamate decarboxylase-reactive peripheral blood lymphocytes from patients with IDDM express gut-specific homing receptor $\alpha_4ß_7$-integrin. *Diabetes* 1997;46:583–588.
70. Vaarala O, Paronen J, Otonkoski T, Åkerblom HK. Cow milk formula feeding induces antibodies to insulin in children—a link between cow milk and insulin-dependent diabetes mellitus. *Scand J Immunol* 1998;47:131–135.
71. Vaarala O, Knip M, Paronen J, Hämäläinen A-M, Muona P, Väätäinen M, Ilonen J, Simell O, Åkerblom HK. Cow milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes* 1999;48:1389–1394.
72. Paronen J, Knip M, Savilahti E, Virtanen SM, Ilonen J, Åkerblom HK, Vaarala O, the Finnish TRIGR Study Group. The effect of cow milk exposure and maternal type 1 diabetes: Cellular and humoral immunization to dietary insulin in infants at genetic risk for Type 1 diabetes. *Diabetes* 2000;49:1657–1665.
73. Arslanian SA, Becker DJ, Rabin B, Atchison R, Eberhardt M, Cavender D, Dorman J, Drash AL. Correlates of insulin autoantibodies in newly diagnosed children with insulin-dependent diabetes before insulin therapy. *Diabetes* 1985;32:926–930.
74. Karjalainen J, Knip M, Mustonen A, Ilonen J, Åkerblom HK. Relation between insulin antibody and complement-fixing islet cell antibody at clinical diagnosis of IDDM. *Diabetes* 1986;35:620–622.
75. Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: The 2-year analysis of the German BABYDIAB Study. *Diabetes* 1999;48:460–468.
76. Kimpimäki T, Kupila A, Hämäläinen A-M, Kukko M, Kulmala P, Savola K, Simell T, Muona P, Ilonen J, Simell O, Knip M. The first signs of ß-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;86:4782–4788.
77. Åkerblom HK, Savilahti E, Saukkonen TT, Paganus A, Virtanen SM, Teramo K, Knip M, Ilonen J, Karjalainen J, Vaarala O, Reunanen A. The case for elimination of cow's milk in early infancy in the prevention of Type 1 diabetes: The Finnish experience. *Diabetes Metab Rev* 1993;9:269–278.
78. Åkerblom HK, Virtanen SM, Hämäläinen AM, Ilonen J, Savilahti E, Vaarala O, Reunanen A, Teramo K, Knip M. Emergence of diabetes associated autoantibodies in the nutritional prevention of IDDM (TRIGR) project. *Diabetes* 1999;48(Suppl 1):A45.
79. Scott FW, Sarwar G, Cloutier HE. Diabetogenicity of various protein sources in the diet of the diabetes-prone BB rat. In: Camerini-Davolos RA, Cole HS, eds. *Prediabetes*, New York: Plenum Press, 1988:277–285.
80. Atkinson MA, Winter WE, Skordis N, Beppu H, Riley WM, MacLaren NK. Dietary protein restriction reduces the frequency and delays the onset of insulin dependent diabetes in BB rats. *Autoimmunity* 1988;2:11–220.
81. Hoorfar J, Scott FW, Cloutier HE. Dietary plant materials and development of diabetes in the BB rat. *J Nutr* 1991;121:908–916.
82. 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;5:439–441.
83. MacFarlane AJ, Burghardt KM, Kelly J, Simell T, Simell O, Altosaar I, Scott FW. A type 1 diabetes-related protein from wheat (*Triticum aestivum*). A cDNA clone of a wheat storage globulin, Glb1, linked to islet damage. *J Biol Chem* 2003;278:54–63.
84. Tuomilehto J, Tuomilehto-Wolf E, Virtala E, LaPorte R. Coffee consumption as trigger for insulin dependent diabetes mellitus in childhood. *Br Med J* 1990;300:642–643.
85. Soltesz G, Jeges S, Dahlquist G, Hungarian Childhood Diabetes Epidemiology Study Group. Non-genetic risk determinants for type 1 (insulin-dependent) diabetes mellitus in childhood. *Acta Paediatr* 1994;83:730–735.
86. Virtanen SM, Räsänen L, Aro A, Ylönen K, Lounamaa R, Åkerblom HK, Tuomilehto J, The Childhood Diabetes in Finland Study Group: Is children's or parents' coffee or tea consumption associated with the risk for type 1 diabetes mellitus in children? *Eur J Clin Nutr* 1994;48:279–285.
87. Haglund B, Ryckenberg K, Selinus O, Dahlquist G. Evidence of a relationship between childhood-onset Type 1 diabetes and low groundwater concentration of zinc. *Diabetes Care* 1996;19:873–875.
88. The EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk of Type I (insulin-dependent) diabetes mellitus. *Diabetologia* 1999;42:51–54.
89. Hyppönen E, Läärä E, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of Type 1 diabetes: A birth cohort study. *Lancet* 2001;358:1500–1504.
90. Fournier C, Gepner P, Sadouk M, Charriere J. In vivo beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D_3 on the induction of experimental autoimmune thyroiditis. *Clin Immunol Immunopathol* 1990;54:53–63.
91. Mathieu C, Waer M, Laureys J, Rutgeerts O, Nouillon R. Prevention of type 1 diabetes in NOD mice by 1,25-dihydoxyvitamin D_3. *Diabetologia* 1994;37:552–558.
92. Myers MA, Mackay IR, Rowley MJ, Zimmet PZ. Dietary microbial toxins and type 1 diabetes—a new meaning for seed and soil. *Diabetologia* 2001;44:1199–1200.
93. Hyppönen E, Kenward MG, Virtanen SM, Piitulainen A, Virta-Autio P, Knip M, Åkerblom HK, The Childhood Diabetes in Finland Study Group. Infant feeding, early weight gain and risk of type 1 diabetes. *Diabetes Care* 1999;22:1961–1965.
94. Hyppönen E, Virtanen SM, Kenward MG, Knip M, Åkerblom HK, The Childhood Diabetes in Finland Study Group: Obesity, increased linear growth and risk of type 1 diabetes mellitus in children. *Diabetes Care* 2000;23:1755–1760.
95. Wilkin TJ. The accelerator hypothesis: Weight gain as the missing link between type 1 and type 2 diabetes. *Diabetologia* 2001;44:914–922.

96. Knip M. Natural course of preclinical type 1 diabetes. *Horm Res* 2002;57(Suppl 1):6–11.
97. The EURODIAB ACE Study Group and the EURODIAB ACE Substudy 2 Study Group. Familial risk of type 1 diabetes in European children. *Diabetologia* 1998;41:1151–1156.
98. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, Bottazzo GF, Gale EAM. Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 1994;43:1304–1310.
99. Kulmala P, Petersen JS, Vähäsalo P, Karjalainen J, Löppönen T, Dyrberg T, Åkerblom HK, Knip M, The Childhood Diabetes in Finland Study Group. Prediction of insulin-dependent diabetes mellitus in siblings of diabetic children—a population-based study. *J Clin Invest* 1998;101:327–336.