Natural History of Type 1 Diabetes

Peter Achenbach, Ezio Bonifacio, Kerstin Koczwara, and Anette-G. Ziegler

The natural history of autoimmune type 1 diabetes in children is associated with the appearance of islet autoantibodies early in life, which is influenced by genetic and environmental factors. Once islet autoantibodies have developed, the progression to diabetes in antibody-positive individuals is determined by the age of antibody appearance and by the magnitude of the autoimmunity, in turn related to the age of the subject. Characteristics that describe the magnitude of the autoimmunity can stage progression to type 1 diabetes in islet autoantibody–positive subjects regardless of genetic background or age. ***Diabetes*** **54 (Suppl. 2):S25–S31, 2005**

Type 1 diabetes is a chronic inflammatory disease caused by a selective destruction of the insulin-producing β-cells in the islets of Langerhans (1). The incidence of type 1 diabetes has consistently increased worldwide during the last decades, especially in children and developed countries (2). Type 1 diabetes is associated with the appearance of humoral and cellular islet autoimmunity (1), and a defective immunoregulation appears to be involved (3). The exact etiology and pathogenesis of type 1 diabetes, however, is still unknown.

The model of the natural history of type 1 diabetes suggests stages that commence with a genetic susceptibility, autoimmunity without clinical disease, and finally clinical diabetes (4). Over the last 15 years, several groups have initiated prospective studies from birth examining the development of islet autoimmunity and diabetes (5–8), providing an opportunity to test such theoretical models in patients developing type 1 diabetes. Findings from these studies have significantly contributed to our current understanding of the pathogenesis of childhood diabetes. We now know when islet autoantibodies first appear in life, some of the genetic factors influencing their development, and which characteristics of islet autoantibodies are most associated with progression to type 1 diabetes. The relevant islet autoantibodies identified so far are autoantibodies to insulin (IAAs), the 65-kDa isoform of GAD (autoantibody to GAD [GADA]), and the protein tyrosine phosphatase–related molecules IA-2 (autoantibody to IA-2 [IA-2A]) and IA-2β (1). Using these autoantibodies, it is possible to trace events that occur during the preclinical phase of type 1 diabetes. Based on this principle, a multi-center study called The Environmental Determinants of Diabetes in the Young (TEDDY) has been launched to examine what environmental factors shape the autoimmunity that leads to type 1 diabetes (9). Information gained from all these studies in pre-diabetes will determine how we can predict type 1 diabetes, identify individuals who may benefit from intervention to halt autoimmunity, and ultimately determine how we may prevent type 1 diabetes. In this article, we will present our current view on the natural history of islet autoimmunity, factors that influence its appearance and progression, and characteristics that are associated with the development of type 1 diabetes.

From the Diabetes Research Institute, Munich, Germany.

Address correspondence and reprint requests to Dr. Anette-G. Ziegler, MD, Diabetes Research Institute, Koelner Platz 1, 80804 Munich, Germany. E-mail: anziegler@lrz.uni-muenchen.de.

Received for publication 2 March 2005 and accepted in revised form 28 April 2005.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

DAISY, Diabetes Autoimmunity Study in the Young; DIPP, Diabetes Prediction and Prevention; GADA, autoantibody to GAD; IA-2, autoantibody to IA-2; IAA, autoantibody to insulin.

PROSPECTIVE STUDIES FROM BIRTH INVESTIGATING THE NATURAL HISTORY OF TYPE 1 DIABETES

The German BABYDIAB study commenced in 1989 to prospectively follow islet autoantibody and diabetes development in newborn offspring of parents with type 1 diabetes (5). By today, it represents the longest-running prospective study from birth examining the risks for islet autoimmunity and type 1 diabetes. A total of 1,642 offspring have been recruited at birth and participated in the follow-up. The Finnish type 1 Diabetes Prediction and Prevention (DIPP) project (6) started in 1994 and follows newborn infants with increased genetic risk in close intervals for up to 10 years. The American Diabetes Autoimmunity Study in the Young (DAISY) (7) follows newborns with a genetically increased risk of type 1 diabetes from the general population and relatives of patients with type 1 diabetes since 1994, the Australian BABYDIAB study (8) follows newborns who have a first-degree relative with type 1 diabetes, and the Prospective Assessment of Newborns for Diabetes Autoimmunity (PANDA) study (10) from Florida follows newborns with a genetically high risk for type 1 diabetes.

EARLY CHARACTERISTICS OF ISLET AUTOIMMUNITY

Children developing type 1 diabetes in early childhood (<10 years of age) have the first signs of islet autoimmunity very early in life, with the majority by 2 years of age (11). Around 4% of offspring of parents with type 1 diabetes in the BABYDIAB study and around 6% of genetically at-risk infants from the general population in the Finnish DIPP study have developed islet autoantibodies by age 2 years (12,13). Children who develop autoantibodies within the first 2 years of life are those who most often develop multiple islet autoantibodies and progress to type 1 diabetes in childhood (Fig. 1) (12). Autoantibodies do not exclusively develop before age 2 years, but children who develop autoantibodies later have a slower progression to multiple antibodies and type 1 diabetes (12).

IAAs are usually the first autoantibodies detected (11). Children who progress to type 1 diabetes have IAAs of high affinity (14) and also develop GADAs concomitantly or soon after the first IAA response. Spreading of the response to IA-2 and IA-2β often follows (11–13,15).

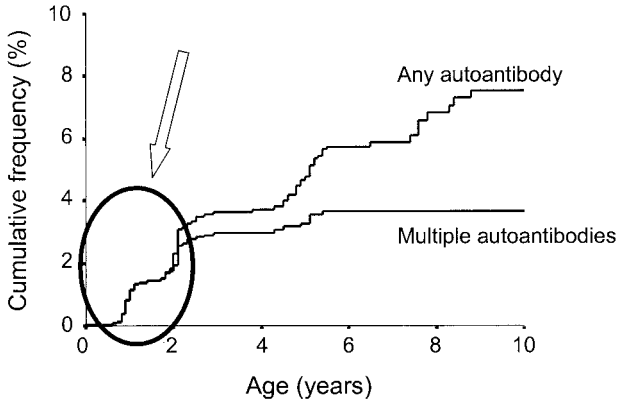

FIG. 1. Life-table islet autoantibody frequencies in 1,642 offspring of parents with type 1 diabetes from the BABYDIAB study. Frequencies are shown for any persistent positive autoantibody (IAA, GADA, and/or IA-2A) and for two or three positive autoantibodies. The majority of children who developed islet autoantibodies within the first 2 years of life developed multiple autoantibodies (circle).

Overall, autoantibodies of the IgG1 subclass are the dominant component of the early humoral immune response against each islet antigen, and other subclasses are usually only detected during high-titer peak IgG1 responses (16).

Spreading of autoimmunity is not only observed between antigens but also within one antigen. With respect to GADAs, epitope spreading is frequent (17). Autoantibody reactivity is initially directed against epitopes within the middle region (residues 235–444) and the COOH-terminal region (residues 440–585) of GAD65, indicating that either a rapid spreading of reactivity or simultaneous immunization against distinct GAD65 regions can occur (17,18). Reactivity to NH_2-terminal GAD65 epitopes in children is less common, weaker, and usually appears after that against central and COOH-terminal epitopes. GAD67 antibody reactivity is also relatively uncommon in children (17). Initial IA-2A reactivity is heterogeneous against the IA-2 juxtamembrane region and protein tyrosine phosphatase domains and is always directed against epitopes that are specific for IA-2. Spreading to IA-2β occurs together with an expansion of the autoimmune response to IA-2 (19).

Once islet autoantibodies appear, they usually persist, although significant fluctuations in antibody titer can be observed during the pre-diabetic phase (16,20). Of the three islet autoantibodies discussed, IAAs are reported to be the least persistent (13,15,21), and not all children who develop IAAs retain IAAs or subsequently develop multiple islet autoantibodies. One reason why IAAs, and indeed GADAs or IA-2As, may not persist is because they may be transferred from the mother with type 1 diabetes during pregnancy (20,22). Depending on the titer of antibodies in the mother, maternal insulin antibodies can persist in the circulation of the child for up to 1 year and maternal GADAs for up to 18 months (20,22). As a consequence, if antibodies are detected in a child early in life, it is important for the correct assignment of diabetes risk to distinguish whether these antibodies are indeed de novo–produced antibodies of the child or rather antibodies acquired from the mother. Antibody titer and different immunoglobulin subclasses may help distinguishing between maternal and nonmaternal autoantibodies in some cases (20).

FACTORS INFLUENCING THE DEVELOPMENT OF ISLET AUTOIMMUNITY

Genetic factors. Islet autoimmunity and type 1 diabetes develop in genetically susceptible individuals, and a major risk factor is an a priori first-degree type 1 diabetes family history (23). Familial aggregation of type 1 diabetes has been recognized for many years, and ~10–13% of newly diagnosed children have a first-degree relative affected with type 1 diabetes (23,24). With respect to family history, risk of developing islet autoimmunity varies depending on which relative(s) have type 1 diabetes. In the Diabetes Prevention Trial 1 (DPT-1), siblings of type 1 diabetic patients developed islet autoantibodies more frequently than offspring or parents of type 1 diabetic patients (25). The risk also depends on the number of relatives with type 1 diabetes. Analysis of the BABYDIAB cohort found that children's risk for islet autoantibodies was markedly increased if both parents or a parent and a sibling had type 1 diabetes compared with a single affected family member (26).

The major type 1 diabetes susceptibility genes are found within the HLA class II region on chromosome 6 (*IDDM1*) (23). HLA genes are thought to contribute as much as 50% of the genetic risk for type 1 diabetes. Remarkable with respect to HLA genotypes is that, whereas several genotypes confer increased risk, other genotypes confer protection (e.g., genotypes containing the HLA DQ6 haplotype) (27,28). In Caucasians, islet autoimmunity and type 1 diabetes are strongly associated with HLA DR3-DQ2 and DR4-DQ8 haplotypes (23), and recent studies from different European countries have confirmed that the HLA DR3-DQ2/DR4-DQ8 genotype is associated with the highest diabetes risk (29–34). This genotype is found in 20–30% of type 1 diabetic patients and in almost 50% of patients diagnosed in early childhood (23,31–34). Islet autoantibodies differ in their association with HLA haplotypes. GADAs are more frequent in patients with HLA DR3-DQ2 (27,35), whereas IAAs and IA-2As are more frequent in patients with HLA DR4-DQ8 (27,35–37). Patients without these haplotypes are more frequently islet autoantibody negative (13,27,36,37).

HLA haplotypes can also be used to identify children who are more likely to develop islet autoantibodies. Results from the BABYDIAB study, the DIPP study, and the DAISY study consistently show that children carrying high-risk HLA genotypes have a higher risk for early and more frequent development of islet autoantibodies in infancy (13,36,37). Among BABYDIAB offspring, the risk of developing islet autoantibodies by age 2 years is 20% in individuals who have the high-risk DR3-DQ2/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotypes compared with 2.7% in offspring without these genotypes, and, overall, 50% of islet autoantibody–positive offspring have at least one of these genotypes (36) (Fig. 2). HLA typing can also help to identify islet autoantibodies that have type 1 diabetes–relevant characteristics (see below). For example, the DAISY study found the development of persistent islet autoantibodies to be associated with the HLA DR3-DQ2/DR4-DQ8 genotype both in relatives of type 1 diabetic patients and in children from the general population, whereas transient islet autoantibodies were not correlated with known genetic risk factors (15,37). The BABYDIAB study showed that the genetic risk factors found in children who developed multiple islet autoantibodies were absent in children who developed single islet autoantibod-

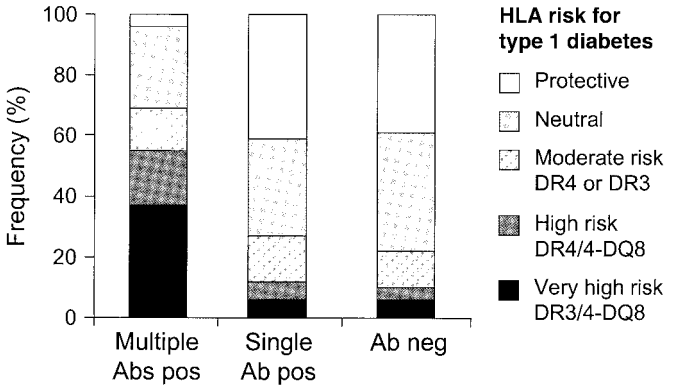

FIG. 2. Frequency of HLA genotypes within the BABYDIAB cohort. Children are grouped according to their islet autoantibody status, and HLA genotypes are grouped according to their conferred type 1 diabetes risk. Children who develop multiple islet autoantibodies (Multiple Abs pos) have HLA genotypes that are found in type 1 diabetes, whereas children who developed single antibodies (Single Ab pos) had HLA genotypes similar to children who were autoantibody negative (Ab neg). Single autoantibody–positive children have few type 1 diabetes risk genotypes.

ies (26,33) (Fig. 3). Similarly, the Australian BABYDIAB study found that HLA DR4-DQ8 and DR3-DQ2 were more prevalent in children who developed persistent multiple islet autoantibodies than in children who were transient or single antibody positive (21). The Karlsburg schoolchildren study found children with multiple islet autoantibodies, but not in subjects with single islet autoantibodies—they had HLA allele frequencies that were similar to frequencies found in type 1 diabetes (38). Finally, high affinity IAAs are associated with high-risk HLA DR4-DQ8 containing genotypes (14), and most IA-2A–positive offspring with the HLA DR3-DQ2/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotypes immediately develop a broad antibody reactivity to multiple epitopes expressed in both IA-2 and IA-2β (19). Clearly, HLA genotype and probably other type 1 diabetes susceptibility genes may affect the magnitude and breadth of the autoimmune response.

A second genetic susceptibility locus has been mapped by a variable number of tandem repeat (VNTR) in the insulin gene (*INS*) promoter region (*IDDM2*). Risk has been suggested to be conferred by different expression of the insulin protein in the thymus, leading to defective central tolerance to the insulin molecule (3,39). In accordance with this, IAAs are less frequent in patients or relatives who have the type 1 diabetes protective *INS* VNTR class I/III or III/III genotypes (33,35). Although genotype variation at *INS* significantly affects type 1 diabetes susceptibility at all HLA risk categories, there is significant heterogeneity in the distribution of *INS* genotypes in patients with different HLA genotypes (40,41). Combining HLA and *INS* genotyping, therefore, will improve type 1 diabetes risk stratification (33), but not in a manner strictly predicted from the multiplicative model.

Modification of risk for islet autoimmunity and type 1 diabetes by the environment is also likely to be genotype specific, as shown for early exposure to cereals (see below) (42,43). Further evidence comes from twin studies that etiological factors other than the genetic background of an individual must play a role as well in type 1 diabetes pathogenesis. Here, the concordance of type 1 diabetes between monozygotic twins is up to 50%, whereas between dizygotic twins it is only ~10% (44). Although such differences in the concordance rates between identical

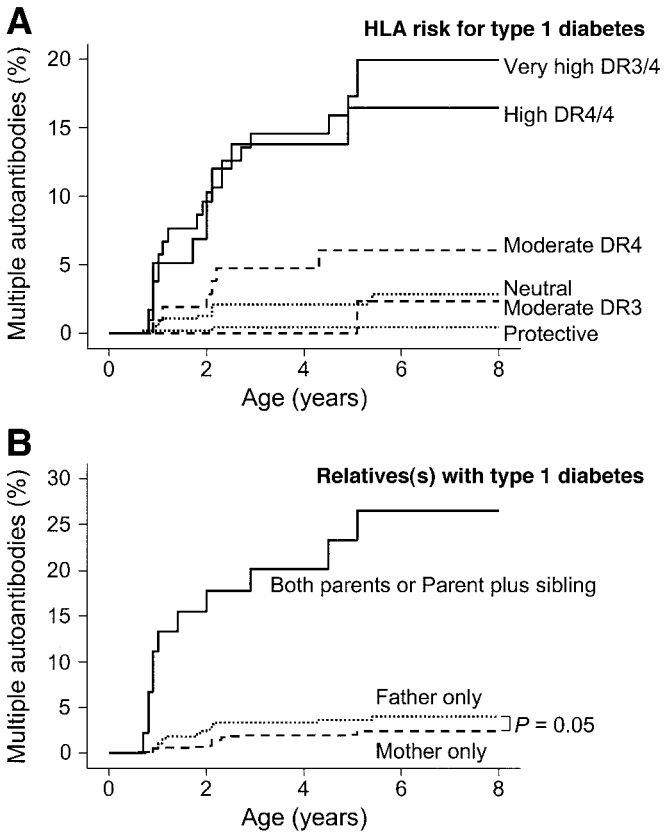

FIG. 3. Cumulative risk for the development of multiple islet autoantibodies in BABYDIAB offspring in relation to HLA genotype (*A*) and type 1 diabetes family history (*B*). Children have a higher risk to progress to multiple autoantibodies if they have either a high-risk HLA genotype or at least two family members affected with type 1 diabetes.

and nonidentical twins clearly underline the impact of genes on the development of type 1 diabetes, they also show that genetic susceptibility alone cannot be the ultimate cause for the disease.

Environmental factors. Environmental agents that are suspected to trigger β-cell autoimmunity in genetically susceptible individuals include dietary factors and common viral infections. By today, however, no single factor has been identified that can induce the process of autoimmune β-cell destruction, and so far available data are partially conflicting.

Dietary factors. Among candidate dietary factors that may influence the development of islet autoimmunity and type 1 diabetes are a short duration of breast-feeding, the uptake of cow's milk proteins during the first months of life, and the early introduction of cereals. It has been suggested by some investigators that breast-feeding may protect against type 1 diabetes (45), whereas early introduction of supplementary milk feeding may promote the development of islet autoantibodies and type 1 diabetes (46). The debate as to whether cow's milk ingestion increases the risk for islet autoimmunity has been controversial (47–50). Prospective studies in at-risk neonates have not demonstrated an increased risk for developing islet autoantibodies in children who were not breast-fed and received cow's milk proteins early in life (48,49). Nevertheless, an interventional trial is currently ongoing committed to exploring the impact of cow's milk proteins in an infant's diet on the development of type 1 diabetes (the TRIGR study) (51). Another candidate factor is the

early introduction of cereals in an infant's diet. In two recent studies, it was suggested that risk to develop islet autoimmunity is increased in children who experienced an exposure to cereal proteins, and particularly gluten, early in life (42,43). Again, a German-wide interventional trial (BABYDIET study) has been initiated to investigate whether delaying dietary gluten influences the development of islet autoimmunity in newborns at genetically high risk for type 1 diabetes (52).

Viral infections. Viruses may induce islet autoimmunity by molecular mimicry between amino acid sequences of viral peptides and islet antigens that can activate autoreactive T-cells, by bystander activation and expansion of previously activated autoreactive T-cells at a local inflammatory site (including the spreading of T-cell responses to other epitopes on islet antigens), and by nonspecific activation of autoreactive T-cells through viral superantigens (53). In animal models, the administration of poly-IC (poly inosinic cytodylic acid), a mimic of viral double-stranded RNA, can trigger insulitis in normal nondiabetic strains (54). Therefore, it is possible that many common RNA viral infections may induce islet autoimmunity in genetically susceptible patients. The most compelling evidence for an association between viral infection and subsequent development of type 1 diabetes is the high prevalence of diabetes among children with congenital rubella infection (55). Acute enterovirus infections have also been associated with the appearance of serum autoantibodies to islet antigens by some investigators (8,56), but others could not confirm such observations (57,58). In a recent study from Finland, combined serum and stool analyses in 878 children of the DIPP cohort revealed a significant higher frequency of enterovirus infections in children at the time of islet autoantibody appearance compared with age-matched children without signs of islet autoimmunity (59).

Maternal transfer of islet autoantibodies. The influence of maternally transmitted islet autoantibodies on the development of islet autoimmunity and type 1 diabetes has been examined in both animal models and humans. In the NOD mouse, removal of maternally transmitted immunoglobulin prevented spontaneous diabetes in offspring mice, suggesting that maternal antibodies present during gestation including islet autoantibodies could be important factors in the pathogenesis of β-cell destruction (60). Further studies in mice looking specifically at whether maternal insulin antibodies influence diabetes development reported controversial findings (61,62), but in neither of these studies could maternal transfer of IAAs be the sole cause for the dramatic protection of diabetes observed by fetal transfer experiments.

In the BABYDIAB study, 86% of offspring from mothers with type 1 diabetes have antibodies to exogenously administered insulin at birth, and 66% of offspring have GADAs and/or IA-2As at birth. The presence or absence of maternal insulin antibodies did not affect the risk of developing diabetes-associated autoantibodies and type 1 diabetes in the child, but offspring with GADAs and/or IA-2As at birth had a significantly lower diabetes risk than offspring who were autoantibody negative at birth. This is particularly the case in offspring without the diabetes-associated HLA genotype DR3-DQ2/DR4-DQ8 (63). Therefore, and in contrast to the data from animal studies, these findings in humans do not support the hypothesis that fetal exposure to islet autoantibodies increases diabetes risk, but rather suggest that fetal exposure to GADAs and/or IA-2As may protect from future endogenous islet autoimmunity and type 1 diabetes (63). Consistent with this observation is the overall decreased risk to develop islet autoimmunity and diabetes in offspring of mothers with type 1 diabetes compared with that of offspring of fathers with type 1 diabetes and nondiabetic mothers (64,65). Data from the DAISY study was consistent with the findings from the BABYDIAB study in that the presence of cord blood islet autoantibodies was not predictive of subsequent development of islet autoimmunity in children who had diabetes-susceptible HLA genotypes (66).

PROGRESSION FROM ISLET AUTOANTIBODY POSITIVITY TO TYPE 1 DIABETES

The sequence of events before type 1 diabetes onset. The first islet autoantibody appearance in BABYDIAB offspring is usually characterized by an early IgG1 peak response to one or more islet autoantigens and, in some children, acute fulminant β-cell destruction, and diabetes onset can occur soon after this initial autoantibody response (16). In most children, first peak antibody levels decline and autoantibodies against other β-cell antigens may arise sequentially over several years, suggesting regulation and spreading of islet autoimmunity in childhood (12,13,15). Developing multiple islet autoantibodies is an important step in the pathogenesis of the disease.

Factors affecting progression to disease

Genes. Genes clearly determine the likelihood of developing islet autoantibodies and progression to multiple islet autoantibodies. It remains controversial, however, if progression from multiple islet autoantibodies to type 1 diabetes is influenced by genetic factors. Most studies have stratified progression to type 1 diabetes using the HLA genotype in autoantibody-positive relatives without distinguishing multiple- from single-antibody positivity. In these studies, the presence of the protective HLA DQB1*0602 allele was associated with decreased progression to type 1 diabetes (27,28), and the high-risk HLA DR3/4 genotype was associated with increased progression to type 1 diabetes (15,21,36,37). As seen in the BABYDIAB cohort, however, these alleles and genotypes only associate with protection or susceptibility for the development of multiple islet autoantibodies and not single islet autoantibodies (26,33). Our own analysis of progression to type 1 diabetes in relatives who have multiple islet autoantibodies finds no striking stratification by HLA genotype or indeed by multiple type 1 diabetes family history (Fig. 4). If true, then it is also likely that family history per se will have no bearing on the rate of progression to type 1 diabetes in multiple islet autoantibody–positive subjects.

Environment. Like genes, the same environmental factors discussed as triggers of islet autoimmunity are also discussed as accelerators or protectors of progression to type 1 diabetes. Few hard data are available, however. Very different incidences in type 1 diabetes between countries that have similar prevalences of autoimmunity is suggestive that environment can influence progression to type 1 diabetes (67,68), but these studies suffer from relatively small numbers of subjects with islet autoantibodies. The BABYDIAB study found that events before the development of autoimmunity could apparently affect progression to type 1 diabetes after the development of islet autoantibodies (69). The bacillus Calmette-Guerin vaccination in the first days or weeks of life had no influence on

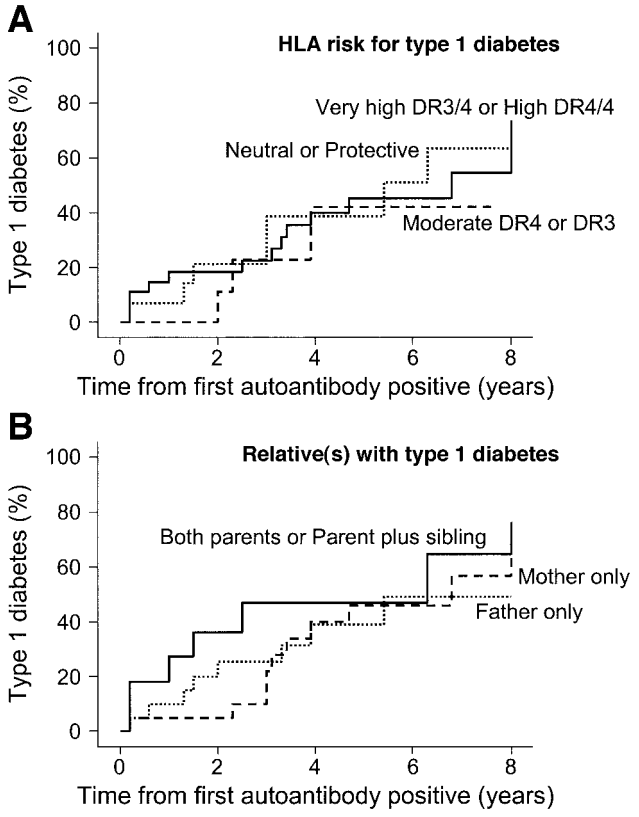

FIG. 4. Cumulative risk for progression to type 1 diabetes in BABYDIAB offspring who developed multiple islet autoantibodies in relation to HLA genotype (*A*) and type 1 diabetes family history (*B*). Neither high-risk HLA genotype nor multiple type 1 diabetes family history significantly increased type 1 diabetes risk in children once multiple autoantibodies have developed.

the development of islet autoantibodies, including multiple islet autoantibodies. However, the bacillus Calmette-Guerin vaccination modified the rate of progression to type 1 diabetes in the autoantibody-positive children (69).

Age. Age affects the risk of progression to type 1 diabetes (12). In the BABYDIAB cohort, almost all offspring who develop islet autoantibodies by age 2 years had IAAs in their first sample and rapidly developed multiple islet autoantibodies. In contrast, only one-third of offspring who develop islet autoantibodies at age 5 or 8 years have IAAs in their first autoantibody-positive sample, and progression to multiple autoantibodies is slow in these children (12). Accordingly, the risk for developing type 1 diabetes can also be stratified on the basis of how early islet autoantibodies develop. Progression to diabetes is significantly faster in individuals who have multiple islet autoantibodies already within the first year of life than in individuals who develop multiple islet autoantibodies at age 2 or 5 years (12). Thus, delaying progression to multiple antibodies may be effective in markedly delaying diabetes onset.

Characteristics of autoantibodies in progressors versus nonprogressors. It is conceivable that the development of diabetes is accompanied by a maturation of the autoimmune response. Supporting this concept, it has been shown in relatives of type 1 diabetic patients that diabetes risk and time of progression to diabetes directly correlates with the titer of cytoplasmic islet cell antibodies and with the number of different islet autoantibodies present (70,71), suggesting that the intensity of the humoral response may reflect the stage of β-cell destruction. It is now well established that subjects with multiple islet autoantibodies have considerably increased rates of progression to type 1 diabetes than subjects with only one islet autoantibody (72–75). Substantial effort has been made to identify other disease-specific characteristics of autoantibodies that will help distinguish who will and who won't develop type 1 diabetes and who will develop type 1 diabetes early and late (14,74). In general, the magnitude of the autoimmune response is an important predictor of type 1 diabetes risk.

Affinity determines progression. The affinity of IAAs has been found to vary considerably between IAA-positive children. IAAs range from high-affinity IgG in most individuals through to low-affinity cold-reactive IgM antibodies in others (14). Children who developed high-affinity IAAs ($K_d > 10^9$ l/mol) have persistent IAAs, develop multiple islet autoantibodies, and have a 50% risk for developing type 1 diabetes within 6 years. In contrast, children who have IAAs of lower affinity infrequently progress to multiple islet autoantibodies or type 1 diabetes. High-affinity IAAs differ from lower-affinity IAAs in their insulin-binding characteristics in a manner consistent with distinct epitope recognition and in contrast to the lower-affinity IAAs (which often do not bind proinsulin); the epitope associated with high-affinity IAAs is also expressed on the proinsulin molecule (14). From these findings, one can postulate that an early exposure to insulin or proinsulin is relevant to disease pathogenesis. The very early appearance of high-affinity IAAs with uniform-binding characteristics in almost all children who subsequently develop multiple autoantibodies or diabetes suggests a consistent mode of immunization.

Progressors have broader autoantibody responses. The breadth of the autoantibody response can be measured by the number of autoantibody epitopes it is directed against, and probably by the subclass usage. Broad multiple subclass autoantibodies are usually synonymous with high titer, but these features can be separate indicators of disease risk also in low titer autoantibody-positive subjects (74). In a recent analysis of autoantibody-positive relatives followed for up to 15 years, the highest risks for type 1 diabetes were associated with high titer IAA and IA-2A responses, with the appearance of antibody subclasses IgG2, IgG3, and/or IgG4 of IAA and IA-2A and antibodies to the IA-2–related molecule IA-2β (74). Using various combinations of these islet autoantibody characteristics, it was possible to stratify diabetes risk from <10% to ~90% within 5 years. In contrast, titer, subclass usage, and epitope specificity of GADAs could not further stratify risk. These data also indicate that there is a hierarchy in the diabetes risk associated with the different islet autoantibodies. IA-2As are associated with a higher risk for type 1 diabetes than GADAs or IAAs (73,74), and within IA-2A–positive subjects, those with IA-2A reacting against IA-2β have a higher diabetes risk than those who are IA-2β antibody negative (74). The study was able to identify risk assessment models that could stratify type 1 diabetes more effectively than the current practice of islet autoantibody number.

CONCLUSIONS

Tracing the natural history of type 1 diabetes has been greatly facilitated by our ability to measure islet autoantibodies and by prospective studies in infants. We have

learned much about the appearance of autoimmunity and the characteristics of autoantibodies that are associated with progression to type 1 diabetes. We are, however, still faced with some difficult but exciting challenges to use this knowledge for disease prevention. We still have little idea of the etiologic mechanisms that trigger autoimmunity and promote progression to disease, nor do we have ready access to the autoreactive T-cells within the pancreas that are responsible for disease or an ability to quantify and characterize these cells. Advances in these areas are necessary if we are to fully understand the autoimmune pathogenesis of type 1 diabetes. An international initiative sponsored by the National Institutes of Health has recently commenced an ambitious study (The Environmental Determinants of Diabetes in the Young [TEDDY] study) to address the early pathogenic mechanisms operating in islet autoimmunity (9). These are long but necessary studies that we hope will provide us with the knowledge of which environmental factors determine the disease process and therefore an avenue to prevent autoimmunity without facing the arduous task of modifying an immune response determined to destroy the β-cell.

ACKNOWLEDGMENTS

This work was supported by grants from Deutsche Forschungsgemeinschaft (ZI 310/12-6) and the Juvenile Diabetes Foundation (JDRF 1-2003-646).

REFERENCES

1. Atkinson MA, Eisenbarth GS: Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 358:221–229, 2001
2. Gale EA: The rise of childhood type 1 diabetes in the 20th century. *Diabetes* 51:3353–3361, 2002
3. Mathis D, Benoist C: Back to central tolerance. *Immunity* 20:509–516, 2004
4. Eisenbarth GS: Type I diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 314:1360–1368, 1986
5. Ziegler AG, Hillebrand B, Rabl W, Mayrhofer M, Hummel M, Mollenhauer U, Vordemann J, Lenz A, Standl E: On the appearance of islet associated autoimmunity in offspring of diabetic mothers: a prospective study from birth. *Diabetologia* 36:402–408, 1993
6. Kupila A, Muona P, Simell T, Arvilommi P, Savolainen H, Hamalainen AM, Korhonen S, Kimpimaki T, Sjoroos M, Ilonen J, Knip M, Simell O: Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. *Diabetologia* 44:290–297, 2001
7. Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, McDuffie RS Jr, Hamman RF, Klingensmith G, Eisenbarth GS, Erlich HA: Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). *Diabetologia* 39:807–812, 1996
8. 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* 49:1319–1324, 2000
9. TEDDY investigators. Denver, Augusta/Gainesville, Seattle, Tampa (U.S.), Munich (Germany), Turku/Tampere/Oulu (Finland), Malmö (Sweden): The environmental determinants of diabetes in the young study (Abstract). In *Programme & Abstracts.* Cambridge, U.K., Immunology of Diabetes Society Conference, 2004, p. 160
10. Bennett Johnson S, Baughcum AE, Carmichael SK, She JX, Schatz DA: Maternal anxiety associated with newborn genetic screening for type 1 diabetes. *Diabetes Care* 27:392–397, 2004
11. 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* 48:460–468, 1999
12. Hummel M, Bonifacio E, Schmid S, Walter M, Knopff A, Ziegler AG: Brief communication: early appearance of islet autoantibodies predicts childhood type 1 diabetes in offspring of diabetic parents. *Ann Intern Med* 140:882–886, 2004
13. Kimpimaki T, Kulmala P, Savola K, Kupila A, Korhonen S, Simell T, Ilonen J, Simell O, Knip M: Natural history of beta-cell autoimmunity in young children with increased genetic susceptibility to type 1 diabetes recruited from the general population. *J Clin Endocrinol Metab* 87:4572–4579, 2002
14. Achenbach P, Koczwara K, Knopff A, Naserke H, Ziegler AG, Bonifacio E: Mature high-affinity immune responses to (pro)insulin anticipate the autoimmune cascade that leads to type 1 diabetes. *J Clin Invest* 114:589–597, 2004
15. Barker JM, Barriga KJ, Yu L, Miao D, Erlich HA, Norris JM, Eisenbarth GS, Rewers M: Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). *J Clin Endocrinol Metab* 89:3896–3902, 2004
16. Bonifacio E, Scirpoli M, Kredel K, Fuchtenbusch M, Ziegler AG: Early autoantibody responses in prediabetes are IgG1 dominated and suggest antigen-specific regulation. *J Immunol* 163:525–532, 1999
17. Bonifacio E, Lampasona V, Bernasconi L, Ziegler AG: Maturation of the humoral autoimmune response to epitopes of GAD in preclinical childhood type 1 diabetes. *Diabetes* 49:202–208, 2000
18. Hoppu S, Ronkainen MS, Kulmala P, Akerblom HK, Knip M: GAD65 antibody isotypes and epitope recognition during the prediabetic process in siblings of children with type I diabetes. *Clin Exp Immunol* 136:120–128, 2004
19. Naserke HE, Ziegler AG, Lampasona V, Bonifacio E: Early development and spreading of autoantibodies to epitopes of IA-2 and their association with progression to type 1 diabetes. *J Immunol* 161:6963–6969, 1998
20. Naserke HE, Bonifacio E, Ziegler AG: Prevalence, characteristics and diabetes risk associated with transient maternally acquired islet antibodies and persistent islet antibodies in offspring of parents with type 1 diabetes. *J Clin Endocrinol Metab* 86:4826–4833, 2001
21. Colman PG, Steele C, Couper JJ, Beresford SJ, Powell T, Kewming K, Pollard A, Gellert S, Tait B, Honeyman M, Harrison LC: Islet autoimmunity in infants with a type I diabetic relative is common but is frequently restricted to one autoantibody. *Diabetologia* 43:203–209, 2000
22. Hamalainen AM, Ronkainen MS, Akerblom HK, Knip M: Postnatal elimination of transplacentally acquired disease-associated antibodies in infants born to families with type 1 diabetes: The Finnish TRIGR Study Group: Trial to Reduce IDDM in the Genetically at Risk. *J Clin Endocrinol Metab* 85:4249–4253, 2000
23. Redondo MJ, Eisenbarth GS: Genetic control of autoimmunity in type I diabetes and associated disorders. *Diabetologia* 45:605–622, 2002
24. Dahlquist G, Blom L, Holmgren G, Hagglof B, Larsson Y, Sterky G, Wall S: The epidemiology of diabetes in Swedish children 0–14 years: a six-year prospective study. *Diabetologia* 28:802–808, 1985
25. Yu L, Cuthbertson DD, Eisenbarth GS, Krischer JP: Diabetes Prevention Trial 1: prevalence of GAD and ICA512 (IA-2) autoantibodies by relationship to proband. *Ann N Y Acad Sci* 958:254–258, 2002
26. Bonifacio E, Hummel M, Walter M, Schmid S, Ziegler AG: IDDM1 and multiple family history of type 1 diabetes combine to identify neonates at high risk for type 1 diabetes. *Diabetes Care* 27:2695–2700, 2004
27. Kulmala P, Savola K, Reijonen H, Veijola R, Vahasalo P, Karjalainen J, Tuomilehto-Wolf E, Ilonen J, Tuomilehto J, Akerblom HK, Knip M: Genetic markers, humoral autoimmunity, and prediction of type 1 diabetes in siblings of affected children: Childhood Diabetes in Finland Study Group. *Diabetes* 49:48–58, 2000
28. Pugliese A, Gianani R, Moromisato R, Awdeh ZL, Alper CA, Erlich HA, Jackson RA, Eisenbarth GS: HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM. *Diabetes* 44:608–613, 1995
29. Hermann R, Bartsocas CS, Soltesz G, Vazeou A, Paschou P, Bozas E, Malamitsi-Puchner A, Simell O, Knip M, Ilonen J: Genetic screening for individuals at high risk for type 1 diabetes in the general population using HLA class II alleles as disease markers: a comparison between three European populations with variable rates of disease incidence. *Diabete Metab Res Rev* 20:322–329, 2004
30. Van der Auwera BJ, Schuit FC, Weets I, Ivens A, Van Autreve JE, Gorus FK: Relative and absolute HLA-DQA1-DQB1 linked risk for developing type I diabetes before 40 years of age in the Belgian population: implications for future prevention studies. *Hum Immunol* 63:40–50, 2002
31. Lambert AP, Gillespie KM, Thomson G, Cordell HJ, Todd JA, Gale EA, Bingley PJ: Absolute risk of childhood-onset type 1 diabetes defined by human leukocyte antigen class II genotype: a population-based study in the United Kingdom. *J Clin Endocrinol Metab* 89:4037–4043, 2004
32. Gillespie KM, Gale EA, Bingley PJ: High familial risk and genetic susceptibility in early onset childhood diabetes. *Diabetes* 51:210–214, 2002
33. Walter M, Albert E, Conrad M, Keller E, Hummel M, Ferber K, Barratt BJ, Todd JA, Ziegler AG, Bonifacio E: IDDM2/insulin VNTR modifies risk conferred by IDDM1/HLA for development of type 1 diabetes and associated autoimmunity. *Diabetologia* 46:712–720, 2003
34. Nejentsev S, Sjoroos M, Soukka T, Knip M, Simell O, Lovgren T, Ilonen J:

Population-based genetic screening for the estimation of type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. *Diabet Med* 16:985–992, 1999
35. Graham J, Hagopian WA, Kockum I, Li LS, Sanjeevi CB, Lowe RM, Schaefer JB, Zarghami M, Day HL, Landin-Olsson M, Palmer JP, Janer-Villanueva M, Hood L, Sundkvist G, Lernmark A, Breslow N, Dahlquist G, Blohme G: Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. *Diabetes* 51:1346–1355, 2002
36. Schenker M, Hummel M, Ferber K, Walter M, Keller E, Albert ED, Janka HU, Kastendiek C, Sorger M, Louwen F, Ziegler AG: Early expression and high prevalence of islet autoantibodies for DR3/4 heterozygous and DR4/4 homozygous offspring of parents with type I diabetes: the German BABY-DIAB study. *Diabetologia* 42:671–677, 1999
37. Yu J, Yu L, Bugawan TL, Erlich HA, Barriga K, Hoffman M, Rewers M, Eisenbarth GS: Transient antiislet autoantibodies: infrequent occurrence and lack of association with "genetic" risk factors. *J Clin Endocrinol Metab* 85:2421–2428, 2000
38. Schlosser M, Wassmuth R, Strebelow M, Rjasanowski I, Ziegler M: Multiple and high-titer single autoantibodies in schoolchildren reflecting the genetic predisposition for type 1 diabetes. *Ann N Y Acad Sci* 1005:98–108, 2003
39. Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, Wickramasinghe S, Colle E, Polychronakos C: Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet* 15:289–292, 1997
40. Laine AP, Hermann R, Knip M, Simell O, Akerblom HK, Ilonen J: The human leukocyte antigen genotype has a modest effect on the insulin gene polymorphism-associated susceptibility to type 1 diabetes in the Finnish population. *Tissue Antigens* 63:72–74, 2004
41. Motzo C, Contu D, Cordell HJ, Lampis R, Congia M, Marrosu MG, Todd JA, Devoto M, Cucca F: Heterogeneity in the magnitude of the insulin gene effect on HLA risk in type 1 diabetes. *Diabetes* 53:3286–3291, 2004
42. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E: Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. *JAMA* 290:1721–1728, 2003
43. Norris JM, Barriga K, Klingensmith G, Hoffman M, Eisenbarth GS, Erlich HA, Rewers M: Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *JAMA* 290:1713–1720, 2003
44. Kyvik KO, Green A, Beck-Nielsen H: Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. *BMJ* 311:913–917, 1995
45. Sadauskaite-Kuehne V, Ludvigsson J, Padaiga Z, Jasinskiene E, Samuelsson U: Longer breastfeeding is an independent protective factor against development of type 1 diabetes mellitus in childhood. *Diabete Metab Res Rev* 20:150–157, 2004
46. Vaarala O, Knip M, Paronen J, Hamalainen AM, Muona P, Vaatainen M, Ilonen J, Simell O, Akerblom HK: Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes* 48:1389–1394, 1999
47. Martin JM, Trink B, Daneman D, Dosch HM, Robinson B: Milk proteins in the etiology of insulin-dependent diabetes mellitus (IDDM). *Ann Med* 23:447–452, 1991
48. 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 beta-cell autoimmunity. Diabetes Autoimmunity Study in the Young (DAISY). *JAMA* 276:609–614, 1996
49. Hummel M, Fuchtenbusch M, Schenker M, Ziegler AG: No major association of breast-feeding, vaccinations, and childhood viral diseases with early islet autoimmunity in the German BABYDIAB Study. *Diabetes Care* 23:969–974, 2000
50. Virtanen SM, Laara E, Hypponen E, Reijonen H, Rasanen L, Aro A, Knip M, Ilonen J, Akerblom HK: Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: a nested case-control study of siblings of children with diabetes: Childhood Diabetes in Finland Study Group. *Diabetes* 49:912–917, 2000
51. Sadeharju K, Hamalainen AM, Knip M, Lonnrot M, Koskela P, Virtanen SM, Ilonen J, Akerblom HK, Hyoty H: Enterovirus infections as a risk factor for type I diabetes: virus analyses in a dietary intervention trial. *Clin Exp Immunol* 132:271–277, 2003
52. Schmid S, Buuck D, Knopff A, Bonifacio E, Ziegler AG: BABYDIET, a feasibility study to prevent the appearance of islet autoantibodies in relatives of patients with type 1 diabetes by delaying exposure to gluten. *Diabetologia* 47:1130–1131, 2004
53. Wucherpfennig KW: Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest* 108:1097–1104, 2001
54. Moriyama H, Wen L, Abiru N, Liu E, Yu L, Miao D, Gianani R, Wong FS, Eisenbarth GS: Induction and acceleration of insulitis/diabetes in mice with a viral mimic (polyinosinic-polycytidylic acid) and an insulin self-peptide. *Proc Natl Acad Sci U S A* 99:5539–5544, 2002
55. Menser MA, Forrest JM, Bransby RD: Rubella infection and diabetes mellitus. *Lancet* 1:57–60, 1978
56. Lonnrot M, Korpela K, Knip M, Ilonen J, Simell O, Korhonen S, Savola K, Muona P, Simell T, Koskela P, Hyoty H: Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. *Diabetes* 49:1314–1318, 2000
57. 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* 46:161–168, 1997
58. Fuchtenbusch M, Irnstetter A, Jager G, Ziegler AG: No evidence for an association of coxsackie virus infections during pregnancy and early childhood with development of islet autoantibodies in offspring of mothers or fathers with type 1 diabetes. *J Autoimmun* 17:333–340, 2001
59. Salminen KK, Vuorinen T, Oikarinen S, Helminen M, Simell S, Knip M, Ilonen J, Simell O, Hyoty H: Isolation of enterovirus strains from children with preclinical type 1 diabetes. *Diabet Med* 21:156–164, 2004
60. Greeley SA, Katsumata M, Yu L, Eisenbarth GS, Moore DJ, Goodarzi H, Barker CF, Naji A, Noorchashm H: Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. *Nat Med* 8:399–402, 2002
61. Koczwara K, Ziegler AG, Bonifacio E: Maternal immunity to insulin does not affect diabetes risk in progeny of non obese diabetic mice. *Clin Exp Immunol* 136:56–59, 2004
62. Melanitou E, Devendra D, Liu E, Miao D, Eisenbarth GS: Early and quantal (by litter) expression of insulin autoantibodies in the nonobese diabetic mice predict early diabetes onset. *J Immunol* 173:6603–6610, 2004
63. Koczwara K, Bonifacio E, Ziegler AG: Transmission of maternal islet antibodies and risk of autoimmune diabetes in offspring of mothers with type 1 diabetes. *Diabetes* 53:1–4, 2004
64. Warram JH, Krolewski AS, Gottlieb MS, Kahn CR: Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N Engl J Med* 311:149–152, 1984
65. Pociot F, Norgaard K, Hobolth N, Andersen O, Nerup J: A nationwide population-based study of the familial aggregation of type 1 (insulin-dependent) diabetes mellitus in Denmark: Danish Study Group of Diabetes in Childhood. *Diabetologia* 36:870–875, 1993
66. Stanley HM, Norris JM, Barriga K, Hoffman M, Yu L, Miao D, Erlich HA, Eisenbarth GS, Rewers M: Is presence of islet autoantibodies at birth associated with development of persistent islet autoimmunity? The Diabetes Autoimmunity Study in the Young (DAISY). *Diabetes Care* 27:497–502, 2004
67. Samuelsson U, Sadauskaite V, Padaiga Z, Ludvigsson J: A fourfold difference in the incidence of type 1 diabetes between Sweden and Lithuania but similar prevalence of autoimmunity. *Diabetes Res Clin Pract* 66:173–181, 2004
68. Marciulionyte D, Williams AJ, Bingley PJ, Urbonaite B, Gale EA: A comparison of the prevalence of islet autoantibodies in children from two countries with differing incidence of diabetes. *Diabetologia* 44:16–21, 2001
69. Huppmann M, Baumgarten A, Ziegler AG, Bonifacio E: Neonatal Bacille Calmette-Guerin vaccination and type 1 diabetes mellitus. *Diabetes Care* 8:1204–1206, 2005
70. Bonifacio E, Bingley PJ, Shattock M, Dean BM, Dunger D, Gale EA, Bottazzo GF: Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet* 335:147–149, 1990
71. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, Bottazzo GF, Gale EA: Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 43:1304–1310, 1994
72. Kulmala P, Savola K, Petersen JS, Vahasalo P, Karjalainen J, Lopponen T, Dyrberg T, Akerblom HK, Knip M: Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes: a population-based study: the Childhood Diabetes in Finland Study Group. *J Clin Invest* 101:327–336, 1998
73. Decochez K, De Leeuw IH, Keymeulen B, Mathieu C, Rottiers R, Weets I, Vandemeulebroucke E, Truyen I, Kaufman L, Schuit FC, Pipeleers DG, Gorus FK: IA-2 autoantibodies predict impending type I diabetes in siblings of patients. *Diabetologia* 45:1658–1666, 2002
74. Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AJ, Bingley PJ, Bonifacio E, Ziegler AG: Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. *Diabetes* 53:384–392, 2004
75. Bingley PJ, Williams AJ, Gale EA: Optimized autoantibody-based risk assessment in family members: implications for future intervention trials. *Diabetes Care* 22:1796–1801, 1999