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.

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).
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 NH2-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-2B 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).

**FIG. 1.** Life-table islet autoantibody frequencies in 1,462 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).

**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 off-spring 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 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-
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 Karlsruhe 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 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 (polyinosinic cytidylic acid), a mimic of viral double-stranded RNA, can trigger insulinitis 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...
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 autoantibodies 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.

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