Environmental Triggers and Determinants of Type 1 Diabetes

Mikael Knip,[1,2] Riitta Veijola,[3] Suvi M. Virtanen,[4,5,6] Heikki Hyöty,[7,8] Outi Vaarala,[9,10] and Hans K. Åkerblom[1]

Type 1 diabetes is perceived as a chronic immune-mediated disease with a subclinical prodromal period characterized by selective loss of insulin-producing β-cells in the pancreatic islets in genetically susceptible subjects. A series of evidence supports a critical role of exogenous factors in the development of type 1 diabetes, such as *1*) the fact that <10% of individuals with HLA-conferred diabetes susceptibility do progress to clinical disease, *2*) a pairwise concordance of type 1 diabetes of <40% among monozygotic twins, *3*) a more than 10-fold difference in the disease incidence among Caucasians living in Europe, *4*) a several-fold increase in the incidence over the last 50 years, and *5*) migration studies indicating that the disease incidence has increased in population groups who have moved from a low-incidence to a high-incidence region. This article discusses the trigger-booster hypothesis claiming that the diabetic disease process is triggered by an exogenous factor with definite seasonal variation and driven by one or several other environmental determinants. In addition, there are a series of modifying factors affecting the fate and pace of the process. Accordingly, progression to clinical type 1 diabetes typically requires the unfortunate combination of genetic disease susceptibility, a diabetogenic trigger, and a high exposure to a driving antigen. *Diabetes* 54 (Suppl. 2):S125–S136, 2005

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 β-cells are still functioning. Environmental factors have been implicated in the pathogenesis of type 1 diabetes both as triggers and potentiators of β-cell destruction (1–3), although the contribution of any individual exogenous factor has not yet been definitely proven. Type 1 diabetes is considered to be a chronic immune-mediated disease with a subclinical prodrome of variable duration. It is characterized by selective loss of insulin-producing β-cells in the pancreatic islets in genetically susceptible subjects. The most important genes contributing to disease susceptibility are located in the HLA class II locus on the short arm of chromosome 6 (4). Nevertheless, only a relatively small proportion, i.e., <10%, of genetically susceptible individuals progress to clinical disease. This implies that additional factors are needed to trigger and drive β-cell destruction in genetically predisposed subjects.

NATURAL HISTORY OF TYPE 1 DIABETES

The clinical presentation of type 1 diabetes is preceded by an asymptomatic period of highly variable duration (5). Aggressive β-cell destruction may lead to disease manifestation within a few months in infants and young children, whereas in other individuals, the process may continue for years (in some cases, even for >10 years) before the eventual presentation of overt disease. The appearance of diabetes-associated autoantibodies is the first detectable sign of emerging β-cell autoimmunity. There are four disease-related autoantibodies that have been shown to predict clinical type 1 diabetes (6). These include classic islet cell antibodies detected by conventional immunofluorescence, insulin autoantibodies (IAAs), and autoantibodies to the 65-kDa isoform of GAD and the tyrosine phosphatase-related IA-2 molecule. The latter three autoantibodies are measured with specific radiobinding assays. The number of detectable autoantibodies is unequivocally related to the risk of progression to overt type 1 diabetes both in family studies and also in surveys based on general population cohorts. In family studies, positivity for three to four autoantibodies is associated with a risk of developing clinical type 1 diabetes in the range of 60–100% over the next 5–10 years. Preliminary studies in the general population indicate that the predictive value of multiple autoantibody positivity is approaching that observed among first-degree relatives (7,8).

Several studies have shown that β-cell autoimmunity may be induced early in life (9,10). Figure 1 presents data from the Finnish Diabetes Prediction and Prevention (DIPP) study showing that the first autoantibodies appear already before the age of 3 months and that ~9% of these children recruited from the general population based on increased HLA DQB1–conferred genetic risk develop persistent positivity for at least one autoantibody by the age of 5 years, whereas close to 4% seroconvert to persistent positivity for multiple (two or more) antibodies by that age (11). These figures demonstrate that a higher proportion of the population develops signs of β-cell autoimmunity than those progressing to clinical type 1 diabetes. Data from the DIPP study indicate that the spreading of the humoral autoimmune response from one epitope to another and
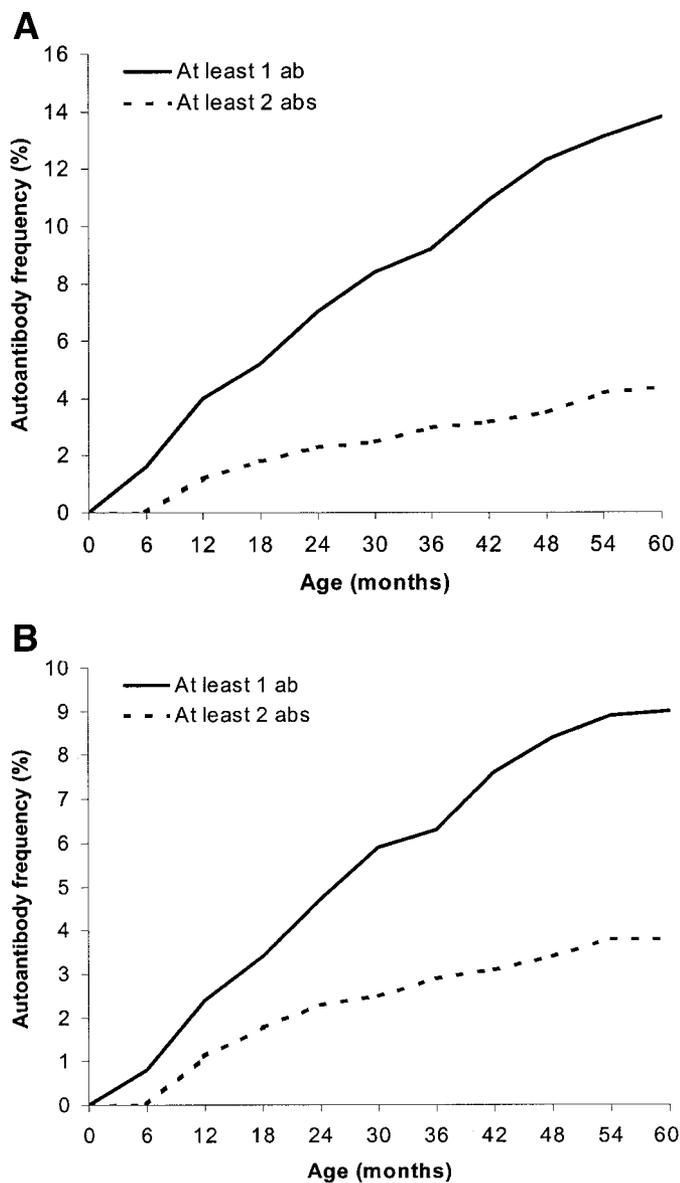
FIG. 1. The appearance of β-cell autoimmunity, defined based on autoantibody positivity in at least one sample (*A*) or positivity in two or more sequential samples (*B*) over the first 5 years of life in 1,006 children with increased HLA DQB1 conferred susceptibility to type 1 diabetes identified from the general population (the Finnish DIPP study, modified from Kukko et al. [11]). Proportion of children positive for at least one autoantibody (ab) specificity and of those positive for at least two autoantibodies (abs) out of four analyzed (islet cell antibodies, insulin autoantibodies, and autoantibodies to the 65-kDa isoform of GAD and the tyrosine phosphatase-related IA-2 antigen) is shown.

from one antibody to another occurs in a relatively short window of time (5,12). If such a spreading does not take place within a year after the appearance of the first autoantibodies, it rarely occurs later. These and other observations imply that positivity for a single autoantibody specificity represents in most cases harmless nonprogressive β-cell autoimmunity, whereas the presence of two or more autoantibodies reflects a progressive process that only rarely reverts (13). Accordingly, positivity for multiple autoantibodies can be used as a surrogate marker of clinical type 1 diabetes in prospective studies (in young children in particular), since the overwhelming majority of young children with multiple autoantibodies will eventu-

ally present with overt diabetes (14). The use of meaningful surrogate markers shortens the time needed for prospective studies on the pathogenesis of type 1 diabetes and for primary intervention studies aimed at preventing genetically susceptible individuals from progressing to multiple autoantibody positivity. The new insights into the natural history of type 1 diabetes have accordingly opened up new possibilities and strategies for assessing the role of environmental factors in the development of diabetes.

ARGUMENTS IN FAVOR OF ENVIRONMENTAL FACTORS IN THE DEVELOPMENT OF TYPE 1 DIABETES

Several lines of evidence support a critical role of exogenous factors in the pathogenesis of type 1 diabetes. Studies in monozygotic twins indicate that only 13–33% are pairwise concordant for type 1 diabetes (15,16), suggesting that there is either acquired postconceptional 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 the lowest annual rate in Europe reported from Macedonia amounting to 3.2/100,000 children under the age of 15 years (17) and the highest rate observed in Finland reaching 54 in 2003 (A. Reunanen, personal communication). This more than 15-fold 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—for example, in Finland, the incidence has increased 4.5-fold from the early 1950s (18). Such an increase cannot be the consequence only of enhanced genetic disease susceptibility in the population but must mostly be due to changes in lifestyle and environment. Migrant studies have been used little in epidemiological surveys of type 1 diabetes. However, available data indicate 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 (2). Accumulating evidence suggests that the proportion of subjects with high-risk HLA genotypes has decreased over the last decades among patients with newly diagnosed type 1 diabetes, whereas the proportion of people with low-risk or even protective HLA genotypes has increased (19,20). These data are compatible with an increased environmental pressure resulting in progression to clinical diabetes with less genetic susceptibility.

A PATHOGENETIC MODEL OF TYPE 1 DIABETES

A series of observations suggest that β-cell autoimmunity may be triggered by an environmental culprit at any age, although a majority of the processes appear to start early in childhood (21). Figure 2 presents a pathogenetic model of type 1 diabetes according to which the genetic disease susceptibility allows the initiation of a β-cell–destructive process resulting in the presentation of clinical type 1 diabetes in some individuals. However, initiation of the process does not necessarily lead to progression of clinical disease. According to our hypothesis, there is a need for a driving exogenous antigen playing the same role as gluten in celiac disease. As a matter of fact, there are striking similarities between type 1 diabetes and celiac disease from a pathogenetic point of view (22, Table 1). Both diseases are characterized by genetic susceptibility determined by both HLA and non-HLA genes. The former
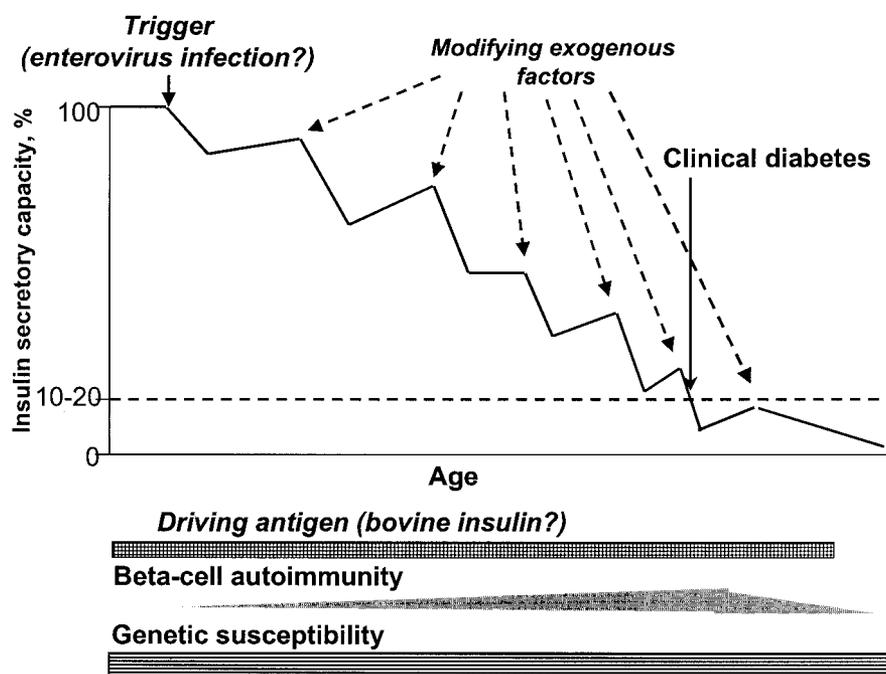
FIG. 2. Progression from genetic susceptibility to overt type 1 diabetes. According to this model, the disease process is triggered by an exogenous factor, driven by another environmental determinant, and modified by a series of environmental factors in individuals with increased genetic disease susceptibility.

explain about half of the familial clustering in both diseases, and accordingly, the other half must be due to non-HLA genes and/or a shared environment. About 20% of Caucasians carry HLA-conferred genetic susceptibility to celiac disease, and the overwhelming majority (>95%) of the population in developed countries are exposed to gluten-containing cereals daily. Despite that, only maximally 1.3% of the population present with clinical disease (23). Accordingly, some other factors in addition to HLA-conferred predisposition and daily gluten intake are needed for progression to overt celiac disease. It seems unlikely that non-HLA genes should totally account for the missing link. It is tempting to speculate that you may need a triggering gastrointestinal infection inducing primary target cell damage and/or a proinflammatory cytokine milieu in the gut epithelium to initiate the disease process subsequently driven by dietary gluten toward clinical celiac disease in genetically predisposed individuals. Previous studies have indicated that adenovirus infections might contribute to the pathogenesis of celiac disease (24,25), but only a few studies have focused on the possible role of microbes in this disease.

In parallel to celiac disease, about one-fifth of Caucasians carry HLA-defined susceptibility to type 1 diabetes, whereas the lifetime cumulative incidence of clinical disease can be estimated to be close to 1%, indicating that only ~5% of individuals with HLA-conferred predisposition progress to overt type 1 diabetes. Our hypothesis holds that progression to clinical diabetes requires the combination of genetic disease susceptibility, a critically timed trigger, and high subsequent exposure to a driving antigen. If any of these determinants is missing or any of the exogenous factors are inappropriately timed, the risk of type 1 diabetes would be minimal even in the presence of the other predisposing elements. Such a model could explain why only a small minority of individuals with HLA-conferred genetic susceptibility to type 1 diabetes do present with overt disease. In addition to the trigger and the driving antigen, there are most likely a series of environmental factors modifying the fate and pace of the β-cell destructive process, some having protective and other predisposing effects.

POTENTIAL TRIGGERS OF β-CELL AUTOIMMUNITY AND TYPE 1 DIABETES

As mentioned earlier, the first signs of β-cell autoimmunity may appear very early in life. We have focused our

TABLE 1
Features of celiac disease and type 1 diabetes

| Characteristic	Celiac disease		Type 1 diabetes		
Genetics	HLA DQ2 (>85%)		HLA DQ8		
	(HLA DQ8)	} Account for ~50%	HLA DQ2	} (>90%) }	Account for ~50%
	Non-HLA genes, account for ~50%		Non-HLA genes, account for ~50%		
Trigger of target cell damage	Infection?		Infection?		
Driving antigen	Gluten		Dietary?		
Autoantibodies	Reticulin antibodies, endomysial antibodies, tissue transglutaminase antibodies		Insulin autoantibodies, GAD and IA-2 antibodies		
Outcome	1 of 15–20 with HLA-conferred susceptibility progresses to clinical disease		1 of 15–20 with HLA-conferred susceptibility progresses to clinical disease		
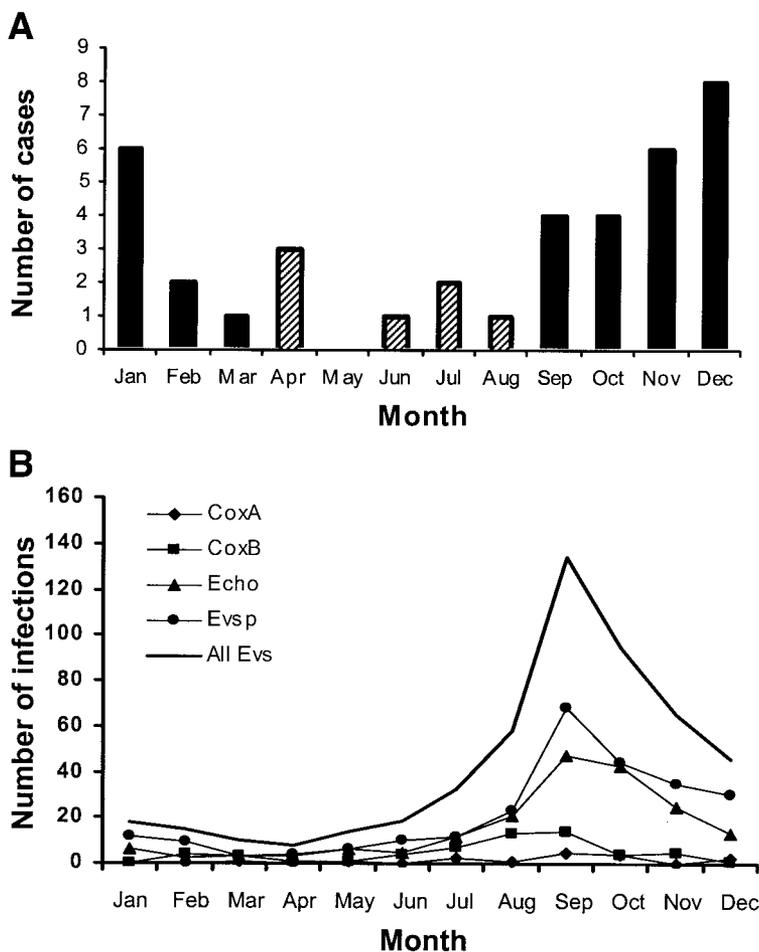
FIG. 3. Seasonal variation in the appearance of the first auto-antibodies in young children with increased HLA-conferred susceptibility to type 1 diabetes from the DIPP study (*A***, modified from Kimpimäki [10]) and the seasonal variation in laboratory-confirmed enterovirus infections in Finland (***B***, Statistics from the National Public Health Institute). All Evs, sum of all enterovirus infections; CoxA, coxsackie virus A; CoxB, coxsackie virus B; Echo, echovirus; Evsp, enterovirus species (serotype not defined).**

research on defining the characteristics of the trigger(s) of β-cell autoimmunity by observing subjects with increased HLA-conferred susceptibility to type 1 diabetes prospectively from birth, with frequent follow-up visits with an interval of 3–6 months up to the age of 2 years and thereafter with an interval of 6–12 months (26). These studies have revealed that there is an unequivocal temporal variation in the appearance of the first diabetes-associated autoantibodies reflecting the initiation of the disease process and paralleling the seasonal variation previously observed in the presentation of clinical diabetes (10,27). Most initial autoantibodies appear during the cold period in the fall and winter but rarely in the spring or in the summer (Fig. 3A). There also seems to be some variation from one year to another in the timing and height of the autoantibody peaks. When studying families with more than one child experiencing seroconversion to autoantibody positivity, we have noticed that the autoantibodies rarely appear simultaneously in seroconverting siblings (28). The first autoantibodies do emerge, however, more often than expected during the same season among such siblings but infrequently in the same year. Based on these observations, we conclude that the trigger of β-cell autoimmunity *1*) has a seasonal pattern, being more common during the cold season; *2*) shows some temporal variation from year to year; and *3*) does not necessarily induce β-cell autoimmunity at the same time in all genetically susceptible siblings within the same family.

Based on the characteristics listed above, some exogenous factors implicated as potential triggers of β-cell autoimmunity may be excluded. These include exogenous factors with a stable or consistently increasing exposure, such as most dietary components in early childhood. The pattern of the autoantibody appearance strongly points to the role of infectious agents with conspicuous seasonal variation as triggers of β-cell autoimmunity. Such variations are typical for viral infections, and the pattern of laboratory-confirmed enterovirus infections in Finland fits well into the proposed role of enterovirus infections as triggers of β-cell autoimmunity (Fig. 3B). In addition to viral infections, one should also consider other environmental variables with seasonal variation. There is definitely seasonal variation in the amount of daylight and sunshine hours, especially in Northern Europe, which has the highest incidence of type 1 diabetes in the world (29). Without oral substitution, the sunlight-dependent synthesis of vitamin D in the skin is the most important source of this immunologically active hormone. Some studies have indicated that the lack of oral vitamin D substitution in infancy increases the subsequent risk of type 1 diabetes (30,31). The following two arguments do, however, speak against the role of vitamin D deficiency as a trigger of β-cell autoimmunity: *1*) there is a general recommendation that all young children should take daily vitamin D drops in Northern Europe, and this recommendation is implemented by >95% of the parents, at least in children up to the age of 2 years; and *2*) there are regions with a low type 1 diabetes incidence in Northern Europe, e.g., Russian Karelia, having an annual incidence rate of 7.8/100,000 children under the age of 15 years in the time period of 1990–1999 compared with a rate of 42 in Finland (32). It has been shown that the mean HbA_{1c} level varies over the

year in children with manifest type 1 diabetes, with the highest values in the fall and winter and with lower levels in the spring and summer (33–35). This may reflect improved insulin sensitivity in the spring and summer because of more physical exercise. Improved insulin sensitivity diminishes β-cell stress, as the workload on the β-cells decreases. However, it is unlikely that there should be substantial seasonal variation in physical exercise in very young children, the target group in whom the seasonal variation in the appearance of the first diabetes-associated autoantibodies has been observed.

Accordingly, we are left with viral infections as the most likely explanation for the seasonal variation in the emergence of the first signs of β-cell autoimmunity. Taken into account the timing and profiles of the autoantibody peaks observed in the Finnish DIPP study, enterovirus infections appear to be the most probable trigger of β-cell autoimmunity. This is supported by our previous observations of a strong temporal relationship between enterovirus infections and the appearance of the first diabetes-associated autoantibodies in two prospective series of young children with increased genetic susceptibility to type 1 diabetes (36–40). The implicated link between enterovirus infections and β-cell autoimmunity has been questioned, since most of the supportive data have come out of Finnish studies. Two other prospective studies (BABYDIAB in Germany and the Diabetes Autoimmunity Study in the Young [DAISY] in Denver, CO) have failed to demonstrate any association between enterovirus infections and β-cell autoimmunity (41,42). There are, however, at least three critical issues with a decisive impact on the ability of any prospective study to provide meaningful observations in this context. The first one is the size of the study and the number of subjects who develop signs of β-cell autoimmunity—factors clearly related to the statistical power of the study (43). All prospective studies have so far included <50 seroconverters, which confers a high risk of missing even major influences of enterovirus infections on β-cell autoimmunity. A second critical consideration is the study design and the sampling interval. Long sampling intervals definitely hamper the possibility to detect enterovirus infections from collected samples. In the BABYDIAB study, coxsackie virus antibodies were measured from serum samples taken at the age of 9 months, 2 years, 5 years, and 8 years, and in DAISY, the samples were obtained at the age of 9 months, 15 months, 2 years, and then annually. In contrast, the DIPP study has a more frequent sampling schedule, collecting samples with an interval of 3–6 months over the first 2 years of life and subsequently with an interval of 6–12 months. The third crucial point is related to the type of samples collected and the methodological arsenal used for the detection of enterovirus infections, taking into account that there are more than 60 different serotypes. The DIPP study used the most extensive strategy, including detection of enterovirus mRNA with polymerase chain reaction from serum and stool samples and analysis of IgA, IgM, and IgG class enterovirus antibodies using both group- and serotype-specific assays. The impact of these differences is reflected by the substantially lower frequency of infections diagnosed, e.g., in the BABYDIAB cohort compared with the DIPP study (7 vs. 81% of the children had had an enterovirus infection by the age of 2 years). It is unlikely that such a difference could be due to a lower frequency of enterovirus infections in Germany, because enterovirus infections actually seem to be more common in the

background population in Germany than in Finland (44). In addition to these prospective studies, a series of cross-sectional surveys have documented that enterovirus infections are more common in patients with newly diagnosed type 1 diabetes than in control subjects (45). For example, enterovirus RNA has been detected in the peripheral blood on average in one-third of the patients with type 1 diabetes but in <5% of the control subjects.

The frequency of enterovirus infections has decreased over the last decades in the background population in developed countries, e.g., in Finland and Sweden (44). Despite that, these countries have a high and increasing incidence of type 1 diabetes among children. This appears to be paradoxical. The paradox can, however, be explained by the so-called "polio hypothesis" introduced by Viskari et al. (46). The polioviruses comprise three serotypes among >60 enterovirus serotypes. When the frequency of acute poliovirus infections started to decrease at the beginning of the last century among the general population in countries with an increasing standard of hygiene, the incidence of paralytic polio being a complication of the acute infection began to increase. This was obviously the consequence of decreased levels of protective maternal poliovirus antibodies transferred transplacentally and through breast milk to the infant, leading to a situation where the risk increased that the infant would get his or her first poliovirus infection at the time of no maternal protection. Similarly, the decreasing frequency of enterovirus infections in the background population would increase the susceptibility of young children to the diabetogenic effect of enteroviruses. The same phenomenon may also contribute to the marked international variation in type 1 diabetes incidence, because enterovirus infections seem to be rare in countries where the rate of type 1 diabetes is high (44).

The tropism phenomenon (the characteristic of a virus to infect a particular tissue or cell type), in which the attachment of the virus to the viral receptors on the cell surface together with other interactions with cellular proteins is a central feature, is thought to explain why some variants of enteroviruses may be diabetogenic and others not (47). It has been proposed that pancreatic β-cell tropic variants of the coxsackie B virus are present in the general population and that they are able to induce β-cell damage in susceptible individuals (48). The biological significance of enterovirus tropism has been demonstrated by the highly specific nature of the motorneuron damage in poliomyelitis, and it is possible that viral tropism could also explain the specificity of β-cell damage in type 1 diabetes. In vitro studies have shown that enteroviruses infect β-cells easily and induce functional impairment and cell death (49,50). Such a capacity seems to be shared by a wide range of serotypes, but the extent of the cellular lesions appears to be characteristic of individual virus strains. Recent studies have shown that enterovirus genome or enterovirus proteins can be detected in pancreatic islets of patients affected by type 1 diabetes or autoantibody-positive subjects (51–53). These findings suggest that enteroviruses have a tropism for islet cells in humans in contrast to the mouse models where enteroviruses infect preferentially exocrine pancreas and only certain strains can cause β-cell damage. They also raise the possibility that patients with type 1 diabetes may have a persistent enterovirus infection in their pancreatic islets.

An Australian study reported a few years ago molecular homology between the VP7 protein of rotavirus and T-cell
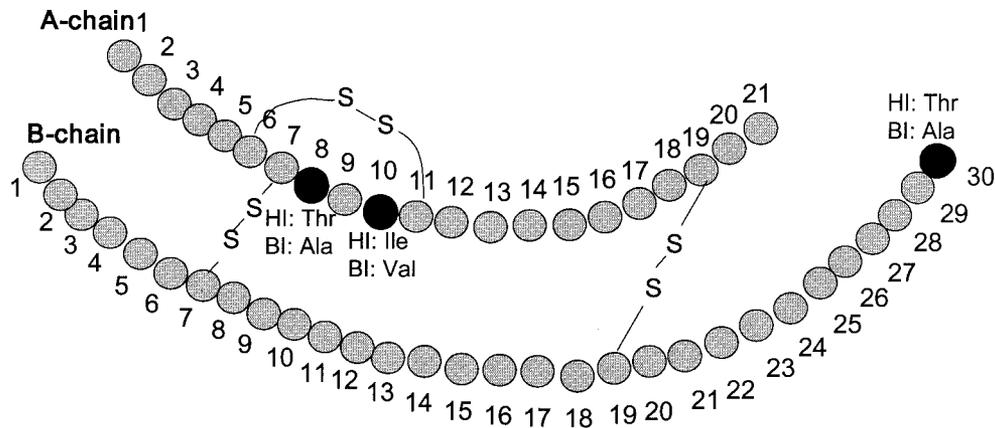
FIG. 4. There is a three–amino acid difference between human and bovine insulin: human insulin (HI) has threonine and bovine insulin (BI) alanine in the A-chain position 8, the A-chain position 10 holds isoleucine in human insulin and valine in bovine insulin, and B-chain position 30 comprises threonine in human insulin and alanine in bovine insulin.

epitopes in the protein tyrosine phosphatase–related IA-2 molecule and in the GAD65 molecule (54). In a prospective study of infants genetically predisposed to type 1 diabetes, the appearance of diabetes-associated autoantibodies was associated with a significant rise in rotavirus antibodies, indicating that rotavirus infections may induce β-cell autoimmunity in genetically susceptible infants (55). A Finnish prospective study showed that ~16% of infants and young children with HLA-conferred susceptibility to type 1 diabetes experienced a rotavirus infection during the 6-month window preceding the detection of the first diabetes-associated autoantibodies, whereas 15% of the control subjects matched for sex, birth date, delivery hospital, and HLA genotype had signs of a rotavirus infection during the corresponding time period (56). That observation does not support the role of rotavirus infections as triggers of β-cell autoimmunity.

Taken together, accumulated data support the hypothesis that a diabetogenic enterovirus infection is the likely trigger of β-cell autoimmunity. This is supported by the observed temporal variation in the appearance of the first diabetes-associated autoantibodies in young children, the profile of which resembles the temporal profile of enterovirus infections in the background population.

POTENTIAL DRIVING ANTIGENS IN PRECLINICAL TYPE 1 DIABETES

There is no direct evidence of an exogenous antigen driving the type 1 diabetes disease process from initial β-cell autoimmunity to clinical disease. Accordingly, this idea is based on indirect data and parallels with celiac disease, and it has to be critically considered. In general terms, one may argue that the driving antigen must represent a relatively common exposure among the population in most developed countries, and one can also assume that there must be some variability in the exposure within and across populations. In addition, one may hypothesize that the candidate antigen must comprise original or processed structural motifs bound by HLA molecules encoded by major histocompatibility complex class II susceptibility genes and presented effectively to T-cells by antigen-presenting cells.

Celiac disease again provides interesting angles of approach to these issues. The causal link between wheat and celiac disease was only uncovered through (unplanned) intervention: the wheat-deficient World War II diet in the Netherlands and its postwar improvement identified a culprit autoimmune disease-causing agent, i.e., dietary gluten (57). Without that experience, gluten would have remained most likely unidentified as the driving dietary antigen in celiac disease. It has recently been proposed that the HLA association in celiac disease, in which HLA DR3-DQ2 is the dominant susceptibility haplotype, can be explained by a superior ability of the DQ2 molecule to bind proline-rich gluten peptides that have survived gastrointestinal digestion and have been deamidated by tissue transglutaminase (58). This brings up two thought-provoking points. First, the most important autoantigen in celiac disease, i.e., tissue transglutaminase, seems to have a biological role in the disease process. Second, the exogenous antigen, i.e., gluten, must be processed in a critical manner (in this case deamidated) before it is bound by the predisposing HLA molecule to be presented to T-cells.

Insulin is a crucial autoantigen in type 1 diabetes because it is the only β-cell–specific autoantigen in postnatal life. IAAs are the first or among the first autoantibodies to appear when the disease process leading to type 1 diabetes is initiated in young children (9,10). The frequency of IAAs is also substantially higher among young children at the diagnosis of type 1 diabetes than among older children and adults (59). Because cow's milk formulas generally are the first and most common dietary source of foreign complex proteins to which an infant is exposed in developed countries, we asked the question whether early exposure to cow's milk formula results in an immune response to bovine insulin present in formulas and differing from human insulin by three amino acids: two in the A chain and one in the B chain (Fig. 4). Our study showed that the infants who were exclusively breast-fed at least up to the age of 3 months had substantially lower IgG class antibodies to bovine insulin than those who were exposed to formula before that age (60). All subjects were observed up to the age of 18 months. The follow-up demonstrated that the IgG class insulin antibodies started to slowly decrease after the age of 3 months as a sign of the development of oral tolerance in those infants who were given formula before the age of 3 months, whereas in those exclusively breast-fed at least to the age of 3 months, the antibody levels increased up to the age of 12 months, after which the antibody titers leveled off (Fig. 5). That antibody profile was expected, since these infants were exposed to cow's milk–based products, mostly formula, at some point
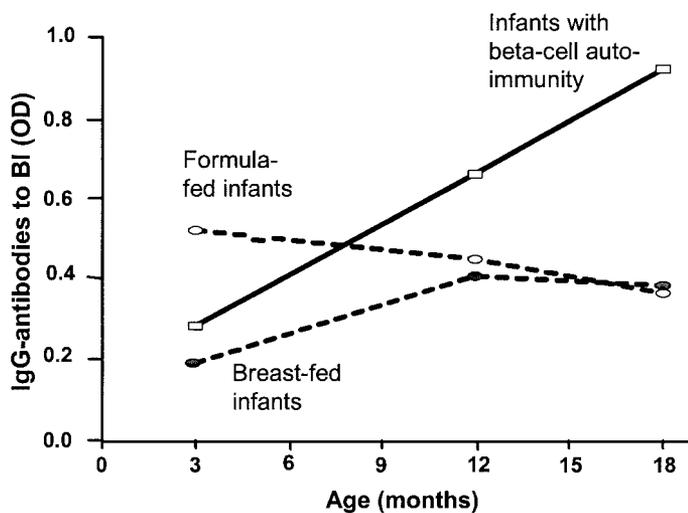
FIG. 5. IgG class antibodies to bovine insulin (BI) in a group of infants exposed to cow's milk–based formula before the age of 3 months (formula-fed infants), in another group exclusively breast-fed at least up to the age of 3 months but subsequently exposed to cow's milk products (breast-fed infants), and in a small group of infants who seroconverted to positivity for two or more diabetes-associated autoantibodies before the age of 12 months (modified from Blomquist et al. [56]). OD, optical density.

during their first 12 months of life. The study cohort included a small series of subjects ($n = 11$) who developed early signs of β-cell autoimmunity. These subjects showed continuously increasing levels of IgG antibodies to bovine insulin. This increase may partly be explained by the appearance of IAAs, since there is a considerable cross-reactivity between bovine and human insulin in the insulin antibody assays. However, the increase in IgG antibodies to bovine insulin remained significant, even after adjustment for the IAA titers. Our observation indicates that those young children who present with early signs of β-cell autoimmunity appear to lack the capacity to develop oral tolerance to bovine insulin. Accordingly, the initial immune response to bovine insulin may be diverted into a response targeting human insulin in such individuals.

Are there then any data suggesting that increased exposure to cow's milk later in childhood might confer increased risk for type 1 diabetes? Data from three population-based case-control studies on cow's milk intake before diagnosis of type 1 diabetes are conflicting. Verge et al. (61) reported that the cow's milk intake had been higher in pre-diabetic children than in control children in New South Wales, Australia. In our nationwide Childhood Diabetes in Finland (DiMe) study, we observed that a high consumption of cow's milk in childhood was associated with a more frequent appearance of diabetes-associated autoantibodies and type 1 diabetes in a prospective cohort of initially unaffected siblings of children with type 1 diabetes (62,63). In contrast, a Swedish retrospective survey indicated that the frequency of milk intake had been lower among children who presented with type 1 diabetes than among unaffected children (64).

Increased circulating antibody levels to cow's milk proteins were reported in children with type 1 diabetes for the first time in 1988, when Savilahti et al. (65) observed that such children had significantly higher levels of serum IgA antibodies to cow's milk and β-lactoglobulin and of IgG antibodies to β-lactoglobulin than age-matched control subjects. This finding has been confirmed by later

studies (2). Dahlquist et al. (66) reported from the Swedish nationwide case-control study that most cow's milk antibody levels tended to be increased in diabetic children when compared with control subjects, the difference being significant for IgA antibodies to cow's milk, BSA, and β-lactoglobulin. In a multiple logistic regression analysis, the authors observed that IgA antibodies to β-lactoglobulin were significantly associated with an increased risk of diabetes at young age, independent of islet cell antibody status and of early weaning to cow's milk–based formula. These observations could be interpreted as supporting the idea that enhanced consumption of cow's milk before the presentation of clinical type 1 diabetes is a risk factor. There are, however, also alternative explanations to the increased circulating cow's milk antibody levels in children with newly diagnosed type 1 diabetes. The immunological reactivity to cow's milk proteins may be enhanced in children who will progress to type 1 diabetes or their gastrointestinal permeability to cow's milk proteins might be increased. The enhanced humoral immune response to cow's milk proteins seem to be specific for type 1 diabetes because elevated levels have not been generally observed in other autoimmune diseases. The active immune response has been reported to be restricted to cow's milk proteins, since patients with newly diagnosed type 1 diabetes have not been shown to have increased antibody levels to other dietary antigens, such as gliadin and ovalbumin (65,67). Bovine insulin antibodies have not been measured in children with signs of β-cell autoimmunity beyond the age of 18 months, but increased levels of bovine insulin-binding antibodies have been detected in children with type 1 diabetes (60). Taken together, the evidence in favor of the role of bovine insulin as a driving antigen in the disease process leading to overt type 1 diabetes is relatively fragmentary, and further research is clearly needed to confirm or exclude this hypothesis. Bovine insulin is definitely present in cow's milk, although the structural components of immunoreactive insulin are poorly characterized in milk.

Gluten or other cereal-derived proteins have been implicated as potential driving antigens in type 1 diabetes. Two small-scale pilot intervention studies have been performed in family members testing positive for diabetes-associated autoantibodies to assess whether gluten elimination modifies the natural course of β-cell autoimmunity. In the German trial, seven autoantibody-positive first-degree relatives were placed on a gluten-free diet for a period of 12 months followed by gluten re-exposure over the subsequent 12 months (68). The autoantibody titers did not change significantly during the gluten-free intervention period nor during the re-exposure period. A total of 17 family members testing positive for at least two diabetes-associated autoantibodies were put on a gluten-free diet for 6 months in an Italian trial (69) and then again on a normal diet for another 6 months. There were no significant changes in the autoantibody titers during the intervention period or during the subsequent 6 months. The first-phase insulin response to intravenous glucose increased in 12 of 14 subjects tested during the gluten-free period and decreased in 10 out 13 retested family members during the re-exposure period. Accordingly, this trial indicated that a gluten-free diet has no effect on the signs of β-cell autoimmunity in first-degree relatives of affected patients, but such a diet may increase the endogenous insulin secretion in family members at increased risk of type 1 diabetes.

From a theoretical point of view, a dietary antigen would fit well into the role as the factor driving the disease process toward clinical type 1 diabetes, since the exposure to most dietary factors tends to be frequent, and still there is some variation in the exposure both within and across populations. Bovine insulin is an attractive candidate, since an immune response initially induced by bovine insulin will cross-react and may target human insulin in the β-cell.

MODIFYING FACTORS

There is a conspicuous interindividual variation in the duration of the preclinical disease process in type 1 diabetes. Although some of this difference may be due to genetic elements (70), there is likely in addition a series of nongenetic host-related and exogenous factors having an impact on the fate and pace of the disease process.

Early exposure to complex foreign proteins in infancy may be a factor that affects the programming of the immune system in such a way that autoimmune responses are favored later in childhood. The gut-associated lymphoid tissue represents the largest immune organ in the human organism, and accumulating evidence suggests that gut-associated lymphoid tissue is involved in the diabetic disease process (71–73). A subclinical intestinal immune activation demonstrated as increased expression of adhesion molecules, HLA class II molecules, and cytokines has been observed in patients with type 1 diabetes without any signs of celiac disease (74,75). Early nutrition has indeed been implicated as a potential risk factor for later type 1 diabetes (76). Cow's milk proteins are the first foreign proteins an infant is exposed to in more than two-thirds of all infants in developed countries. A series of retrospective studies have implied that breast-feeding protects from type 1 diabetes, but there are also reports suggesting no protective effect and even some indicating a predisposing effect of breast-feeding (2). In 1994, Gerstein (77) conducted a meta-analysis of all acceptable retrospective studies available to assess whether early feeding practices had any influence on the risk of type 1 diabetes. That meta-analysis showed that breast-feeding for a shorter period than 3 months was associated with a 1.4-fold risk for type 1 diabetes, whereas exposure to cow's milk before the age of 4 months conferred a 1.5-fold disease risk. Prospective and cohort studies on the association between early feeding and the appearance of β-cell autoimmunity, i.e., diabetes-associated autoantibodies, have also provided partly conflicting results. A cohort study from Colorado did not observe any effect of breast-feeding/early exposure to cow's milk on the appearance of β-cell autoimmunity similarly to two prospective studies: one from Germany and the other from Australia. In contrast, a Finnish birth cohort study reported that short exclusive breast-feeding and early exposure to cow's milk predisposed young children to progressive β-cell autoimmunity in the form of positivity for all four autoantibody specificities analyzed (3).

Two recent prospective studies have indicated that early exposure to cereals may increase the risk of seroconversion to positivity for diabetes-associated autoantibodies (78,79). The American report suggested that both early (before the age of 4 months) and late (at the age of 7 months or later) exposure to cereals were associated with an increased risk of β-cell autoimmunity, whereas the German study implied that an increased risk was related to exposure to cereals before the age of 3 months. In addition, the American survey indicated that both gluten-containing and non–gluten-containing cereals conferred an increased risk for β-cell autoimmunity. Neither of the studies reported any data on the amount and type of cereals the infants were exposed to at various ages. Early exposure to cereals is against generally accepted recommendations on infant nutrition in all developed countries and occurs only rarely. Accordingly, one may ask whether early exposure to cereals is a proxy of other baby care practices possibly predisposing to type 1 diabetes. Dietary gliadin may however trigger intestinal inflammation in subjects with type 1 diabetes likely because of the presence of the HLA DQ2/DQ8 genotype capable of binding gliadin-derived peptides (75). Wheat gliadin could thus affect the immune activation stage of the gut immune system in individuals at genetic risk for type 1 diabetes and modify the autoimmune process toward β-cell destruction.

A pilot intervention study conducted mainly in Finland determining whether weaning to a highly hydrolyzed formula over the first 6–8 months of life decreases the cumulative incidence of diabetes-associated autoantibodies indicated that such a dietary intervention resulted in a decreased frequency of positivity for islet cell antibodies and at least one diabetes-associated autoantibody by the age of 6 years (80). This is the first indication that it may be possible to manipulate spontaneous β-cell autoimmunity in humans by avoiding early exposure to complex foreign proteins. Based on the experiences from the pilot study, a randomized double-blinded trial proper was initiated in 2002 to answer the question whether it is possible to reduce the frequency of diabetes-associated autoantibodies and/or clinical type 1 diabetes by the age of 6 years and the cumulative incidence of type 1 diabetes by the age of 10 years by weaning to a highly hydrolyzed formula over the first 6–8 months of life. This Trial to Reduce IDDM in the Genetically at Risk (TRIGR) will recruit 2,032 infants with at least one affected family member and HLA-conferred susceptibility to type 1 diabetes by the end of 2006, and accordingly the final answer will be available at the earliest in year 2013, illustrating the need for long follow-up periods in prospective and intervention studies exploring the association between early feeding and later type 1 diabetes.

As mentioned earlier, vitamin D has been implicated as a risk factor for type 1 diabetes. The daily requirement of vitamin D cannot be met in the infant period without supplementation, particularly not in countries located far from the equator, which have decreased daylight in the wintertime. A European retrospective multicenter survey indicated that vitamin D supplementation during infancy was associated with a reduced risk of type 1 diabetes (30). A Finnish birth cohort study showed that vitamin D supplementation in the infant period was related to a decreased risk of type 1 diabetes, whereas suspicion of rickets by the age of 2 years increased the risk of later diabetes (31). These observations suggest that vitamin D supplementation in the infant period confers partial protection against type 1 diabetes.

Increased weight gain in infancy has repeatedly been reported to be a risk factor for type 1 diabetes later in childhood (3). A Finnish study showed that those children who presented with type 1 diabetes had been not only heavier but also taller in infancy (81). Increased height and weight later in childhood turned out to be definite risk factors for type 1 diabetes as well (82). Accelerated linear

growth and weight gain result in an enhanced β-cell load and increasing insulin resistance. It has been shown experimentally that active β-cells are more prone to cytokine-induced damage than resting cells. This implies that rapid growth induces β-cell stress. According to the accelerator hypothesis presented by Wilkin (83) a few years ago, 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 are the pace of progression to overt disease and the fact that individuals who present with type 1 diabetes carry genetic susceptibility to autoimmunity.

WHY IS THE INCIDENCE OF TYPE 1 DIABETES RISING AMONG CHILDREN IN DEVELOPED COUNTRIES?

There has definitely been an increase in the incidence of childhood type 1 diabetes in most industrialized countries after World War II (18). Whether this reflects a population-wide increase or is a consequence of decreasing average age at disease presentation is controversial. A recent Swedish study reported that there was no significant change in the overall incidence of type 1 diabetes over a 16-year period in the Swedish population below the age of 35 years, but there was a shift toward a younger age at diagnosis both among males and females (84). In contrast, there seems to be a concomitant increase in incidence as well as a decrease in average age at diagnosis among the population below the age of 30 years according to preliminary Finnish data (A. Reunanen, personal communication).

We already pointed out that the frequency of enterovirus infections has lately decreased among the general population in Northern Europe, resulting in a situation where infants have an impaired protection against acute enterovirus infections early in life (44). This may lead to more invasive enterovirus infections, some of which might lead to the induction of the diabetic disease process as a complication. If we accept the hypothesis that bovine insulin could be a driving antigen in type 1 diabetes, one can speculate that the increasing processing of milk products may have some impact on the immunogenicity of bovine insulin in commercial milk products. Children in the developed world grow linearly and gain weight faster now than some decades ago. They also reach adult height at a younger age than earlier. The average BMI in 15-year-old Finnish children increased, for example, over a 12-year period from 20.0 kg/m^2 in 1980 to 21.3 kg/m^2 in 1992 (M.K., personal communication). This is a conspicuous increase, and if such a trend would continue over the next decades, the mean BMI would be 25 kg/m^2 in this age-group in 2030. Rapid growth and weight gain induces β-cell stress and could thereby induce an earlier presentation of clinical type 1 diabetes and also an increasing incidence by expanding the proportion of susceptible individuals progressing to overt disease. The hygiene hypothesis postulating a relationship between allergic diseases and a childhood environment that leads to decreased pathogen exposure has gained considerable popularity as an explanation for the increasing prevalence of asthma and atopy (85). Recently, the hygiene hypothesis has been implicated to also explain the increasing incidence of autoimmune diseases such as type 1 diabetes (86). It is conceivable that a decreased microbial load in early life may have a major impact on the programming of the immune system (gut-associated lymphoid tissue in particular). However, this field needs more epidemiological research to assess the possible association between specific infections and defined autoimmune and allergic diseases.

CONCLUSIONS

The identification of exogenous factors triggering and driving β-cell destruction offers potential means for intervention aimed at the prevention of type 1 diabetes. Therefore, it is important to pursue studies on the role of environmental factors in the pathogenesis of this disease. Environmental modification is likely to offer the most powerful strategy for effective prevention of type 1 diabetes, 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 comparative European survey (87). The preliminary results of the second pilot study of the TRIGR (Trial to Reduce IDDM in the Genetically at Risk) project, suggesting that it is possible to manipulate the spontaneous appearance of β-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 (80).

It is important to keep in mind that there are gene-environmental interactions operating in all phases of the disease process of type 1 diabetes, not only in the induction of β-cell autoimmunity. The delineation of such interactions is challenging and requires both new methodological tools and study cohorts large enough for the analyses of interactions. So far, there are only a few examples of gene-environmental interactions implicated to play a role in the development of type 1 diabetes. The findings in two case-control studies suggested that there might be an interaction between increased genetic disease susceptibility, based on HLA class II alleles, and an early introduction of supplementary milk feeding during infancy on the risk of type 1 diabetes (88,89). A Finnish study indicated that the increased relative risk associated with childhood milk consumption may be higher among siblings of affected children carrying HLA class II genotypes conferring strong or moderate disease susceptibility than among those with low or decreased risk genotypes (63). HLA risk alleles for type 1 diabetes have also been linked to the appearance of autoantibodies subsequent to enterovirus infections (37) and to increased immune responsiveness to enterovirus antigens (90). Another consideration that has to be taken into account is that there may be interactions between two or more environmental factors. However, extensive study cohorts are needed to identify such interactions. A recent Finnish study implied that two environmental risk factors for type 1 diabetes, i.e., an early enterovirus infection and early exposure to cow's milk, can, when present concomitantly, boost the immune response to bovine insulin in infants with increased HLA-defined disease predisposition (91).

The scientific challenges in the near future are to define the most likely environmental culprit(s) and booster(s) of β-cell autoimmunity and to delineate how exogenous factors affect the natural history of type 1 diabetes. A new consortium comprising six prospective birth cohort studies (the German BABYDIAB study, the American DAISY,

and the Finnish DIPP study among others) and observing at-risk individuals from birth through signs of β-cell autoimmunity to clinical disease provides an optimal setting for successful explorative 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 for a 5-year period (2003–2007) and has started to recruit participating families in fall 2004. We also have 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 (92,93).

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